Growth, phenological, and yield response of upland rice (*Oryza sativa* L. cv. Nerica 4®) to water stress during different growth stages

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Highlights

- Water stress during early reproductive stage increases thermal time to maturity.
- Delaying of flowering and anthesis is not dependent on tiller abortion.
- Spikelet sterility with stress during tillering is due to limited assimilate availability.
- Recover ability and highly elastic growth is characteristic of early reproductive stress.
- Considerable water savings without substantial yield penalty is possible in some stages.

Abstract

Rice (*Oryza sativa* L.) grown in uplands is exposed to variable soil water conditions and unpredictable periods of water stress (WS). The study was conducted to determine the impacts of WS imposed at different phenological stages of upland rice on growth, phenology, recovery of source size, yield and water use efficiency (WUE). The popular cv. Nerica 4® grown in Africa was sown under a rain-out shelter for two seasons. Treatments included a well-watered control (CT) and stress imposed by withholding water for the duration of different stages: tillering (Ti), panicle initiation (PI), anthesis (AT) and grain filling (GF). Name codes used for treatments were thus: CT, STi, SPI, SAT and SGF. When water was withheld, soil water content in the 0 – 0.6 m soil layer dropped to approximately 50 % of plant available water, while stomatal conductance of the abaxial leaf surface and leaf area index decreased significantly, suggesting that severe stress was experienced. Growing degree days to reach the different growth stages were roughly equal in both seasons, even though sowing was in the mid-summer of 2013/2014 and early summer of 2014/2015, respectively. Time to reach peak tillering could not be explained by temperatures and cumulative solar radiation during growth. The onset of reproduction was highly significantly ($p < 0.0001$) delayed by water stress, independent of whether tiller abortion occurred or not. Findings suggest that lower plant densities are recommended to cope with stress during PI, to reduce water loss and control unproductive tillers at harvest. It is concluded that stress during late reproductive stages, unlike during PI, does not alter crop duration and has a negligible effect on water loss and WUE. Farmers with limited irrigation water can try to avoid WS by making sure they irrigate during PI and save water during later reproductive stages.

Key words: Delayed development, plant available water, water stress, thermal time, water use efficiency, NERICA®

1. Introduction

About 11% of the global rice (*Oryza sativa* L.) cultivated area is in uplands (non-flooded, unsaturated soils) (IRRI, 2002). Growing rice in non-puddled, unsaturated and well-drained soils (upland systems) (Kato and Katsura, 2014) is gaining popularity over flooded rice for various merits. This share is likely to increase, particularly because of growing recognition to save water in rice systems (Bouman, 2001; Kato et al., 2006). Growth in rice production and acreage in sub-Saharan Africa (SSA) is estimated at 7% per annum and can be ascribed to the release of improved New Rice for Africa (NERICA®) cultivars (ARC, 2007). These progenies are the result of interspecific crossings between African indigenous upland rice (*Oryza glaberrima* Steud) and Asian lowland rice (*Oryza sativa* L. Japonica) and were developed for low input systems (Jones et al., 1997). The yield of rice in rainfed conditions is comparatively lower in uplands (1.5 – 2.5 Mg ha^{-1}) than for lowlands (2.5 – 4.5 Mg ha^{-1}) and approximately 30% lower under similar water supply conditions (ARC, 2007; Kato and Katsura, 2014). This yield difference among Japonica rice cultivars was marginal when water supply was unlimited (Kato et al., 2009), meaning that high potential cultivars need full irrigation. Although water saving technologies, including aerobic systems, have reduced water inputs compared to conventional flooded rice (Tuong et al., 2004), levels of water application are still quite high. The irrigation strategy practiced in aerobic rice systems is to raise soil water content to about field capacity if rainfall is insufficient (Bouman, 2001). Investigations are therefore needed to determine the minimum water requirements for maximum rice productivity.

In most parts of Africa, upland rice is rainfed (Kijoji et al., 2014) and periods of WS are unpredictable due to poor rainfall distribution. Response of rice to WS generally varies with duration, intensity of stress (Heinemann et al., 2011) and most importantly, the growth stage when stress occurs. The three main growth phases of rice are the vegetative, reproduction and ripening stages, which are subdivided into 10 principle growth stages (Fageria, 2007), and which overlap even within a single plant because the rice crop makes tillers of different chronological ages. Stress in rice plants was reported to develop at $\Psi_{soil} < -86$ kPa for lowland conditions (Bouman et al., 2001) and at $\Psi_{soil} < -100$ kPa in potted upland soil (Asch et al, 2005), but sensitivity of physiological processes such as transpiration and leaf expansion to WS differs along the crop cycle (Devatgar et al., 2009; Heinemann et al., 2011). Mechanisms to cope with WS in *Oryza sativa* L. are thus well documented for pot, growth chamber and lysimeter studies, although such conditions may not represent field conditions well (Parent et al., 2010; Kijoji et al., 2014). For instance, Ψ_{soil} between -60 and -140 kPa at 0 – 25 cm depth, which are reported as threshold values for lowland rice growth in anaerobic soils (Bouman et al., 2001), may be atypical of upland conditions; where (i) surface soil can be dryer than -600 kPa (Jensen et al., 2008) and (ii) roots can exploit soil layers deeper than 30 cm (Lilley and Fukai, 1994).

Drought effects on rice growth depends on the timing thereof. Stress between germination and flowering was reported to delay development in lowland and in upland rice (Wopereis et al., 1996; Boonjung and Fukai, 1996), but the delay in development was much more pronounced in direct-seeded upland rice compared to transplanted lowland rice (Kijoji et al., 2014). Water stress during the ripening stage reportedly also hastens development (Dingkuhn and Le Gal, 1996). The duration of these phases can also be altered by excess water (Dingkuhn and Asch, 1999) and it remains to be investigated if upland rice is tolerant to some degree of soil saturation. Most studies, such as the one by Boojung and Fukai (1996), did not quantify thermal time, which makes it difficult to assess how sensitive the phenology of upland rice is to WS. Research that generates information on changes in growing degree day (GDD) requirements for different stages under water limited conditions will be useful for optimising crop production systems that entirely depend on rainfall, of which the distribution is usually uneven.

In addition to changes in the duration of different developmental stages, drought affects rice plants in various other ways. Stress between flowering and grain filling increases spikelet sterility (Matsuo et al., 2010), and during panicle initiation (PI) it inhibits panicle exertion (Okada et al., 2002), but Asch et al. (2005) reported that when stressed during vegetative growth, dry matter partioning was not affected. Stress at one stage can also have cumulative effects on subsequent components, for instance tiller abortion with stress during early growth (Wopereis et al., 1996) may reduce panicle number (associated with tiller number) and the final spikelet number per unit area. No investigation considered the duration of developmental stages and demonstrated such effects with WS at all above stages in a single field study. Thus, relative yield loss due to stress during different phenological stages and ideal traits to cope with stress at each growth stage have not been studied well before. With respect to alterations in phenology, it has not been established if delay in flowering under vegetative stress may be a result of new tillers developing after relieve of severe stress (tiller abortion), tiller inhibition without death after mild stress, or due to alteration in dry matter partitioning between plant organs. It is noteworthy that rice tillering is spread over time. Delays in phenology has been related to size of source or canopy, and recovery, dry matter partitioning and growth stage in relation to leaf number (Boonjung and Fukai; 1996; Prasertsak and Fukai, 1997; Bouman et al., 2001), with some contrasting reports. An aspect that has not been well reported is whether recovery of source capacity (e.g. canopy size) after stress will be to the same level as for a well-watered control, and whether it will affect the final grain yield. Clarification of source-sink relations is needed since WS during vegetative growth is known to slow development of rice. The crop therefore continues to grow (accumulates dry matter) but at a decreasing rate. It is noteworthy that crop development is different from growth. A crop that has recovered from stress can be of the same canopy size and similar height as a well-watered one, but at a different developmental stage. It is also important to identify desirable traits for yield improvement with stress during different developmental stages in view of variable and cumulative effects of WS on growth and changes after recovery. There is lack of information on effects of drought stress during different developmental stages on phenology (thermal time accumulation), sink-source relations when the stressed crop attains a similar development stage as a well-watered crop, and water use efficiency (WUE). This information is scarce because of a paucity of field studies on WS in rice, especially in unsaturated uplands (Kato et al., 2006), despite the significance of the crop. The overall objective of this study was to determine the effects of water stress on upland rice (cv. NERICA 4®) crop growth and phenology, and to identify the contribution of plant characteristics to yield at each developmental stage. The specific objectives were: (i) to evaluate dry matter partitioning and leaf N content when a crop has recovered from WS, compared to a well-watered control and (ii) to quantify water use and WUE when a rice crop was stressed at different developmental stages.

It is envisaged that this research will inform upland rice growers on best management practices to minimise yield loss under water stress and breeders on traits for adapting rice to water limitations.

2. Materials and methods

2.1. Planting material

Upland rice cultivar NERICA 4® (WAB450-I-B-P-91-HB) (Jones et al., 1997; Ndjiondjop et al., 1998) seed was acquired from the National Crop Resources Research Institute (NaCRRI) in Uganda. Cultivar NERICA 4® is widely adopted in West and East Africa (Africa Rice Centre, 2007) and was chosen because of its high grain yield, estimated at around 4.7 Mg ha^{-1} under rainfed conditions, and because it is still the top-ranked cultivar in tropical rice producing areas such as Uganda (Lamo et al., 2000; Imanywoha et al., 2004). Furthermore, farmers prefer NERICA 4® to other NERICA cultivars for its heavy grains and medium growth duration.

2.2. Study site and rain-out shelter experimental set-up

The trial was conducted in a rain-out shelter on the Hatfield Experimental Farm of the University of Pretoria, South Africa (located at 25° 45' S, 28° 16' E and 1370 m a.s.l.), from December 2013 to June 2014 (season 1) and from October 2014 to April 2015 (season 2). The sowing dates are within the planting window of summer crops in South Africa and other parts of SSA. Daily solar radiation, minimum and maximum air temperatures and relative humidity, and wind speed were recorded, and short grass reference evapotranspiration (ETo) was calculated by an automatic weather station located approximately 100 m from the rain-out shelter. Vapour pressure was calculated from relative humidity and temperature. Daily weather parameters during stress periods in the two years are presented in Table 1.

Table 1. Mean maximum, and minimum temperatures, mean vapour pressure deficit and mean reference evapotranspiration between stress periods during two growing seasons at Hatfield experimental farm, Pretoria.

		Parameter			
Year	DAE[†]	T_{max} (ºC)	T_{min} (ºC)	Average VPD (kPa)	Average ETo (mm day^{-1})
2013/2014	1 – 42	28.7	16.3	1.47	4.65
	43 – 66	29.8	17.2	1.52	4.78
	66 – 95	26.2	16.5	0.98	3.27
	95 – 117	26.1	13.4	1.16	3.46
	117 – 144	24.6	9.1	1.16	2.86
Average		27.3	14.8	1.28	3. 93
2014/2015	1 – 51	28.5	14.1	1.66	4.56
	51 – 75	28.0	16.6	1.22	4.79
	78 – 100	30.1	16.8	1.57	4.95
	100 – 120	31.1	16.9	1.75	4.84
	120 – 138	30.0	15.8	1.71	4.56
Average		27.4	15.0	1.29	4.59
F-test		*	*	ns	*

[†]days after emergence on 15th December 2013 and 17th October 2014.
The periods before first stress treatment are 0 – 42 and 0 – 60.
*significant at $p < 0.05$ for paired data of parameters between years.
Ns, not significant.
Mean values calculated for the period from first day of withholding water to the last day of stress.

The soil at the site is a deep Hutton (MacVicar et al., 1977), loamy, kaolinitic, mesic, Typic Eutrustox (Soil Classification Working Group, 1991), with an effective depth of 1 m. Soil characteristics over the top 0.6 m depth were: pH (water) 5.8 ± 0.14, 0.5 ± 0.06% C, 4.3 ± 2.4 mg kg^{-1} mineral N, 15.9 ± 11.01 mg kg^{-1} available P (Bray I), 95.6 ± 39.7 mg kg^{-1} K, 1377 ± 48 mg kg^{-1} Ca, 159.8 ± 29.4 mg kg^{-1} Mg, 10.2 ± 1.6 mg kg^{-1} Na, 1400 ± 40 kg m^{-3} dry bulk density, 0.3 ± 0.02 $m^3 m^{-3}$ field capacity, 0.2 ± 0.02 $m^3 m^{-3}$ permanent wilting point, 59.7 ± 1.2% sand, 33.3 ± 2.9% clay and 7.0 ± 2.8% silt. Soil characteristics of the 0 – 0.6 m depth were relatively similar, except for the 0.4 – 0.6 m layer, where available P (3.74 mg kg^{-1}) was substantially lower and pH water (5.9) was slightly higher than in the top 0 – 0.4 m layer. Soil mineral N (NH_4-N + NO_3-N) also varied greatly with depth, 6.8 mg N kg^{-1} at 0 – 0.2 m, 4.1 mg N kg^{-1} at 0.2 – 0.4 m and 2.0 mg N kg^{-1} in the 0.4 – 0.6 m layer. In the second season, the experiment was laid out on the opposite side of the rain-out shelter, which was rested in the first season.

2.3. Agronomic practices

Seeds of Nerica 4® were manually sown directly at about 0.03 m depth in dry soil. Sowing was done on 5 December 2013 (mid-summer) and 7 October 2014 (early summer) in the respective seasons. An inter-row spacing of 0.25 m was used in plots measuring 3.75 m × 2.75 m and separated by 0.75 m walkways. Before sowing, N:P:K [4-3-4(30)+Zn] fertilizer was applied at a rate of 54 kg N, 40.5 kg P and 54 kg K per hectare and incorporated into the soil. Limestone Ammonium Nitrate (28% N) was top dressed on 43 and 46 days after emergence (DAE) in seasons 1 and 2 at a rate of 66 kg N ha^{-1} so that total N applied was equivalent to 120 kg N ha^{-1}. Fertiliser rates were based on guidelines for NERICA provided by the Africa Rice Centre (ARC) and timing of top dressing was done to optimize N uptake (ARC, 2007; Fageria, 2007). Prior to implementation of drip irrigation, sprinklers were used to irrigate about 15 mm weekly from sowing to 10 DAE in season 1 and to 7 DAE in season 2. Typical quality of the irrigation water used was: EC (12 – 41 mS m^{-1}), pH (7.7 – 8.2) and TDS (20 – 160 mg L^{-1}). All other chemical properties were within allowable levels for safe use (AQUA Earth, University of Pretoria, 2017). Plants were thinned at 20 and 22 DAE in the respective seasons, leaving 25 plants per metre row length, to achieve a spacing of 0.25 m × 0.04 m, equivalent to a population of $1x10^6$ plants ha^{-1}. Soil water deficit in the 0 – 0.4 m soil layer was replenished to field capacity twice weekly (except for water stress treatments) until a month before harvest, using a high-density drip irrigation system. Profile soil water content (θ) was estimated at 0.2 m depth intervals from readings of a neutron probe water meter (Model 503DR CPN Hydroprobe; Campbell Pacific Nuclear, California, USA) that was calibrated for the site. Weeds were regularly removed by hand and rice plants across plots were uniform before imposing treatments. Malathion® (active ingredient *O*,*O*-dimethyl phosphorodithioate) was sprayed once for aphids and Tiforine® (active ingredient azoxystrobin) for rice blast, according to manufacturer instructions. Symptoms of the disease were mild and aphid damage was minimal because spraying was done at an early stage of incidence.

2.4. Identification of developmental stages

Visual observations were made to characterize crop phenology. The Zadoks decimal code for cereals was used to describe the developmental stages (Zadoks et al., 1974; Fageria et al., 2010) before and after introduction of stress treatments. Developmental stages of rice as described by IRRI (2013) were identified based on morphological features, for instance mean tiller number during the active tillering stage and grain colour and texture during physiological maturity. The panicle initiation stage was marked by the observation of a furry tip of panicle primordium above the growing points under a light microscope. Four main stems were weekly randomly sampled from three well-watered plots from the day of second tiller appearance (about 21 DAE) until peak tillering. Stems were cut just above the crown, hydrated in glycerol for 24 hours and dehydrated in sequential concentrations of ethanol for subsequent days (James and Tas, 1984). Longitudinal sections were prepared and examined under a microscope. The number of folded and open leaves counted under a microscope was used to confirm stages.

2.5. Experimental design and water stress treatments

The trial was laid out as a completely randomized block design (CRBD) with three replications. The five water treatments were randomly allocated to each replicate, giving a total of 15 plots. All plots were well irrigated until a growth stage (GS) of interest was reached, whereafter water was withheld completely to induce WS from the onset of the following development stages (DVS): tillering, panicle initiation, anthesis or 50% flowering and grain filling. The five treatments were as follows:

(i) CT (well-watered control): Irrigating twice weekly (2 – 4 day interval) to replenish the soil water content in the top 0 – 0.4 m soil layer to field capacity.

(ii) STi (stress during tillering or early vegetative stage) or DVS 0.4: from GS 2.3 (two tillers per plant on average) to GS 6.0.

(iii) SPI (stress during panicle initiation or early reproductive stage) or DVS 0.53: from GS 6.0 (panicle primordium visible under a microscope) to GS 10.3.

(iv) SAT (stress around anthesis) or DVS 0.90: from GS 10.3 (50% of the stems have flowered, flowers may just be emerging for some tillers) to GS 11.2.

(v) SGF (stress around grain filling stage II or soft dough stage) or DVS 1.0: from GS 10.2 (grains are turning from green to brown and are not milky anymore) to GS 11.3 or DVS 2.0 (physiological maturity (PM), flag leaf drying and 90% grains are brown).

Initiation of the stress treatments above was based on crop development under well-watered conditions. Irrigation in all plots was terminated on the same day during both seasons. The periods of stress (days after emergence) in season 1 were: STi (43 – 66), SPI (66 – 95), SAT (95 – 117) and SGF (117 – 144) and in season 2 were: STi (51 – 75), SPI (78 – 101), SAT (101 – 121) and SGF (121 – 138). Due to one incident in season 2 when the rain-out shelter failed to close during a rainfall event, stress was ended prematurely during tillering and slightly delayed in panicle initiation. Temporary portable rain-out structures were then constructed and placed over each of the plots that was stressed during PI whenever there was a threat of rain. The structures were used for five days only until the shelter was repaired.

2.6. Plant growth analysis, yield and leaf N uptake

Plant height and number of tillers was monitored weekly on 10 plants in the centre row of each plot. Plant height was measured from the ground surface to the tallest green leaf. Tillering was monitored at least weekly from the date of first tiller appearance (GS 2.0) at 19 and 17 DAE in the respective seasons, but more frequently in the earlier stages before anthesis. Tillers were also counted on sampled plants during biomass measurements. For destructive growth analysis sampling, 14 plants were sampled weekly (from 30 to 130 DAE) from an area of 0.5 m x 0.25 m per treatment by carefully cutting off the plants at ground level. In some occasions, biomass harvests were done after 10 days from the previous date of harvest. Leaf blades were separated from the stems and passed through an LI-3100 leaf area meter (LiCor, Lincoln, Nebraska, USA) to determine leaf area and to calculate leaf area index (LAI). Stems, leaves, panicles and grains were separated and DM yields were determined after oven drying the samples at 65 –70 ^{o}C for at least 48 hours or until constant mass.

At final harvest, aboveground dry matter (ADM) yield was determined by cutting off all the plants at ground level from a net plot area of 1.75 m x 1.50 m (excluding border rows). Total biomass was weighed fresh for moisture content determination and grains were separated from the stover after threshing. The mass of grains (less empty grains) and stover was weighed on a scale (0.1 kg precision). Sub-samples of the stover (90 – 120 g) and grain (~20 g) from each plot were oven dried at 65 ^{o}C for at least 48 hours. Total dry biomass yields were then calculated from the fresh yields and dry matter contents of the different components. Grain yield was adjusted to a 12% moisture content for comparison with commercial harvests.

To assess N uptake of stress-recovered and well-watered crops, flag leaves from the main stems of 25 plants in each plot were sampled 25 – 30 days after heading (DAH) and before grain filling. This stage marks peak N accumulation in rice flag leaves, which is critical during grain filling (Shiratsuchi et al., 2006). Leaves were dried and analysed for total N content using the Dumas method in N Pro-Rapid Nitrogen / Protein Analyzer equipment (Dumatherm®, C. Gerhardt GmbH & Co., Königswinter, Germany). Yield components were assessed by considering plants sampled from a 0.75 m x 0.5 m subplot. Stems were counted and recorded as either non-productive tillers (without panicles) or tillers with panicles. Ten panicles were randomly selected from each plot and their lengths measured. The spikelets in each panicle were detached and counted to determine the number of spikelets and panicle size. The mean number of spikelets from 10 panicles was then expressed per unit area. Spikelets were floated in water to separate empty and full grains, oven dried and weighed. Full grain ratio and sterility were computed as the ratio of full or empty grain mass to the total spikelet mass. Full grains were counted using a seed counter (Numigral®, Triplette and Renaud, Paris, France) and the mass of 1000 grains was weighed. The mass was standardized to 12% moisture content to determine the 1000 grain mass.

2.7. Measurement of radiation interception, water stress and soil water content

Interception of Photosynthetically Active Radiation (PAR, 0.4-0.7μm) by the canopy was determined at least three times every month by measuring photosynthetic PAR above and below the canopy at ground level with a 1 m long Decagon Sunfleck Ceptometer (Decagon Devices, Pullman, Washington, USA). Four points were measured diagonally across rows in each plot and the average value was computed. Profile soil water content (θ) at 0.2 m depth intervals was estimated from the readings of a neutron probe (Model 503DR CPN Hydroprobe, Campbell Pacific Nuclear, California, USA) that was calibrated for the site. At the start of the experiment and before planting, soil samples were taken at 0.2 m depth increments using an auger. Composite samples from each depth were analysed for soil chemical and physical properties. A soil water retention curve (SWRC) for the top 1 m soil layer was also determined using a WP4-T Dewpoint Hygrometer (WP4-T PotentiaMeter®, Decagon Devices, Inc., Pullman, Washington, USA) with an accuracy of ± 0.1 MPa after calibration with the gravimetric method (van Genuchten et al., 1991). The SWRC was also plotted on a log-scale (log-pressure head, cm) using RETC® software for describing hydraulic properties of unsaturated soils (Scientific Software Group, Salt Lake City, Utah, USA). The curves for different depths were used to calculate the actual values of Ψ_{soil} during dry periods. Soil water pressure head (h) was estimated from values of θ (mm), using a graph of θ vs. h, and h was then converted into soil Ψ_{soil} using the following equation:

$$\Psi_{soil} = \rho_w \times g \times h$$

where; ρ_w = the density of water (1000 kg m^{-3}), g = acceleration due to gravity (9.8 m s^{-1}) and h (m). Pressure head is a negative value. Field capacity (FC) and permanent wilting point (PWP) were then estimated from the SWRC. Evapotranspiration (ET) was calculated using the soil water balance equation (Allen et al., 1998):

$$I + P = ET + \Delta S + D + R$$

where; *I = Irrigation, P = Precipitation, D = Drainage and R= Runoff.* P, D and R were considered negligible. A high-density drip irrigation system with water meters was used to apply the applicable water amount to each treatment. Irrigation water was summed from sowing to maturity.

During stress periods irrigation water was completely withheld (except for one event when the shelter failed to close in 2014/15). The change in storage (ΔS) was calculated as the difference in θ between consecutive neutron probe readings. During stress periods, the first neutron probe readings were taken about three days after the last irrigation. This was considered as the start of stress when calculating the initial θ. Soil water content calculated at the end of the water stress period was taken as terminal θ. Stomatal conductance (g_s) on the upper (adaxial) and lower (abaxial) surfaces of the uppermost fully expanded leaf was occasionally measured using a Decagon SC-1 Leaf Porometer (Decagon Devices, Inc., Pullman, Washington, USA). Four replicate stomatal conductance measurements per plot were taken at around 11:45 – 15:20 (range of time for all readings made in both seasons) on the same plants from the start to the end of stress periods. A measurement in a plot under stress was followed by a measurement in a well-watered plot to avoid any bias that could arise by delayed timing of measurements. Stomatal response to WS was later expressed as the mean relative value, that is, the ratio of mean g_s under stress to mean g_s for the CT to normalise variations in atmospheric demand between stages during stress. It was not possible to take frequent measurements of g_s due to unavailability of the porometer and therefore the number of measurements during stress was not equal between growth stages.

2.8. Computation of crop growth parameters

Crop growth parameters were calculated using equations described by Watson, (1952); Lopes et al. (2010) and Fageria et al. (2010). Crop dry matter accumulation rate (g m^{-2} d^{-1}) was calculated as the increase in ADM from the harvested land area per unit time. The total one-sided leaf area (m^2) was divided by the sampling area (m^2) to obtain the leaf area index (LAI) on days of growth analysis sampling. Leaf area duration (LAD) between harvests was calculated as the area under the LAI curve over time, which mathematically is an integral of LAI over time. Thermal time to reach different growth stages was calculated by accumulating daily growing degree days (^{o}Cd) from sowing, using a base temperature of 8.2 ^{o}C for African rice and crosses thereof (Shrestha et al., 2011). Dry masses of individual plant parts were summed to obtain ADM.

The apparent contribution of photosynthesis or reserve assimilates from pre-reproductive stages to grain filling (DM_c) was calculated using the equation by Yoshida (1972):

$$DM_c = ADM_m - ADM_{xi}$$

where ADM_m is the aboveground dry matter yield at maturity and ADM_{xi} is the aboveground dry matter yield at either pre-flowering or pre-anthesis in the case of this study. The apparent contribution was then expressed as a percentage of (ii) ADM_m and (ii) the final grain yield. Grain harvest index (HI) was computed as the ratio of grain mass (fully developed grains) to total ADM on a dry mass basis. Sink size was calculated as the product of the mass of 1000 grains and number of grains per metre square (Kato et al., 2006). Dry matter efficiency (DME) was calculated as the HI divided by the number of days to crop maturity (Watson, 1952). Dry matter efficiency was calculated because of delay in physiological maturity for one treatment. The relative decline in growth and yield was calculated as the difference in the parameter between a water stress treatment and the CT divided by the yield for the CT (stress index). Water use efficiency was calculated during stress periods and for the entire season. During stress, WUE was calculated as the difference in ADM (kg m^{-2}) between the start and end of stress divided by ET (mm) and for the entire season as grain yield (in kg ha^{-1} on dry mass basis) divided by total ET (mm).

2.9. Statistical analysis

The General Linear Model (GLM) Procedure in SAS® 9.3 version 6.1.7061 for Windows (Cary, NC, SAS Institute Inc., 2012) was used to perform statistical analyses. Correlation analysis were performed on selected growth variables and derived parameters during stress, for crop growth rate and final yield components under stress and well-watered conditions to determine the degree of relationship and their contribution to variation in yields. Pearson's correlation coefficient (r) was used to indicate the most consistently related yield component to grain yield. Analysis of variance (ANOVA) was performed with treatments and seasons as factors, and growth, yield and water status indicators as variables. Means for main and interaction effects were separated using Tukey's Studentized Range test at 0.05, 0.01, and 0.001 levels of significance. In order to test for statistical differences in weather parameters that determine crop water use between seasons, the F- test (two – sample for variances) was applied for every pair of daily temperatures, ETo and VPD.

3. Results

3.1. Weather conditions, crop phenology and development

The measured mean maximum and minimum air temperatures of 27.4 ^{o}C and 14.9 ^{o}C (season 1) and 29.2 ^{o}C and 15.6 ^{o}C (season 2) were optimal for rice growth, especially during critical stages of booting to flowering. Evaporative demand during the experimental period was fairly similar between years. Average reference evapotranspiration from sowing to physiological maturity (PM) (period of approximately 149 days) was 3.93 and 4.59 mm day^{-1} in seasons 1 and 2, respectively. Table 1 further shows that the seasonal means were similar, although variances for seasonal averages were significant (except for VPD). Unequal variances in the weather parameters is expected since sowing was on different Julian calendar days of the year (DOY). The similar VPD between seasons and during periods of water stress, suggests similar intensities of stress. The duration of stress periods per growth stage was approximately equal for each season, except for the SGF treatment in season 2, which was 10 days shorter, as grain filling coincided with hotter weather conditions.

Thermal time requirements to reach the different growth stages were similar between seasons for the well-watered control. Growing degree days to emergence was 140 ± 0.8 °Cd, 389 ± 1.0 °Cd to first tiller appearance and 1780 ± 24 °Cd to grain filling, averaged across years. At the time of imposing WS, plants were already past the first two stages while for grain filling, GDD was similar across treatments. Growing degree days to reach peak tillering (DVS = 0.2) differed between seasons under well-watered conditions (Table 2). Peak tillering stage in both years was characterised by the same mean number of tillers. Approximately the same GDDs were required in both seasons to reach first flower appearance (1219 ± 68 °Cd) and anthesis (1544 ± 36 °Cd). For tillering stage, maximum tiller number was attained around flowering (71 DAE) in season 1 and slightly earlier, at around booting (53 DAE) in season 2 (Table 1). This was equivalent to 368 °Cd more in the 2013/2014 season than in 2014/2015. Stress imposed before first flower appearance delayed booting, flowering and anthesis highly significantly ($p < 0.0001$). In particular, stress during early reproductive growth increased GDDs to booting, first flower and anthesis stages more than stress during tillering (Table 2). The increase in thermal time to first flower (197 ± 2 °Cd) and to anthesis (120 ± 45 °Cd), averaged over the two seasons, was therefore considerable due to stress during PI. In contrast, the GDD to anthesis with stress during tillering was almost the same (+45 °Cd) as for well-watered plants to reach the same stage, in spite of considerable delay in appearance of the first flowers for STi, compared to the well-watered control. Stress during anthesis and grain filling did not affect development (except accelerated maturity for SGF) as much as other treatments, when compared to CT. The interaction effect of stress and season on onset of developmental stages, thus GDD, was highly significant ($p < 0.0001$), with slightly longer durations under stress in season 2 than season 1. Again, there was little variation in GDD for CT between years. Stress generally did not alter the time to maturity and GDDs were similar to that under well-watered conditions, except in season 2 when maturity for SPI was about 23 days (~285 °Cd) longer than for CT. Leaf area index and FI of solar radiation during 120 – 139 DAE were significantly higher for treatments that were stressed during Ti and PI than for the CT (results not shown).

Table 2. Growing degrees days of cv. Nerica 4 to reach selected developmental stages for a well-watered control and for stress treatments during tillering and early reproductive stage.

	Thermal time (°Cday) under treatments		
Year, stages observed	CT	STi	SPI
2013/2014			
Peak tiller[†]	1201^a	1505.61^b	1201^a
Panicle initiation	1111^a	1267^b	1111^a
Mid-booting	1229^a	1378^b	1404^b
First flower	1267^a	1415^b	1463^c
Anthesis	1518^a	1606^b	1581^b
Maturity	1947^a	1947^a	2041^a
2014/2015			
Peak tiller[†]	833^a	861^a	833^a
Panicle initiation	1192^a	1206^b	1192^a
Mid-booting	1241^a	1385^b	1489^c
First flower	1358^a	1489^b	1569^c
Anthesis	1581^a	1609^b	1732^c
Maturity	2099^a	2086^a	2384^b

Note: Means followed by same letter within a row are not significantly different at $p = 0.0001$.

[†] DVS = 0.2 was the only stage with different GDD values between seasons. Within seasons, variation in GDD to peak tiller between plots that were subjected to water stress was negligible and in 2014/2015, it was very small.

3.2. Crop growth and recovery- flowering duration, tillering and plant height

Although plants exposed to the SPI treatment took longer to flower, in season 2 some flowering was observed during the stress in all replicate plots. This was not anticipated but it might have been that thermal time to flowering accrued while still under stress and plants extracted water from deeper soil layers. Figure 1 indicates that flowering duration, measured as a percentage of stems with flowers on each sampling date, was significantly ($p < 0.001$) longer for stress during PI than for stress during Ti. Stress during flowering (SAT) did not affect flowering rates. Plot-to-plot variation in ontogeny of plants and in flowering rates was negligible between replicates.

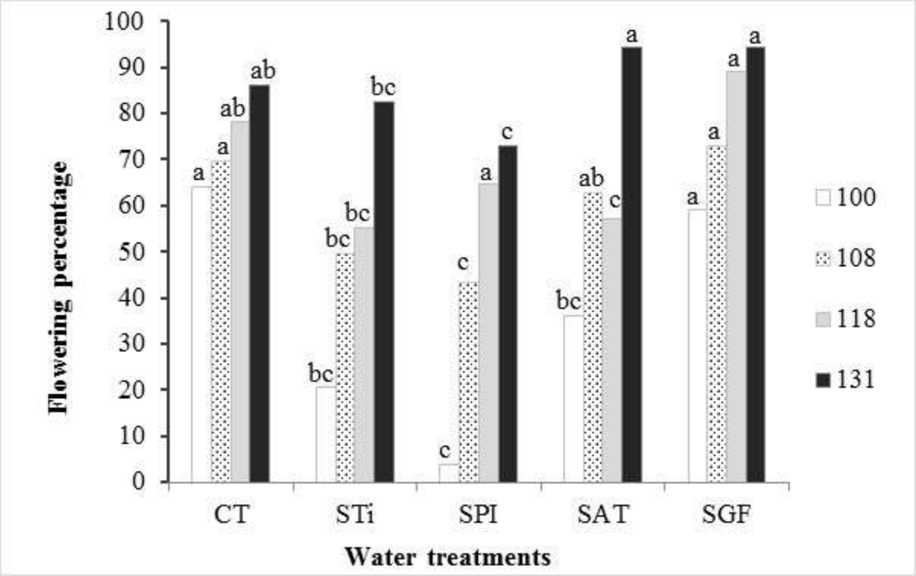

Figure 1. Flowering proportion under stress treatments compared to a well-watered control (CT), from 100 to 131 DAE. Flowering in plants stressed during late stages, SAT and SGF treatments, is similar to that for CT. Bars followed by the same letter at each DAE are not significantly ($p > 0.05$) different by Tukey's Studentized *post hoc* test.

Most plant growth parameters were affected by stress and the interaction of treatment and season on growth during stress was also significant ($p < 0.05$), except for plant height. Results of both seasons are shown in Figure 2. Tillering was suppressed by stress in season 1 only, but mean number of tillers for SPI was similar to CT (Figure 2a). It is apparent in Figure 2a and Table 2 (season 1) that first peak of tiller growth for SPI was at 71 DAE and after the relieve of stress, it peaked again at around 108 DAE. However, in season 2 tillering ability was not significantly ($p > 0.05$) suppressed by stress, relative to CT and tillers were not aborted. As a result, the number of tillers per plant and tillers m^{-2} under early reproductive stress was similar to that of CT in season 2 (data on latter not presented). This can probably be explained as follows: by the time water was withheld for SPI plots in season 2, plants had already attained maximum tiller number and consequently there was virtually no tiller abortion due to stress, as can be seen in the growth analysis results presented in Figure 2b.

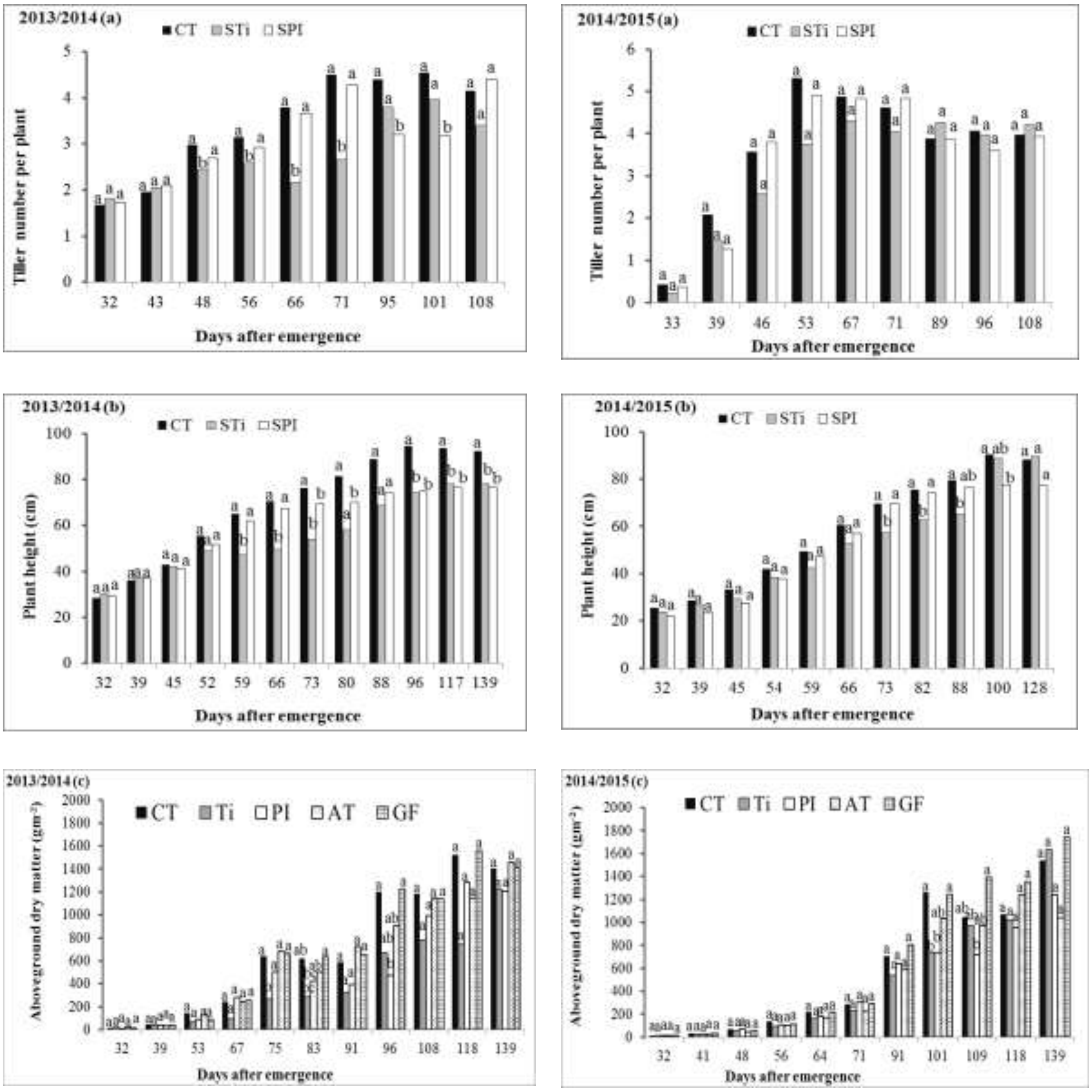

Figure 2. Changes in (a) tiller number and (b) plant height over time with stress during tillering (STi) and early reproductive (SPI) stages and (c) aboveground dry matter yield for all treatments, in comparison to well-watered control in the seasons. Means followed by same letters on the same day after emergence are not significantly ($p < 0.05$) different by Tukey's Studentized Range test.

During plant growth analysis, significant differences in tillers m^{-2} between SPI and CT were detected only at the termination of stress (95 DAE), and the first flowers had already appeared in plants under stress. The average tiller number (grand mean = 4.13±0.42 per plant) in stressed and well-watered treatments was similar at 118 DAE, but the number of panicles m^{-2} at harvest was significantly ($p = 0.028$) lower for STI and SPI (in season 2 only). Therefore, more tillers did not produce panicles under stress compared to well-watered conditions, because of more mutual shading by the canopy of the recovering crop, i.e. larger green LAI and FI values than CT post anthesis.

Plant height increased, albeit at a decreasing rate, for the rest of the stress period of STi. Plant height was reduced by 30% in season 1 and by 17.8% in season 2 for the STi treatment, compared to only 20.8% and 14.3% for the SPI treatment relative to CT, respectively. Reduction in green LAI was substantial compared to reduction in leaf dry matter during stress for the SAT and SGF treatments, unlike for the STi and SPI treatments (Table 3). Thus, these changes mean that specific leaf area (SLA, $m^2 kg^{-1}$) greatly reduced for especially SAT treatments.

Table 3. Decline in soil water content, mean ratio of stomatal conductance in stressed to unstressed control, and relative reduction (%) in growth parameters at the start and end of stress periods.

	2013/2014				2014/2015			
Parameter	STi	SPI	SAT	SGF	STi	SPI	SAT	SGF
Soil water content† (mm)	19.4	13.7	33.7	17.2	38.8	43.9	16.03	25.0
Adaxial g_s ratio†	0.99	0.74	0.77	0.94	0.82	0.49	(1.26‡)	< 0.2
<u>Relative reduction (%)</u>								
Aboveground dry matter	61.5^{ns}	61.0***	24.6**	(0.5‡) ^{ns}	18.9*	41.5*	1.5*	1.3*
Leaf dry matter	61.1*	45.7*	5.1 ^{ns}	3.1^{ns}	18.5**	20.5**	(15.2‡)	(18.6‡)
Leaf area index	87.2**	47.8**	86.7**	81.3**	48.4**	75.7**	80.8**	82.2**
Plant height	30.8***	20.8***	0.1^{ns}	0.003^{ns}	17.0*	14.0^{ns}	0.6^{ns}	0.006^{ns}
Tillers m^{-2}	35.8**	30.4^{ns}	17.8^{ns}	0^{ns}	6.8^{ns}	2.6^{ns}	0.6^{ns}	0^{ns}

* Significant at $p < 0.05$, ** significant $p < 0.01$, *** significant at $p < 0.001$.
ns, not significantly different from the control at p=0.05 level of probability.
Relative reduction (%) is the difference in the parameter between a stress treatment and the control as fraction of the control.
(‡) represent an increase rather than a decrease as values of abaxial gs during stress and leaf DM at the end of stress for SAT and SGF treatments were slightly greater than the CT.
† Soil field capacity in top 0.6 m layer = 168 mm.

Crop recovery from stress was more visible for tiller number than plant height, as at harvest tiller densities were similar, but plants significantly shorter for stress treatments than the CT. Aboveground biomass yield was not affected during stress periods, but several days after resumption of irrigation, differences relative to the control were significant. Pearson correlation analysis revealed that biomass production was well explained ($p < 0.05$; $r = 0.48$) by LAD during stress periods. The overall relationship between biomass yield and LAD for an entire season was strong in season 1 ($p < 0.05$; $R^2 = 0.78$), but weak in season 2 ($R^2 = 0.30$).

3.3. Soil water status and consumption trends, leaf stress symptoms and stomatal conductance

The initial θ before onset of stress was similar across all treatments, with small differences ranging 2 – 5 mm (2013/2014) and 2 – 7 mm (2014/2015) per soil layer (Figure 3). Soils for all treatments then dried to the following ranges (percent plant available water remaining per layer): 34 – 45% at 0 – 0.2 m, 49 – 51% at 0.2 - 0.4 m and 55 – 69% at 0.4 – 0.6 m on average across seasons. The amount of soil water depleted was generally similar between treatments, but in 2014/2015 depletion from the 0.4 – 0.6 m soil layer for STi and SGF was approximately 7 mm less than other treatments (results not shown). The estimated Ψ_{soil} at termination of stress in each season using the SWRC ranged as follows: 0.18 – 0.22 MPa in season 1 and 0.30 – 0.58 MPa in season 2 at 0 – 0.2 m, 0.11 – 0.13 MPa in season 1 and 0.11 – 0.19 MPa in season 2 at 0.2 – 0.4 m and 0.17 – 0.22 MPa in season 1 and 0.11 – 0.09 MPa in season 2 at 0.4 – 0.6 m depth.

Soil water consumption during stress for some treatments and under well-watered CT for the same period is shown in Figure 3. Changes in θ were limited to the 0 – 0.4 m soil layer throughout the growth period, except during early reproductive stress when a decline in θ was measured in deeper layers. Stress during this stage had a unique trend (Figure 3) characterised by (i) a fairly stable level in θ at 0 – 0.2 m layer and (ii) a considerable decline in θ in the 0.4 – 0.6 m layer. The trends in θ for SPI suggest little water was extracted at shallower depths on the basis of roughly equal available θ (40 mm) during the first days of stress, relative to the CT. Later, as the soil became dryer, roots grew deeper into sub-soil layers. During stress at anthesis, θ for the surface layer was virtually constant in season 2, while in season 1, there was a sharp decline for the first 12 days after anthesis, where after θ

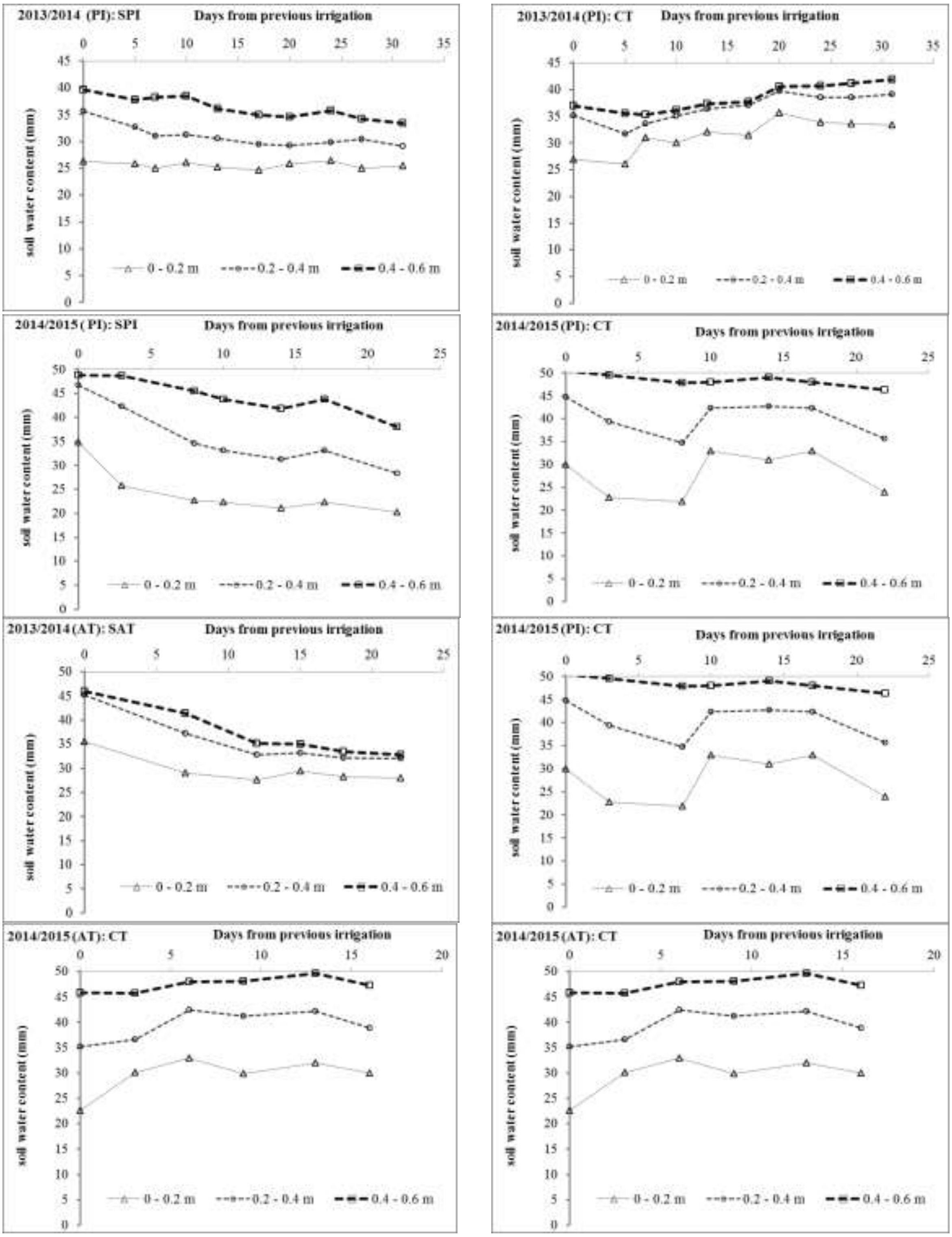

Figure 3. Trends in soil water content in different soil layers (at 0.2 m increments down to 0.6 m depth) during panicle initiation (PI) and anthesis (AT) stages, during stress (S) and in a well-watered control (CT) during the same period in two growing seasons. Treatments codes SPI and SAT are indicated. Patterns for other treatments were not unique from the CT and thus not presented.

remained virtually constant for the rest of the flowering period. The sharp decline in θ shortly after anthesis observed under CT in season 1 as well represents a critical period of water extraction. This initial decline indicates increased root activity, while the fairly constant trend later could mean that water uptake was limited due to root death. Otherwise, if it is not root death, the θ pattern for CT during anthesis should at least be on a downward trend at all depths. There were moderate to strong linear relationships ($R^2 = 0.44 – 0.97$) between the soil water depleted (mm) during stress periods and some yield components at harvest during both years. Mean grain yield decreased ($R^2 = 0.86$ and 0.71) and spikelet sterility increased ($R^2 = 0.44$ and 0.97) linearly with increase in amount of soil water depleted from each treatment during years. Correlation between the number of panicles m^{-2} at harvest, and Δθ during stress was negative ($p < 0.0019$, $R^2 = 0.43$) only in 2013/2014, and Δθ for 0 – 0.6 m layer was positive ($p < 0.017$, $R^2 = 0.63$) in both years. These two relationships showed that panicle number (which differed significantly between treatments) at least partly depended on the amount of water extracted during stress.

Initial stress symptoms were mild, e.g. wilting after termination of irrigation water, but later (about seven days of stress) more severe symptoms were observed, e.g. leaf size was reduced. Symptoms of stress were distinct in each stage: leaf senescence in tillering stage, leaf rolling into bristle-like shapes in early reproductive stage, leaf wilting and gradual drying of flag leaves in anthesis and accelerated leaf drying in grain filling stage.

Stress during most developmental stages reduced g_s on abaxial (lower) leaf surfaces and consequently the relative ratios of mean g_s under stress: mean gs for CT. It was unexpected to measure slightly higher g_s values on abaxial leaf surfaces from four replicate plants with stress during anthesis than corresponding measurements for the CT (thus g_s ratios were greater than 1.0). This was common during most occasions when stomatal conductance was measured. Under well-watered conditions, the gs values on adaxial (206 – 443 mmol m^{-2} s^{-1}) and abaxial (143 – 384 mmol m^{-2} s^{-1}) surfaces of the flag leaf were roughly similar, suggesting equal distribution of stomata on both surfaces - gs values were either higher on abaxial or on adaxial surfaces. Stomatal conductance values for adaxial (upper) surfaces were generally higher under stress than under well-watered conditions, probably because of inward leaf rolling (results not shown). The upper leaf surface was thus less exposed to the atmosphere due to modification of the microclimate around the leaf. The frequency of g_s measurements was irregular between stages (due to unavailability of equipment and unfavourable weather), thus only initial and terminal measurements were used to calculate gs ratios to indicate severity of stress. The g_s ratios for abaxial flag leaf surfaces ranged between 0.8 and 0.9 after the initial withholding of irrigation, to between 0.41 and 0.35 at termination of stress, in all treatments. However, during stress at Ti, g_s did not decrease as much as during other stages (g_s ratio of 0.79 towards termination of stress), relative to the CT.

3.4. Growth, dry matter yield and leaf N content per developmental stage

Comparing growth attained under stress and well-watered conditions at the same developmental stage, LAI was only different at first flowering but shortly after anthesis, values of LAI were surprisingly similar. In fact, after the alleviation of stress during early reproductive growth (SPI), LAI exceeded that of CT in the subsequent developmental stages (Table 4). This phenomenon was also apparent in season 2, even when tiller abortion did not occur. Increase in canopy size was thus not as a result of new tillers emerging after stress relieve, as was likely in season 1. Further, when plants were at the same developmental stage of grain filling, LAI for SPI was again higher than that of CT. Plants in the SPI treatment were also able to maintain higher fractions of intercepted radiation than the well-watered control. This overgrowth (increase in LAI and FI) after the relieve of stress suggests that highly elastic growth occurs with stress during the early reproductive stage. This kind of 'elastic' growth was not apparent with stress during tillering, because the great reduction in LAI (Table 3) for SPI was due to wilting as opposed to senescence.

At first flower appearance, plants which had been stressed attained lower ADM than the well-watered CT. Aboveground dry matter yield for STi and for SPI were half and two thirds of that achieved by the well-watered CT, respectively. Thus, when the crop was stressed during early growth (STi), the source at first flower appearance was comparatively smaller than when stressed later at early reproductive stage (SPI) in both years. However, later on the ADM yield at anthesis was similar between the two treatments (Table 4). The differences in ADM then diminished towards maturity, such that the SPI treatment achieved almost 100% of the ADM value recorded by CT. During both

Table 4. Mean aboveground dry matter yield and leaf area index in different developmental stages under different water treatments.

Year	Growth Stage[‡]	Aboveground dry matter (g m^{-2})					Leaf area index (m^{-2} m^{-2})				
		CT	STi	SPI	SAT	SGF	CT	STi	SPI	SAT	SGF
2013/2014	PI	242.2^{a}	266.3^{ab}	282.1^{b}	243.5^{a}	256.1^{a}	0.46^{a}	0.46^{a}	0.53^{a}	0.53^{a}	0.45^{a}
	Ff	640.3^{a}	324.2^{b}	395.2^{c}	682.5^{a}	662.7^{a}	2.05^{a}	1.94^{b}	1.66^{b}	1.46^{a}	1.76^{a}
	AT	1205.1^{a}	779.3^{b}	993.3^{b}	1146.5^{a}	1227.8^{a}	3.61^{a}	1.59^{c}	2.31^{d}	2.89^{ab}	3.87^{a}
	GF	1523.9^{a}	743.1^{b}	1282.7^{c}	1149.0^{c}	1559.0^{a}	2.22^{a}	1.25^{b}	2.58^{c}	0.48^{d}	2.33^{a}
	M	1404.0^{a}	1218.4^{b}	1211.0^{b}	1458.7^{a}	1411.7^{a}	2.12^{a}	1.24^{b}	2.11^{a}	2.00^{a}	0.39^{b}
2014/2015	PI	283.7^{a}	230.2^{b}	302.8^{a}	223.2^{b}	291.3^{b}	0.82^{a}	0.54^{b}	1.43^{c}	0.69^{ab}	1.19^{d}
	Ff	703.9^{a}	539.9^{b}	718.2^{a}	593.7^{a}	804.8^{a}	2.16^{a}	1.71^{b}	0.25^{c}	1.88^{d}	2.07^{ad}
	AT	1260.5^{a}	736.4^{b}	736.8^{b}	1032.5^{a}	1243.1^{a}	1.97^{a}[†]	1.82^{a}	1.73^{a}	1.74^{a}	1.82^{a}
	GF	1073.2^{a}	1019.0^{a}	1036.3^{a}	1234.4^{c}	1080.9^{a}	0.98^{a}	1.41^{b}	1.61^{b}	0.18^{c}	1.10^{a}
	M	1719.6^{a}	1635.5^{b}	1240.2^{c}	1389.4^{d}	1741.3^{ab}	0.38^{a}	0.80^{b}	0.75^{b}	0.51^{c}	0.007^{d}

[‡] Stages; PI, panicle initiation; Ff, first flower; AT, anthesis; GF, grain filling; M, physiological maturity were observed on each replicate per treatment. A developmental stage was delayed or advanced, depending on when stress was imposed.

Means followed by the same letter within a row are not significantly different at p=0.01 level of probability.

[†] LAI under CT in season 2014/2015 was maximum on 03 February 2015, 8 d after anthesis. The value on 26 January 2015 (start of anthesis) was 1.41 m^{-2}m^{-2}.

years, ADM at final harvest was statistically similar ($p > 0.05$) between the well-watered CT and stress treatments, although on average over years, ADM for CT (13.11 Mg ha^{-1}) was higher than for SPI (9.54 Mg ha^{-1}), STi (10.09 Mg ha^{-1}), SAT (11.27 Mg ha^{-1}) and SGF (11.53 Mg ha^{-1}). It is noteworthy that maximum tillering for STi was delayed by approximately 24 and 14 days in the two seasons (meaning a longer tillering duration), relative to the control. It can be argued that plants under STi and SPI compensated for ADM loss through increase in leaf number per plant following longer vegetative growth duration than CT. Number of tillers was not a distinguishing contributor to biomass between stress treatments and well-watered conditions.

As was reported in previous sections, development was slowed down by WS, so it was important to clarify the indicators of source size when the crop attained the same developmental stages under different treatments. The contribution of reserve assimilates pre-flowering (16.1 ± 1.9%) and pre-anthesis (54.3 ± 0.07%) to final ADM under well-watered conditions (and when not under stress) was identical between years. Pre-flowering and pre-anthesis reserves contributed more to final ADM under stress, than for CT, although results were variable between seasons. For example, 45.5 ± 9.5% of the final crop yield for STi and 51.6 ± 9.5% for SPI was contributed from pre-anthesis assimilates, while the contribution was only 42.5 ± 3.2% (averaged across seasons) under well-watered conditions. This contribution to grain yield for STi and SPI was exceptionally high. It is clear that the grain dry mass yield for these two treatments at harvest was on average 2 – 4 times the ADM at anthesis and approximately 4.6 times for CT (Tables 4 and 5). Considering that the proportion of ADM at anthesis to the final grain yield is similar for STi and CT, results suggest that partitioning of dry matter rather than the source size at the same developmental stage affected final yield.

When plants were unstressed (except for plants of SAT, which were under stress at sampling time) and at the same development stage, leaf nitrogen uptake (g 100 g^{-1} d.w. basis) was higher in plants that were previously stressed than in plants of the control. Mean flag leaf N content for SPI (3.74%) and for STi (3.07%) were significantly ($p < 0.0001$) higher than for CT (2.80%) and SAT (2.70%). The significant increase in flag leaf N concentration after stress over well-watered conditions at 101 – 106 DAE (plants for STi and SPI had fully recovered) was consistent in both seasons. As leaf sampling was done during the stress period of SAT treatment, a lower leaf N content was thus expected. Total leaf N was not measured but based on leaf dry matter per unit area at sampling date, which was similar across treatments. We can thus assume that leaf N content was higher after relieving stress than without stress.

Table 5. Rice yield components at final harvest as affected by water stress treatment

Treatment	Grain yield[†] (Mg ha^{-1})	Harvest index (fraction)	1000 grain mass (g)	Panicle length (cm)	Spikelet sterility (%)	Spikelets m^{-2} (x10^{3})	Shoots m^{-2}
2013/2014							
STi	1.04	0.11^{a}	22.47	19.91^{b}	43.90^{a}	28.71	304
SPI	1.94	0.15^{a}	22.36	20.13^{b}	28.53^{ab}	26.84	345
SAT	4.08	0.30^{a}	23.15	24.24^{a}	21.94^{ab}	40.09	312
SGF	3.56	0.27^{a}	23.65	23.23^{ab}	26.63^{ab}	44.61	374
CT	4.40	0.32^{a}	23.68	23.75^{a}	21.28^{b}	40.73	357
p-value	ns	*	ns	**	*	ns	ns
2014/2015							
STi	6.49^{ab}	0.41^{ab}	24.87	21.65^{a}	3.98^{ab}	49.21^{ab}	444
SPI	1.58^{d}	0.18^{b}	20.18	17.71^{b}	12.11^{ab}	35.32^{b}	456
SAT	3.56^{c}	0.36^{ab}	21.10	21.19^{a}	15.84^{a}	47.95^{ab}	412
SGF	5.23^{bc}	0.40^{ab}	23.82	21.33^{a}	7.71^{ab}	38.77^{ab}	348
CT	7.16^{a}	0.48^{a}	22.85	21.57^{a}	3.80^{b}	57.43^{a}	466
p-value	***	*	ns	***	*	*	**

* Significant at $p < 0.05$, ** significant $p < 0.01$, *** significant at $p < 0.001$.

ns, not significant.

Table 6. Relationships between selected rice yield components and grain yield at harvest

Parameter	Range	Pearson value	p-value
Panicle length (cm)	17.9 – 25.3	0.53	**
Spikelet sterility (%)	1.99 – 49.10	-0.74	***
Spikelets m^{-2} (x 10^3)	13.9 – 64.4	0.76	***
Panicles m^{-2}	160 – 496	0.53	**
Harvest index (%)	8.0 – 54.0	0.89	***
Dry matter efficiency (g g^{-1} d^{-1})	0.06 – 0.39	0.62	***

* Significant at $p < 0.05$, ** significant $p < 0.01$, *** significant at $p < 0.001$.

3.5. Relationship between grain yield, yield components and growth parameters

Water stress significantly ($p < 0.05$) affected grain yield and some yield components, except 1000 grain mass, sink size and tillers m^{-2}, which are not presented. The treatment × season interaction effect on yield components was highly significant ($p < 0.001$) (Table 5). However, in season 1 grain yield was reduced by bird damage, which resulted in lower overall yields. Growth analysis before ripening of grains (some grains had not filled yet) showed higher yield overall than was measured at final harvest. Mean grain yields (on dry mass basis) at the last growth analysis were: STi: 2.18, SPI: 2.38, SAT: 3.65, SGF: 3.59 and CT: 5.46 Mg ha^{-1}. It is therefore likely that final grain yield was underestimated in season 1, considering that stover yield was similar for both seasons. Grain yield components were not considerably affected by WS during the anthesis and grain filling stages. When stress was imposed during Ti, the number of spikelets per panicle, estimated spikelet number per unit area and panicle length significantly reduced and spikelet sterility increased ($p < 0.05$) (Table 5). Stress during PI had a severely negative effect on panicle length and HI, but to a lesser extent on spikelet sterility, and this trend was consistent in both seasons. Thinner panicles were also observed at harvest, notably for plants that were stressed during PI. Sterility of spikelets was common across all WS treatments, but based on mean full grain ratio values for the two seasons, spikelet sterility was more pronounced for SPI than for SAT. The high percentage of unfilled grains in season 1 was common to all water treatments, inclusive of the control, but sterility could not be associated with ambient weather conditions, as temperatures were generally favourable during booting (Table 1).

The most highly correlated yield components with grain yield were spikelets per unit area and spikelet sterility (Table 5). Pearson correlation analysis, therefore, showed that variation in grain yield was explained best by the number of spikelets per panicle, as the number of panicles m^{-2} was quite similar. Several growth parameters like ADM, plant height and LAI or LAD were also related to grain yield (results not shown). However, there were no good relationships between LAD, grain yield and sink indicators or yield components. The mass of 1000 grains was positively related ($p = 0.018$, $r = 0.44$) to LAD, while dry matter accumulation rate was weakly related to panicle length ($p = 0.019$, $r = 0.43$) and to 1000-grain mass ($p = 0.009$, $r = 0.47$).

3.6. Water use and water use efficiency of treatments

Since treatment means did not differ significantly between seasons, water use related parameters were averaged across the two seasons (Table 7). Water applied as irrigation was considerably lower under stress, compared to well-watered conditions, with minimal yield penalty, especially if water was withheld during GF. The amount of water irrigated to stress treatments was on average 130 mm per season less than for the well-watered control. Water used as ET was higher under stress, notably for STi (+72 mm) and SPI (+ 53 mm), than under well-watered conditions. The higher ET under stress could be mostly from soil evaporation (E) and less transpiration, because of smaller canopies during stress periods (due to leaf rolling and senescence), which exposed the soil surface to ambient conditions. Water use efficiency for grain yield (kg ha^{-1} mm^{-1} or kg m^{-3}, yield per ET) did therefore not increase under stress (Table 7), compared to well-watered conditions. Water use efficiency was higher in season 2 than in season 1 due to a higher grain yield response, especially for two treatments. For the respective seasons, WUE values for CT were 0.88 and 0.66 kg m^{-3} and 0.46 and 0.30 kg m^{-3} for SAT. Stress during grain filling did not affect WUE as much as during Ti and PI. Mean values averaged across seasons for SGF (0.64 kg m^{-3}) and for CT (0.75 kg m^{-3}) were similar, while for STi and SPI WUE values were very different from the CT (Table 7). These overall results indicated that the crop was less efficient in utilising water as water inputs were reduced. Results on WUE and water input indicate a possibility of reducing irrigation water with minimal yield loss if water is withheld during late developmental stages.

Table 7. Mean values (± s.e.) across seasons of water use parameters for of cv. Nerica 4 during stress periods and at final harvest.

Treatment	WUE_g (kg m^{-3})	ET^1 (mm)	Irrigation (mm)	‡WUE_b during stress (kg m^{-3})	†WUE_b at end of stress (kg m^{-3})	DME (kg kg^{-1} d^{-1})
CT	0.75^a	627	818	na	na	na
STi	0.53^b	555	573	6.81±1.85	$11.65±8.46^b$	$0.22±0.02^{ab}$
SPI	0.25^{bc}	574	602	10.82±1.85	$23.46±8.46^b$	$0.12±0.02^b$
SAT	0.57^b	609	704	12.39±2.06	$43.16±9.46^{ab}$	$0.24±0.02^a$
SGF	0.64^a	590	642	11.34±2.06	$65.06±9.46^a$	$0.27±0.03^a$
p value	**	ns	na	ns	**	**

Note: Means for values in a column followed by the same letter are not significantly different.
* Significant at $p < 0.05$, ** Significant at $p < 0.01$) and *** Significant at $p < 0.001$.
ns, not significant.
na, not applicable.
DME, dry matter efficiency.
^{1}ET was computed from sowing to final harvest (~150 d) and not to maturity.
WUE_g for grain yield.
WUE_b, water use efficiency for biomass yield during stress calculated as, ‡WUE_b is change in biomass per unit of ET during stress and †WUE_b is biomass at termination of water stress divided by water used as ET from sowing to end of stress.

4. Discussion

4.1. Water stress effects on crop performance

Growth was clearly affected by WS during each stage. However, the lack of significant reduction in ADM indicates that not only water stress, but also factors such as tiller number, leaf expansion and rooting affect biomass production (Asch et al., 2005; Kato et al., 2006). Below-ground biomass was not measured in the present study. The initial decrease in ADM (about eight days after withholding water) during tillering was because plants were source limited at this stage, due to fewer leaves, which limited DM production (Dingkuhn et al., 1989).

Constant soil water regimes or staggering of planting dates have previously been used to achieve WS in screening trials. When the staggering sowing dates was used and a delay in reproduction was considerable in a study by Boonjung and Fukai (1996), unfavourable temperatures was blamed for confounding WS effects on rice growth. This unwanted effect did not occur in our study, despite delay to reproduction for SPI. Besides, the daily temperatures during sensitive growth stages of rice were within the optimum range for all treatments (Shrestha et al., 2011). This study used progressive drying of the soil to achieve intermittent WS, which is typical of natural field conditions (Vadez et al., 2014) and resulted in fairly equal initial θ. Using a similar water regime in potted rice, Okada et al. (2002) also found that roots were able to exploit water reserves from sub-soil layers. When rice plants maximise soil water uptake during stress, enhancement of biomass production occurs during stress (Blum, 2009). These two mechanisms (increased rooting and optimal water use) explain the lack of significant response in ADM for most of the stress periods during tillering and early reproductive stages.

In terms of desirable traits to cope with stage-specific stress, tillering ability was not critical in determining biomass and grain yield under stress. Comparing tillering and biomass responses in each season (Figure 2), ADM was not significantly affected, whether tillers were aborted or not. Again, comparing the responses between treatments at a specific time point, for instance at 95 DAE, mean tiller number was lower for SPI than for STi, although total biomass was similar. Furthermore, despite similar tiller number m^{-2} at harvest, stressed treatments had less productive panicles than the control.

This study established that when WS occurs before flowering, there is no strong linkage between tillering ability and delay in reproduction stage. Although tillering was slightly suppressed by stress in the early reproductive stage relative to the control (Figure 2) and no tillers were aborted on individual plants in season 2, the delay in flowering was still considerable. Conversely, tiller abortion was apparent with stress during tillering stage, although not significant in season 2 (Figure 2). Otherwise, if an association between tiller abortion and delay to flowering and anthesis exists, then increase in GDD to first flowering and anthesis under SPI should have been negligible, at least in 2014/2015 when peak tillering stage occurred days before stress. Further, flowering occurred during the stress period for SPI in 2014/2015 without additional tillers thereafter. These two evidences thus also rule out the possibility of new tillers increasing GDD to reproduction under stress. We suggest this to be a result of preferential partitioning of assimilates to the roots, even though below ground biomass was not sampled. The proposition is, however, based on the increase in water uptake from sub-soil layers under SPI and findings by Price et al. (2002), who associated deep rooting in rice with more investment of C into the roots.

Explanations in literature for the changes in the time to peak tillering in rice under non-limiting conditions are rare. Clerget and Bueno (2013) reported that average number of tillers of potted-grown lowland rice cultivars changed with sowing dates and it was related to duration of the vegetative phase. In our study, stress resulted in an extension of the time to reach peak tillering and therefore an extension of the vegetative phase, but the maximum number of tillers was still similar between treatments. Asch et al. (2005) reported that peak tillering in upland rice cultivars CG14 and WAB 56-104 (which are parents of cv. NERICA) grown in chambers occurred around flowering. Our findings show that the time to peak tillering under well-watered (and non-limiting) conditions can also considerably change with season. It was difficult to explain why plants attained peak tillering (development stage 0.2) (Zadoks et al., 1976) earlier in season 2, as daily temperatures between sowing and about 60 DAE were similar in both seasons. More investigation for possible causes of changes in thermal time to peak tillering is needed.

We expected that biomass production will be lower when stressed in the early reproductive stage (SPI), rather than tillering (STi) stage because of a greater reduction in LAD and also because stress during PI enhances rooting depth (Okada et al., 2002). The positive correlation between ADM and LAD and the lowest mean values of ADM and LAD for SPI confirmed this expectation. Furthermore, we anticipated that the longer the delay to reproduction stage under stress, the higher the biomass would be at reproductive stage. Findings do not support this latter speculation because the delay of the reproductive stage was considerable, while the differences in biomass between STi and SPI were small.

Delay in flowering due to WS has been reported in previous studies in relation to soil water content, number of leaves produced and canopy size. For instance, Boojung and Fukai (1996) reported a delay in flowering for rice plants stressed at full canopy (‘late vegetative growth’). Bouman et al. (2001) also stated that a delay in flowering occurs when the soil is too dry for plants to produce leaves. Their explanation can be interpreted as stress during late vegetative growth (full canopy) delays flowering because few leaves are produced thereafter. In contrast to our study, plants were still producing new leaves at the time of imposing stress. The SPI treatment started with a canopy intercepting 45% of solar radiation (about half the maximum FI) and LAI of 0.38 m^2 m^{-2} (about 17% of the maximum value). These results confirmed that the delay in flowering occurred irrespective of the number of leaves attained or canopy size at that stage.

High grain yield in the present study was due to the high contribution (42%) of pre-anthesis assimilates to grain dry mass. Yoshida (1972) reported that, depending on the rice cultivar, 20 – 40% of pre-anthesis assimilate contribution is needed to attain high grain yields. The introduction of stress during PI resulted in considerable yield loss due to lower panicle size (shorter panicles plus lower number of spikelets on the panicle). Similar results, including pronounced spikelet sterility, has been reported with stress during panicle initiation in lowland rice (Garrity and O'Toole, 1994; Bouman et al., 2001; Hsiao et al., 2002; Kato et al., 2006; Kumar et al., 2006). The new finding in our study is that spikelet sterility in rice is also common with stress during tillering, as was observed in 2013/2014 when tiller abortion was severe. Tiller abortion could have resulted in low assimilate supply for STi because spikelet sterility of more than 20% indicates that assimilate supply from photosynthetic tissues (source) to the grains limited yield (Fageria, (2007). In contrast, negligible effects of WS during late reproductive stages on grain yield, yield components and WUE implies an opportunity to save irrigation water. It is important to highlight the adaptation to stress during anthesis that may have led to better grain yield under SAT treatment than was expected. Results of Table 3 showed that stress during anthesis resulted in the greatest reduction in SLA, compared to other treatments. This increase in leaf thickness indicative of high SLA indicates preferred allocation of assimilates to the stems than to leaves (Asch et al., 2005). Consequently, plants for SAT treatment could have remobilised assimilates more than those for SPI.

There is a great potential for yield improvement of upland rice and to save water under irrigated systems. Results from this study showed that yield components and HI were not affected by water stress, while irrigation water could be saved by withholding water during anthesis and grain filling (Table 7). Water use efficiency did not decline significantly for treatments that were water stressed during anthesis and grain filling, because although total ET increased considerably for these two treatments, grain yield was not much affected. Fertility of rice grains in our study was less affected by WS, compared to values reported in literature under well-watered conditions. Kato et al. (2009) documented grain fertility values of 68–73% (27– 32% sterility) in lowland *O. indica* and *O. japonica* cultivars, while Arai-Sanoh et al. (2014) reported at least 80% fertility in *O. japonica* cultivars. Grain sterility in upland rice is generally higher than in lowland rice, with a difference of 5 – 10% on average between the two rice systems under well-watered conditions (Kato et al., 2006; Fageria, 2007). Since canopy shading likely contributed to pronounced sterility for the SPI and STi treatments, low plant densities may reduce sterility in situations vulnerable to stress during tillering and panicle initiation.

4.2. Implications for coping with stress under water-limited conditions

Rice is very sensitive to mild soil water deficits, partly because its root system is largely confined to the top (about 0.2 – 0.3 m) surface soil layers (Lilley and Fukai, 1993; Okada et al., 2002; Kato and Okami, 2010). In anaerobic soils, stress in lowland rice can develop at Ψ_{soil} below -86 kPa at shallow depths, which is only slightly below the lower limit of the optimum range reported for lowland rice (Bouman et al., 2001). Soil matric potentials estimated in our study during stress were lower than these generic values, indicating that the crop can adapt to drier soil conditions. Growth and morphological responses to stress, namely changes in leaf morphology, tillering ability and regeneration, deep rooting and increased leaf N uptake before grain filling of upland rice during and after stress in different developmental stages were distinct. These are useful traits in the adaptation of upland rice to water-limited environments. It is noteworthy that in most upland rice growing areas in SSA, where NERICA genotypes are popular, that WS is prevalent and can occur at any stage during crop growth (Jones et al., 1997; ARC, 2007). This makes it important to consider stage-specific responses and adaptations to WS. Leaf rolling associated with reduction in leaf transpiring surface (Wopereis et al., 1996) was peculiar during early reproductive stress, while leaf senescence was marked when stress occurred during tillering. Greater reductions in leaf area or LAI were measured with stress during tillering stage than stress during early reproductive stage. These results demonstrate that coping with stress during tillering or which occurs around this stage will be better for cultivars which retain some green leaves (‘stay green’ trait) than otherwise. This could be beneficial in minimising water loss as E, because our results showed that fractional interception of radiation was greatly reduced during stress periods and seasonal ET was thus higher (due to higher E) under stress than under well-watered conditions.

Crop recovery after stress had negative and positive impacts on the final yield with respect to stage. ‘Bounce back’ ability and highly elastic recovery of canopy growth, green LAI and FI was observed, irrespective of whether tillering abortion occurred under stress. Thus, the low number of productive panicles m^{-2} under early reproductive stress, despite similar tillers m^{-2} at harvest as CT, could be improved by lowering plant density. This result also suggests that a low tillering ability would be desirable for coping with stress during the early reproductive stage. As the crop recovered to larger green canopies than the CT post-flowering stage (following resumption of irrigation), mutual shading likely affected flowering of some existing tillers. The slower recovery of plants after stress during tillering than during early reproductive stage resulted in shorter plants and consequently also shorter panicles. Improving recovery in plant height if stress occurs around early growth stages seems to be important for yield enhancement and is more convenient for manual harvesting. Harvesting using sickles is common among smallholder farmers and is difficult in short plants.

Changes in soil water content over time at different depths has been used in several studies to indicate the impact of drought on the extent of root activity (Kondo et al., 2000; Lopes et. al., 2010; Yoyoongwech et al., 2013). The observed deeper extraction of water during early reproductive stage, when root depth is likely at its peak for well-watered rice (Kato and Okami, 2010), could have helped to avoid leaf senescence (maintenance of leaves despite rolling) in contrast to stress during tillering. Increase in rooting depth in well-watered conditions has been shown to increase leaf N concentrations 20 days after heading (approximately 98 DAE), with higher N levels in deep than shallow rooting lowland rice cultivars (Arai-Sanoh et al., 2014). Arai-Sanoh et al. (2014) attributed this difference to absorption of nutrients from lower soil layers by deep rooting cultivars. In our study soil mineral N at sowing, particularly NO_3-N in the 0.4 – 0.6 m layer, where deep rooting was observed for SPI, was very low and plants did probably not benefit from that. These findings have implications on agronomic practices, breeding objectives and cultivar selection by farmers for improving rice yield under water stress. Further investigations are needed on allowable depletion levels for minimal yield loss in irrigated upland rice and on plant density optimisation to control unproductive tillers during early reproductive stress.

5. Conclusions

Thermal time to the onset of specific developmental stages (except for the time to peak tillering) in upland rice was generally stable over seasons under stress-free conditions. However, upland rice phenology was very sensitive to water stress during early reproductive growth. Stress during this stage increased growing degree days greatly, not only to reach subsequent developmental stages, but also to reach maturity and thus it altered crop duration. Delay in flowering was not related to the effect of water stress during vegetative and reproductive stages on tiller development. Even without suppression of tillers or death of apical buds under stress, GDDs to flowering and anthesis still increased. Water stress before flowering resulted in a greater recovery of source size (canopy size) than for a well-watered crop at the same developmental stage. This ‘bounce back’ ability is specific to stress during early reproduction and is not related to tiller number during and after the relieve of stress. Grain yield loss under stress was largely as a result of fewer grains per panicle and spikelet sterility. Results suggest that improvements in spikelet number may be achieved through manipulating plant density. In rice, growing areas that are prone to a high risk of stress during early reproductive stages, low plant densities may be recommended to minimise excessive unproductive tillers at harvest. Stress during anthesis and grain filling resulted in no substantial yield and water use efficiency penalty, while a considerable amount of irrigation water could be saved. The yields achieved in our study match typical grain yields reported for lowland rice systems, suggesting that there is considerable room for increased upland rice yields. The information gained in this study will help upland rice farmers to optimise water management practices, for example to irrigate only during a critical stage and save water during late reproductive stages. Dryland farmers can minimise risks of high water losses by opting for low plant densities and selecting planting dates that reduce chances of early reproductive stress.

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