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Soil temperature related N dynamics in high-altitude rice production systems of Rwanda

Master Thesis

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Abstract

In high-altitude environments, the thermal window for rice production remains small due to cold stress during early/late season. Soil, water and ambient air temperature determine the development and growth rates of rice and thus the stage specific requirements for water and nutrients. However, farmers often follow broad-based fertilizer recommendations which do not consider effects of elevation or growth-stage specific crop nutrient requirements. Thus, synchrony between crop nutrient supply and actual crop demand is sub-optimal.

To evaluate effects of root zone and ambient air temperature related nitrogen dynamics in high-altitude rice production systems of Rwanda, two experimental sites (high-altitude, Rwasave; low-altitude, Bugarama) along a temperature gradient were selected for field experiments. Experiments were designed as split-split plot with rice variety (Chhomrong, IR-64) at the main level, nitrogen rate (80 kg ha^{-1}, 160 kg ha^{-1}) at the sub-level, and the application or omission of basal nitrogen (+/-) at the sub-sub-level. Nitrate-N and ammonium-N in soil and water fractions were evaluated 1 day before and up to 5 days after fertilizer applications at transplanting, mid-tillering, panicle initiation, and heading; similarly, SPAD and leaf color chart values were recorded on the same days. SPAD and leaf color chart (LCC) were corrected for specific leaf weight (SLW) to more accurately reflect actual crop nitrogen status.

Within variety and nitrogen rate, the omission of basal nitrogen did not significantly reduce final yield or yield specific parameters for two contrasting rice varieties compared to the application of basal nitrogen at both experimental sites, respectively. In Rwasave, the percent of applied urea recovered as nitrate-N and ammonium-N in the soil and water fractions were found to be greatest in the soil nitrate (NO_3^-) fraction at MT, and lowest in the water ammonium (NH_4^+) fraction at HD. Significant differences were found in mean SPAD/SLW and mean LCC/SLW values, respectively between two rice varieties at two nitrogen application rates, with or without basal nitrogen application, at different growth stages and both sites.

These results suggest that application of basal nitrogen could be shifted to later phenological growth stages to more appropriately synchronize crop nutrient supply with actual crop demand without reducing final grain yield or specific yield parameters. Additionally, crop responses to fertilizer applications differed significantly between variety, nitrogen rate, and application of basal nitrogen.

Statutory Declaration

I, Isaac Ryan Vincent, born on 20 September 1989, matriculation number 645711, hereby declare that the attached Master's thesis has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

Supervisors: Prof. Dr. Folkard Asch, Tobias Hartmann

Thesis topic: Soil temperature related N dynamics in high-altitude rice production systems of Rwanda

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Stuttgart, Germany

31 July 2018

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Isaac Ryan Vincent

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List of Abbreviations

B	*Basal*
CH	*Chhomrong*
C_{leaf}	*Leaf Canopy Chlorophyll Content*
DAF	Days AFter Fertilizer
DAT	*Days After Transplanting*
DTT	*Daily Thermal Time*
DW	*Dry Weight*
GDD	*Growing Degree-Days*
GS	*Growth Stage*
HA	*High Altitude*
HD	*Heading*
IR	*IR-64*
K	*Potassium*
KCl	*Potassium Chloride*
LA	*Low Altitude*
LCC	*Leaf Color Chart*
masl	*Meters Above Sea Level*
MT	*Mid Tillering*
N	*Nitrogen*
$N0_3^-$	*Nitrate*
N_{DW}	*Nitrogen Dry Weight, Nitrogen Dry Weight*
NH_4^+	*Ammonium, Ammonium*
NO_3^-	*Nitrate*
NUE	*Nutrient Use Efficiency*
P	*Phosphorus*
PI	*Panicle Initiation*
RAB	*Rwanda Agriculture Board*
SLA	*Specific Leaf Area*
SLW	*Specific Leaf Weight*
SPAD	*Soil Plant Analysis Development*
T_a	*Air Temperature*
T_{base}	*Base Temperature*
T_D	*Day Temperature*
T_{max}	*Maximum Air Temperature*
T_{min}	*Minimum Air Temperature*
T_N	*Night Temperature*
T_{opt}	*Optimum Temperature*
TR	*Transplanting*
T_s	*Soil Temperature*
TSP	*Triple Super Phosphate*
T_w	*Water Temperature*

Introduction

In recent decades, rice has become the most rapidly growing food commodity in Sub-Saharan Africa playing a key role in providing food security for the poorest categories of rural and urban populations (Africa Rice Center, 2018). With the proportion of Africans living in urban areas expected to increase to nearly 48% by 2030, rice consumption in Africa is projected to continue growing for the foreseeable future. In order to meet the growing demand for rice, yields must increase by 25% over the next 20 years (Dobermann & Fairhurst, 2000).

Rising temperatures as an effect of climate change lead to higher altitudes being increasingly exploited for rice production formerly ill-suited for rice cultivation due to low temperatures. The thermal window for a rice crop in high altitudes (1600 masl and above) remains small, with cold stress early and/or late in the season being a severe constraint (F. Asch, personal communication, December 12, 2016). Temperature determines the development and growth rates of rice and thus the stage specific requirements for water and nutrients. Thus far, fertilizer application recommendations are quite general and have been developed based on data extrapolated from dissimilar sites with regards to indigenous soil fertility. This means that within an agro-ecological zone effects of elevation on site specific climate are not taken into consideration when estimating nutrient demands of crops grown in higher altitudes and thus in different thermal environments. Both the phenology-specific requirements and the physio-chemical characteristics of the nutrient in question determine the dynamics of fertilizer use in temperature limited systems and need to be synchronized to achieve optimal fertilizer use efficiency. Nutrient management practices typically disregard the effects of temperature in estimating crop nitrogen (N) requirements and in determining the crop specific dosage. Consequently, insufficient and inappropriate fertilizer management practices often result in poor fertilizer nutrient use efficiency (NUE). This accounts for one-half to two-thirds of the gap between the actual and potential rice yields in farmers' fields (Fageria & Baligar, 2005).

Models estimating N demand based on the phenological stages of the crop exist and are being used, however, these models are still neither sensitive enough to accurately estimate the effect of altitude on phenology nor are effects on soil nutrient dynamics or crop uptake dynamics due to the root zone thermal environment integrated into the models.

1. Hypothesis & Objectives

The underlying hypothesis of this study is that rice genotypes respond differently under different climatic conditions. Based on this assumption, the following hypotheses have been formulated:

1. Nutrient uptake dynamics are driven by temperature and genotype across altitudinal gradients.
2. Nutrient dynamics are linked to crop phenological growth stages.
3. Differences in the development rate of crop phenological growth stages result from genotype x environment (climate) interactions.

Based on the above hypotheses, the following objectives have been formulated:

1. To identify the optimum N application rate and split for different rice cultivars in two different agro-ecological zones.
2. To determine the evolution of soil nitrate-N and ammonium-N.
3. To determine N uptake and partitioning within the plant.

2. Literature review

2.1. Phenology and yield components of rice

Rice (*Oryza sativa*) genotypes are short-day plants whose crop duration is influenced by their sensitivity to photoperiod and temperature (Dingkuhn, Sow, Sambs, Diack, & Asch, 1995). Depending on variety and environmental conditions rice requires three to six months to complete all phenological growth stages (Mae, 1997). Juskiw, Jame and Kryzanowski (2001) defined phenology as the development of a plant through successive growth stages, from germination to physiological maturity. The rate of occurrence of different phenological growth stages during the growing season and the effect of temperature can be predicted using accumulated heat units or growing degree-days (GDD). GDD represent cultivars' characteristics of thermal requirements and photoperiod sensitivity. GDD during a development stage is the sum of daily thermal time (DTT) during the period. The concept of heat units has been applied to correlate the phenological development of different crops to predict grain yield and phenological maturity (Swan, Schneider, Moncrief, Paulson, & Peterson, 1987). GDD are based on the concept that real time to attain a phenological stage is linearly related to temperature in the range between base temperature (T_{base}) and optimum temperature (T_{opt}) (Bobade, Chandrawans, Patel, & Kausik, 2018).

Among other factors such as high water-use efficiency and a high harvest index (Richards, 1991) crop phenology is a major factor influencing adaptation and yield of annual crops. Knowledge of crop development patterns and phenology can be used to improve management practices during the crop growth cycle to obtain higher yields (Fageria & Knupp, 2013). Additionally, knowledge of occurrence of growth stages can be used in physiological studies to identify critical periods sensitive to environmental factors, and to adopt appropriate strategies to reduce biotic and abiotic stress factors (Fukai, 1999).

Yoshida (1981) delineated rice phenology into two basic growth stages: vegetative and reproductive. Fageria, Baligar and Jones (1997) further sub-divided the reproductive stage into pre-heading and post-heading periods. The latter stage is also referred to as the ripening period. Hence, agronomically, the life history of rice can be regarded in terms of three phenological stages: vegetative, reproductive, and ripening (Fageria et al., 1997).

The vegetative stage is extended from germination to panicle primordia initiation, and is characterized by an increase in plant height, tiller number, root length or weight, and shoot dry weight. The number of tillers, hence, the number of panicles is determined during the vegetative period (Fageria, 2007). The reproductive stage begins with the differentiation of panicle primordia and extends up to flowering (Counce, Keisling, & Mitchell, 2000). The reproductive stage is characterized by culm elongation, emergence of flag leaves, booting, heading and flowering. Spikelet number, an important yield component, is mainly determined during this period (Fageria, 2007). The ripening stage is characterized by grain filling and senescence of leaves. During this stage, actual yield is largely determined.

Yield is defined as the amount of specific substance produced (e.g., grain, straw, total dry matter) per unit area (Soil Science Society of America, 1997). In the case of rice, yield refers to the weight of cleaned and dried grains harvested per unit area (Fageria, 2007). In any given environment, final yield is the result of yield components and yield associated parameters developed in different development phases and growth stages (Shrestha, Asch, Dusserre, Ramanantsoanirina, & Brueck, 2012). Performance of individual genotypes in any given environment reflects the cumulative environmental effects on the different processes involved in building the final yield (Shrestha et al., 2012). Yield components and yield associated parameters include number of panicles per unit area, number of spikelets per panicle, weight of spikelet and spikelet sterility or filled spikelets (Fageria, 2007). In addition, shoot dry weight, grain harvest index, and nitrogen harvest index are also positively associated with grain yield. Yield potential is determined by the number of tillers formed during the vegetative growth phase, the number of panicles induced at the end of the vegetative stage, the number of spikelets formed in each panicle during panicle development, the number of fertile spikelets determined during the booting and flowering stage, and the final individual grain weight determined during the grain filling phase (Zamski & Schaffer, 1996). Yoshida (1981) reported that expected final grain yield (kg ha^{-1}) could be computed based on the following equation:

$$Grain\ yield\ = \left(\frac{spikelet\ number}{m^2}\right) \text{x}\ 1{,}000\ grain\ weight\ (g)\ \text{x}\ filled\ spikelets\ (\%)\ \text{x}\ 10^{-5}$$

Yoshida (1981) and Krishnan, Ramakrishnan, Raja Reddy and Reddy (2011) further noted that all yield components are strongly influenced by the climatic conditions the plant experiences during the respective phases in which the components are developed. Final yield of a given cultivar, therefore, depends on the interactions between the genotype, its responses to environmental conditions, and management practices (Krishnan et al., 2011). Hence, under the same management practices, the interaction between environmental characteristics and genotype is the sole determinant of varietal performance (Dingkuhn, Luquet, Kim, Tambour, & Clement-Vidal, 2006).

2.2. Nitrogen management in rice production

N is one of the most essential nutrients for rice production. It is required by rice plants in large quantities for optimal yield (Fageria, dos Santos, & Cutrim, 2009). N is a constituent of numerous important compounds including amino acids, proteins (enzymes), nucleic acids, and chlorophyll (Traore & Maranville, 1999). N is also one of the most yield-limiting nutrients in crop production in all agroecological zones throughout the world (Fageria et al., 2005). The main reasons for N deficiency in annual crops such as rice are due to low recovery efficiency (Fageria, Moreira, & Coelho, 2011; Ali, Thind, Sharma, & Singh, 2015). Low recovery efficiency of N is associated with several transformational and loss mechanisms (Ali et al., 2015) via nitrate leaching, denitrification, ammonia volatilization and soil erosion. A major cause for low NUE is related to poor synchrony between soil N supply and crop demand (Raun & Johnson, 1999). Furthermore, inefficient splitting of N applications, including the use of excess N during early vegetative growth stages of rice contributes to low NUE (Thind, Kumar, Gupta, Kaul, &

Vashistha, 2012). The magnitude and nature of N losses vary depending on the timing, rate and method of N application, source of N fertilizer, soil chemical and physical properties, climatic conditions and crop status (Zhang & Wang, 2005).

Current N fertilizer recommendations for irrigated rice generally consist of fixed rates and timings for large rice growing areas having similar climate and landforms (Thind et al., 2012). Broad-based, blanket fertilizer recommendations do not take into account field-to-field variability of indigenous soil N supply, disregard the effects of temperature in estimating crop nutrient requirements, and are unresponsive to temporal variation in crop N demand (Thind et al., 2012). Furthermore, effects of elevation on site specific climate are not taken into consideration when estimating nutrient demands of crops grown in higher altitudes. Farmers aim to improve fertilizer applications with regards to crop, planting date, and region rather than following blanket N recommendations. Improvements in N management for rice include: (i) adjusting early N applications to match the relatively low demand of young rice plants; (ii) varying rates and distribution of fertilizer N within the growing season to more appropriately synchronize crop demand for supplemental N. In this respect, the leaf N status serves as a sensitive indicator of the actual crop demand for N during the growing season (Huang et al., 2008). Several methods are available for assessing the N status of crops. One method is plant tissue analysis, which determines Kjeldahl-N (Gholizadeh, Saberioon, Borůvka, Wayayok, & Mohd Soom, 2017). Plant tissue analysis is a direct and accurate method. However, it is time consuming and trained operators are required. Other methods exist for site-specific assessment of the N status of crops based on leaf and canopy optical properties (Gholizadeh et al., 2017). In farmers' fields or on experimental field stations, sophisticated measurements for monitoring crop nutrient status based on tissue analysis or leaf canopy optical properties are often not available. However, the nutrient status of the rice crop is an important indicator to guide fertilizer application strategies for nitrogen. Therefore, it is important to quickly and accurately diagnose leaf N status using diagnostic tools**.** As leaf color is a good indicator of leaf N content, the leaf color chart (LCC), developed by the International Rice Research Institute (IRRI) serves as a visual and subjective indicator of plant N deficiency (Thind et al., 2012). With four to six color panels of different shades of green, the LCC is an inexpensive diagnostic tool for monitoring the greenness of a rice leaf as an indicator of the plant N status (Alam, Ladha, Khan, Khan, & Buresh, 2005). However, this method does not take into to account differences in leaf greenness that are due to variety, plant type, or leaf thickness. The Minolta Soil Plant

Analysis Development (SPAD) meter (Konica Minolta Sensing Inc., Tokyo, Japan) is a second diagnostic tool used to assess the relative greenness of plants in a quick, non-destructive manner. SPAD readings provided by the hand-held spectrophotometer positively correlate leaf chlorophyll and leaf N concentration (Debaeke, Rouet, & Justes, 2006). As with the LCC, SPAD readings do not account for leaf thickness or leaf weight. Peng, Garcìa, Laza and Cassman (1993) showed that prediction of leaf N concentration on a dry-weight basis (N_{DW}) improved with a simple calculation correcting SPAD for specific leaf weight (SLW). Site-specific and real-time N management strategies based on crop N status expressed as leaf greenness measured by chlorophyll meter (SPAD) and/or LCC are being actively introduced in many countries (Kumar, Vashistha, Thind, & Gupta, 2016).

2.3. Soil and water temperature influence on nitrogen uptake

Rice is widely grown as an annual crop in many countries throughout the world in both temperate and tropical zones, from latitudes ranging between 53°N to 40°S (Lu & Chang, 1980). Hence, rice production is subject to supra and sub-optimal temperatures. Knowledge of the influence of temperature on nutrient uptake of rice during the growth cycle is an important aspect of mineral nutrition and improving total grain yield. While the present study focuses on soil temperature and irrigation water temperature-related N dynamics in a high altitude rice production system, numerous studies have been conducted on micro/macro nutrient uptake and partitioning throughout different phenological growth stages of rice plant (Sarwar et al., 2013; Julia, Wissuwa, Kretzschmar, Jeong, & Rose, 2016; Yoneyama, Fukuda, & Kouchi, 1989; Shimazaki, Satake, & Watanabe, 1963; Mikkelsen, 1970; Guindo, Wells, & Norman, 1994).

The amount of N uptake by rice plants is one of the key factors determining biomass production and grain yield (Shimono, Fujimura, Nishimura, & Hasegawa, 2012). In cool climates, temperatures below an optimal value of approximately 25°C can inhibit N uptake and affect biomass production and grain yield (Yoshida, 1981). Rice is most often grown under flooded conditions, with the shoot meristem and the root zone both located under water, such that water temperature can have a larger influence on plant growth and N uptake than air temperature (Matsushima, Tanaka, & Hoshino, 1964; Stuerz, Sow, Manneh, & Asch, 2014). Several studies have examined the response of N uptake to low air and water temperatures during various growth stages (Shimono et al., 2012; Nagasuga, Murai-Hatano, & Kuwagata, 2011; Shimono, Okada, Kanda, & Arakawa, 2007). However, it is still unclear whether the underlying mechanisms responsible for decreased biomass production and grain yield are caused by the integrated effects of low temperature on the root zone and shoot meristem near the soil surface or the effect on root system alone (Nagasuga et al., 2011).

N uptake is determined by three main factors: the soil N supply, root N uptake ability and N demand of the plant (Shimono et al., 2012). N supply comes from both fertilizer and the mineralization of soil organic matter. Toriyama (1994) showed that soil N mineralization processes can be slowed by low water temperatures. Root N uptake ability can be reduced by low root-zone temperatures as a result of reduced enzymatic activities as well as ammonium and nitrate transporters at the root surface (Engels, 1994). Shimono et al. (2012) showed that plant N demand under low water temperature is growth-stage dependent.

In temperate zones and high-altitude regions, low temperature stresses cause an estimated annual grain loss of about 10% (Garg et al., 2002). Yield loss due to low temperature stress have been reported in Australia, the United States, Senegal, China, Bangladesh, India, and Nepal (Shimono et al., 2007). Low temperatures can reduce germination (Basnayake, Sihathep, Sipaseuth, Sonekham, & Senthonghae, 2003), cause poor establishment (Sasaki, 1979), delay phenological development and increase spikelet sterility (Farrell et al., 2001). While low temperature stress affects all growth stages of rice, from germination to grain maturity (Ha, Mitchell, & Fukai, 2017), the most critical stage for low temperature effect occurs during the booting stage, prior to panicle emergence (Gunawardena, Fukai, & Blamey, 2003). Many studies have been conducted to determine the physiological mechanism responsible for low temperature-induced spikelet sterility (Shimono et al., 2007). Basnayake et al. (2003) reported that high spikelet sterility is often associated with failure of the developing pollen grains, rather than damage caused to the developing stigma. A low number of viable pollen at flowering stage limits pollination, thus resulting in sterility (Shimono et al., 2007). Duration of cold exposure is also an important factor that determines the effect of low temperature on rice yield. Under flooded rice conditions, spikelet sterility is determined by both irrigation water (T_w) and air (T_a) temperature. The panicle is the plant organ most sensitive to low temperature, and has a large influence when determining spikelet sterility (Shimono, Hasegawa, Moriyama, Fujimura, & Nagata, 2005).

3. Material and Methods

3.1. Locations and experimental design

Rwanda has a temperate tropical highland climate, characterized by two rainy seasons. The first rainy seasons occurs February to June and the second from September to December. These are separated by two dry seasons. The long dry season lasts from June to September and a shorter, less severe dry season from December to February (King, 2007). To evaluate effects of root zone and ambient air temperature related N dynamics, two experimental sites differing in altitude along a temperature gradient were selected for field experiments.

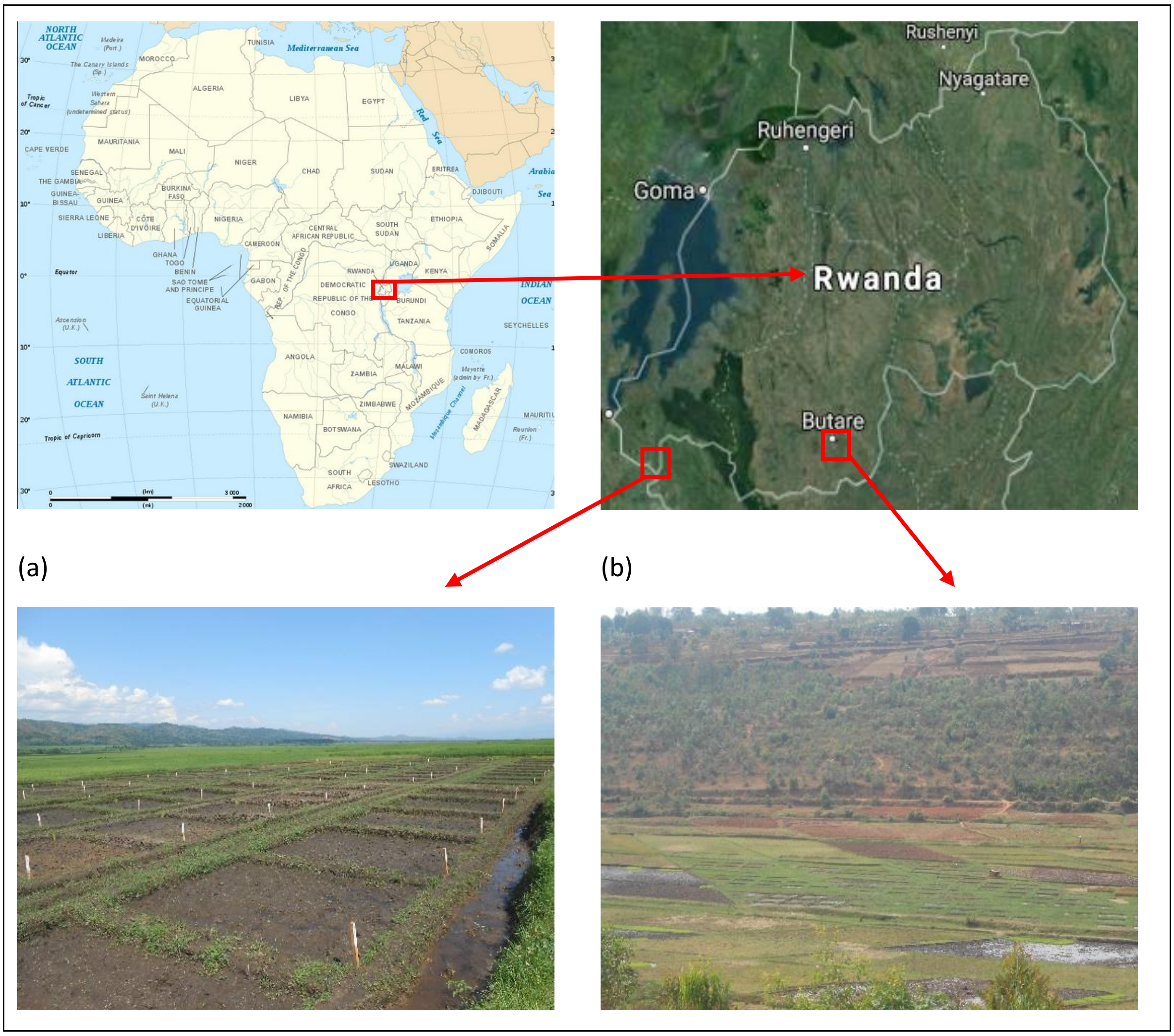

Figure 1. Map of Rwanda and the location of (a) low altitude Bugarama marshland, Bugarama (41°50’S, 29°00’E; 900 m altitude), (b) Rwasave marshland, Butare (2°36’S, 29°43’E; 1600 m altitude) experimental sites.

Field experiments consisted of late season sowing dates of two contrasting rice varieties in mid-May 2017. Experiments were conducted at the Rwanda Agriculture Board (RAB) research stations at (i) high altitude (HA) Rwasave marshland, Butare (2°36’S, 29°43’E; 1600 m altitude), Huye district, in the Southern province of Rwanda; (ii) low altitude (LA)

Bugarama marshland, Bugarama (41°50'S, 29°00'E; 900 m altitude), Rusizi District, Western Province, near the borders of Burundi to the east and the Democratic Republic of Congo to the west (**Fig. 1**).

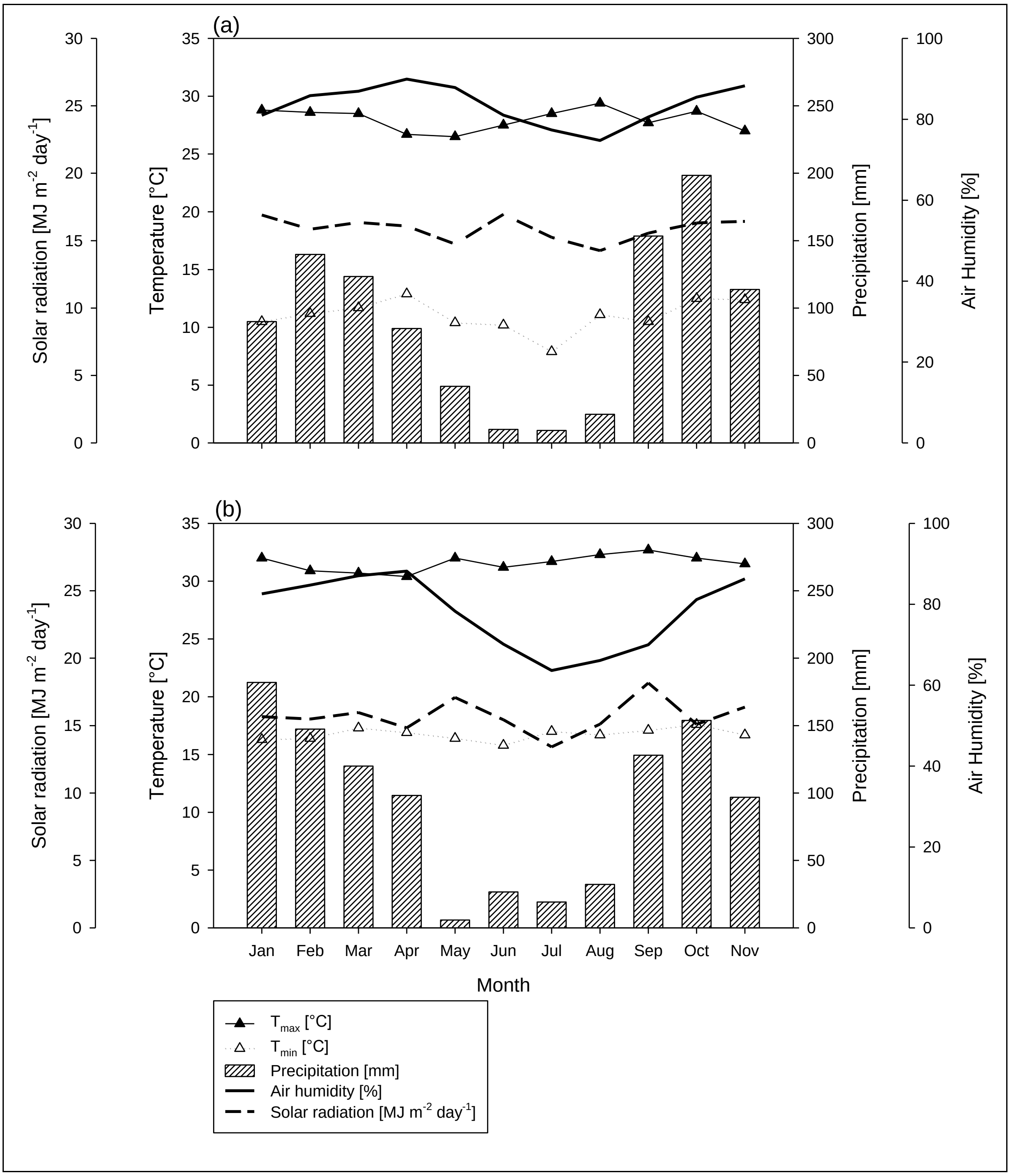

Figure 2. Recorded meteorological data taken by the Rwanda Agricultural Board micrometeorological stations at (a) HA, (b) LA research stations from January to November 2017.

The experiments were designed as split-split plot with N rate at the main level, variety at the sub level, and basal application at the sub-sub level arranged in a randomized complete block design with three replications. Climatic data were recorded using Tinytag Plus 2 data loggers (Tinytag Plus 2 models TGP-4017, Gemini Data Loggers, UK) at both HA and LA locations.

During the cropping season, average monthly minimum (T_{min}) and maximum air temperature (T_{max}) ranged between 7.9–10.2°C and 26.5–29.40°C, respectively with 986 mm of total rainfall in HA (**Fig. 2**). Average daily humidity (%) in HA decreased from April (89.9%) prior to transplanting of seedlings to August (74.4%). During the period April–August, total monthly precipitation also decreased from 84.4 mm to 21 mm, respectively. In LA, average monthly T_{min} and T_{max} ranged between 15.8–17.1°C and 31.2–32.7°C, respectively and received 1167 mm of total rainfall during the cropping season. Average daily humidity (%) in LA decreased from April (88.1 %) prior to transplanting of seedlings to July (63.5%). During the period April–July, total monthly precipitation also decreased from 154 mm to 4 mm, respectively. The recommended sowing date in HA is between early to mid-February, during the long rainy season, and again during the short rainy season between mid-August to mid-September. Sowing occurred on 19/04/2017 in both HA and LA. Seedlings were transplanted from seed beds on 11/05/2017 and 10/05/2017 in HA and LA, respectively.

HA had sandy clay loam soil of pH (KCl) 4.6. Soils in HA are predominantly Humic Ferralsols with characteristically high iron content, low supply of plant available nutrients, and low phosphorus (Van Wambeke, 1974). LA had sandy clay loam soil of pH 6.1 (KCl). Soils in LA are classified as Eutric Vertisols, having high clay content causing soils to swell when wet and crack when dry. Specific soil properties are presented in **Table 1**.

Table 1. Specific soil properties at two research stations taken prior to field experiments in 2017.

Location	pH [a]	Organic C [b]	Total N [b]	Total P [b]	Exch. K [c]	%Sand	%Silt	%Clay	Soil texture class
LA	6.1	18.8	1.8	0.5	132.2	48.97	22.63	28.4	Sandy clay loam
HA	4.6	32.6	2.4	0.4	-	60.52	9.82	29.66	Sandy clay loam

[a] KCl; [b] g kg^{-1}; [c] mmol kg^{-1}

GDD at both locations was calculated as shown in **EQUATION 1**.

$$GDD = \left(\frac{Tmax + Tmin}{2}\right) - Tbase,$$

where T_{max} and T_{min} are the daily maximum and minimum air temperature, respectively. T_{base} is the reference base temperature of 10°C. Duration of each growth stage was calculated as the number of days from one fertilizer application to the next.

3.2. Genotypes and crop management

Two contrasting genotypes, Chhomrong (CH) and IR-64 (IR) were selected for this study (**Table 2**). Chhomrong is a traditional, short duration *japonica* landrace adapted to middle and high altitudes of Rwanda and Nepal. Chhomrong is a high tillering, cold tolerant genotype rapidly defused after its release in 2006 in the HA of Madagascar (Shrestha et al., 2012).

Table 2. Characteristics of the Oryza sativa genotypes used in the study. *Abbreviation*: temp, temperate; trop, tropical; trad, traditional; imp, improved; intern, internal.

Variety name	Sub-species	Type	Growing altitude	Country of origin	Days to maturity	Special property
Chhomrong	temp japonica	Trad	high	Nepal	133	cold tolerant
IR-64	trop indica	Imp	mid	Philippines	117	intern. check

IR-64 is an improved, short duration *indica* genotype bred by the IRRI and released in the Philippines in 1985. IR-64 was chosen as an internal check variety as it is well suited to irrigated and rainfed lowland areas (Khush & Virk, 2005). IR-64 is tolerant to moderate levels of salinity, alkalinity, and phosphorus deficiency.

Soils at HA and LA were hand ploughed before transplanting (TR). Each plot size was 12 m² (3 m x 4 m) in HA and LA. Hill to hill spacing was 20 cm x 20 cm in HA and LA. Plots were separated by bunds. Fertilizer phosphorus (P) and potassium (K) was applied as a basal dose at a rate of 30 kg ha^{-1} triple super phosphate (TSP) and potassium chloride (KCl), respectively. Urea (46% N) was split as top dressing at basal (B), mid tillering (MT), panicle initiation (PI), and heading (HD) at a rate of 20 kg ha^{-1}, 20 kg ha^{-1}, 20 kg ha^{-1}, 20 kg ha^{-1}, respectively, for those treatments receiving a total of 80 kg ha^{-1} N with (+) basal application. For treatments receiving 80 kg ha^{-1} N without (-) basal N applications, urea (46% N) was applied at a rate of 40 kg ha^{-1}, 20 kg ha^{-1}, 20 kg ha^{-1} at MT, PI and HD, respectively. Urea (46% N) was applied as top dressing at B, MT, PI, HD at a rate of 20 kg ha^{-1}, 60 kg ha^{-1}, 60 kg ha^{-1}, 20 kg ha^{-1} respectively for those treatments receiving a total of 160 kg ha^{-1} N (+) basal application. For treatments receiving 160 kg ha^{-1}N (-) basal N applications, urea (46% N) was applied at a rate of 80 kg ha^{-1}, 60 kg ha^{-1}, 20 kg ha^{-1} at MT, PI and HD, respectively (**Table 3**). In HA, splits were applied at 0, 34, 69, and 124 days after transplanting (DAT). DAT in HA correspond with calendar days 11/05/2017, 13/06/2017, 18/07/2017, and 11/09/2017 respectively. In LA, splits were applied at 0, 31, 54 and 85 DAT. DAT in LA correspond with calendar days 11/05/2017, 10/06/2017, 03/07/2017, 03/08/2017. Manual weeding was done as required. Systematic fungicide (Carbenstor-500 SC) was applied at a rate of 1 L ha^{-1} to control leaf blast (*Pyriculariase*) when symptoms occurred.

Table 3. N rate, split, and amount applied at B, MT, PI, and HD in HA and LA.

		N application (kg ha-1)			
N rate	Split	B	MT	PI	HD
80 kg ha-1	(-) basal	0	40	20	20
	(+) basal	20	20	20	20
160 kg ha-1	(-) basal	0	80	60	20
	(+) basal	20	60	60	20

3.3. Sampling, measurement and data analysis

In HA, leaf chlorophyll content was determined at MT, PI and HD one day prior to fertilizer application and five consecutive days after each fertilizer application using a SPAD-502 (Konica Minolta Sensing Inc., Tokyo, Japan) based on measurements of leaf transmittance at 650 nm (red) and 940 (infra-red) wavelengths (Gholizadeh et al., 2017). At each growth stage, triplicate measurements were taken on both sides of the mid rib, one near leaf base and one near leaf tip for the first and third fully expanded leaves of four plants per plot (**Fig. 3**). This method was intended to overcome the effect of non-uniform distribution of chlorophyll in the leaves, and accordingly to produce more representative data. All measurements were then averaged to determine the mean leaf canopy chlorophyll content (C_{leaf}) of each plot. Total leaf area of the first and third fully developed leaves were combined using the ruler method, multiplied by correction factors to provide a more accurate leaf area. Following published procedures (Peng et al., 1993), SPAD values were corrected for leaf thickness. This way, SPAD values represented the N status of the plant and were used to calibrate LCC to actual crop N status. Dry weight (DW) was determined after oven-drying at 70°C to constant weight. SLW was calculated as the ratio of DW (mg) to leaf area (m^2). Specific leaf area (SLA) was calculated as the ratio of leaf area (m^2) to (mg).

The first and third fully expanded leaves were placed on top of the LCC, and the color of the middle part of the leaf was matched with greenness of the panels on the LCC. The leaf being measured was kept under shade of body to avoid color variance caused by sun. LCC values were recorded to the nearest 0.5 value. In this way, 8 LCC classes were derived to more accurately link greenness values provided by LCC to actual N content in the leaves. To analyze effects of variety*rate interaction in HA, LCC class values were regressed against SPAD/SLW values. In HA, SPAD measurements and LCC values were taken on the same days. In LA, LCC values were taken at MT, PI, and HD on 1, 4, and 8 days after fertilizer (DAF), respectively. SPAD and LCC data was used to compare 48 plots at both HA and LA, respectively. In LA, SPAD measurements were not taken. Regression equations for estimating SPAD/SLW measurements were based on values obtained in HA.

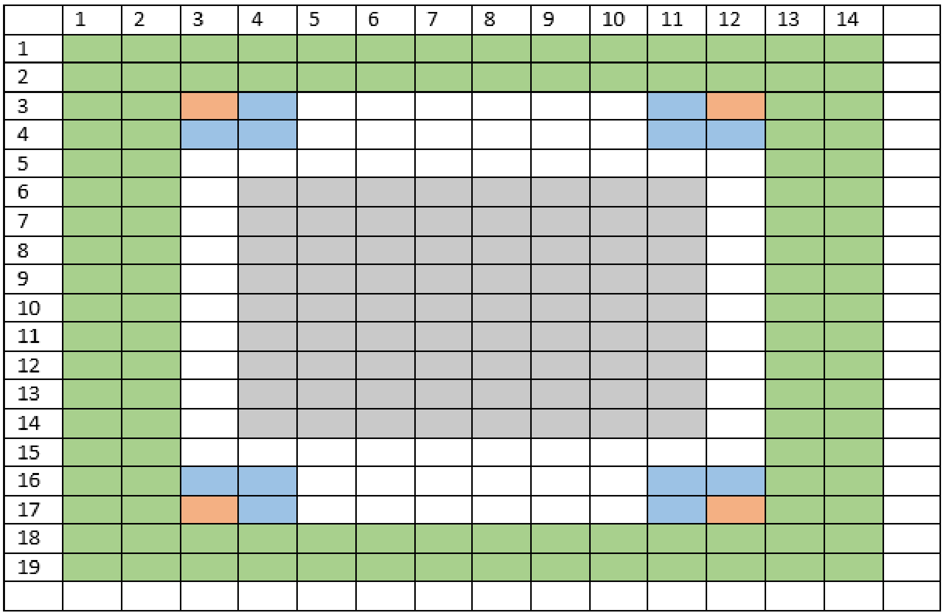

Figure 3. Schematic representation of 3x4 m^2 plot with 266 hills (14 x 19 rows) of a given genotype. Green areas, border rows for destructive observations of SLA and SLW; Orange area, SPAD and LCC measurements; Blue area, soil and water sampling; Gray area, hills for final harvest.

Following treatment sampling procedures detailed by Dobermann & Fairhurst (2000) soil samples were taken from the first 20 cm in regular intervals; that is, one day prior to fertilizer application and five consecutive days after each fertilizer application. Two soil samples were collected from each plot (**Fig. 3**). Mean values were calculated to represent each plot. Soil nitrate and ammonium-N were analyzed using Reflectoquant® reflectometric methods with quick-test strips. Ammonium-N in irrigation flood water was measured in the top 5 cm of each plot according to similar procedures up to four consecutive days after each fertilizer application in LA and up to five consecutive days after each fertilizer application in HA. Percentage change in N recovered as soil nitrate ($N0_3^-$) and soil ammonium (NH_4^+) was calculated for each treatment as the difference between amount recovered 1 DAF and 5 DAF at each growth stage. At PI and HD stages, N recovered as NH_4^+ in water were below detectable limits at 5 DAF. Therefore, changes in percentage of NH_4^+ in water fraction were analyzed at 4 DAF.

Temperature of the flooded irrigation water was taken using IR Crop Temperature Meter (Spectrum Technologies, Inc., Aurora, IL, USA). Soil temperature data was taken using a Digital Stem Thermometer (Omega Engineering, Inc., Norwalk, CT, USA). Significant differences in SPAD/SLW, SLA, SLW and DW content between treatments for each growth stage, and soil and water nitrate and ammonium were compared using two-tailed *t*-tests with an error probability, alpha = 0.05 using Microsoft Excel (2007). In LA, delta SPAD/SLW was calculated at 4 DAF at MT, PI and HD growth stages. In HA, delta SPAD/SLW was calculated at 5 DAF at MT, PI and HD growth stages.

4. Results

4.1. Growing degree-days and temperature differences at three growth stages in HA and LA

Based on **Table 4**, a greater amount of GDD were accumulated in LA than in HA between TR–MT and between MT–PI in fewer days. Between growth stage (GS) TR–MT an average of 8.5 GDD were accumulated per day in HA, compared to 15.3 GDD in LA. Between GS MT–PI an average of 8.2 GDD were accumulated per day in HA, compared to 14.8 GDD in LA. The amount of GDD accumulated per day in HA increased slightly from MT–PI to PI–HD, from 8.2 to 9.3 GDD, respectively. In LA, GDD accumulated per day did not differ between MT–PI (14.8) and between PI–HD (14.4).

Table 4. Rice crop growth stage duration (days) and growing degree-days (°C day day-) at HA and LA.

Location	Growth stage	Duration[a] (days)	Growing degree-days[b] (°C $^{day-}$)
HA	TR-MT	33	280.4
	MT-PI	34	278.1
	PI – HD	54	499.9
	Accumulated	*121*	*1058.4*
LA	TR-MT	31	474.7
	MT-PI	23	341.0
	PI – HD	31	446.3
	Accumulated	*85*	*1262*

[a] Days from one fertilizer event to the next; [b] Sum of daily average temperature minus T_{base} (10°C).

Table 5. Average daily T_{min}, T_{max}, T_s, and T_w at three GS in LA and HA. Average temperatures represent time period 1 day before fertilizer application until 8 days DAF application in LA, and 1 day before fertilizer application until 5 DAF application HA.

Location	Growth Stage	T_{min}	T_{max}	T_s	T_w
HA	Mid Tillering	12.7	26.6	21.6	26.5
	Panicle Initiation	12.4	25.0	21.6	25.0
	Heading	17.9	24.8	21.2	26.2
LA	Mid Tillering	18.0	30.8	23.5	22.7
	Panicle Initiation	19.0	30.2	24.4	24.7
	Heading	17.2	29.8	23.8	23.8

In HA, average daily T_{min} was lowest during PI followed by MT and HD stage, respectively (**Table 5**). Average daily T_{max} was also lowest during PI in HA. Soil temperature (T_s) did not differ greatly between three GS in HA. Average daily T_w was lowest in HA during PI, followed by HD and MT stages, respectively. In LA, higher average daily T_{min} were recorded during MT and PI (18.0°C, 19.0°C) than in HA. At HD, recorded average daily T_{min} was lower in LA than in HA (17.2°C, 17.9°C, respectively). In LA, average daily T_{max} was highest at MT, followed by PI and HD stages, respectively. In LA, average daily T_s was highest at PI, followed by HD and MT stages, respectively. Daily average T_w was also highest at PI, followed by HD and MT stages, respectively.

4.2. Nitrogen content recovered in soil and water fractions in HA

Percentage change in N recovered as soil $N0_3^-$ and soil NH_4^+ was calculated for each treatment as the difference between amount recovered 1 DAF and 5 DAF at each GS. At PI and HD stages, N recovered as NH_4^+ in water was below detectable limits at 5 DAF. Therefore, changes in percentage of NH_4^+ in water fraction were analyzed at 4 DAF.

During MT, N recovered as $N0_3^-$ in soil increased by 99% for CH80- (**Fig. 4a**). During the same period, N recovered as $N0_3^-$ in soil for CH80+ increased by 10%. N recovered as NH_4^+ in soil for CH80- decreased by 53%, while N recovered as NH_4^+ in soil for CH80+ decreased by 65%. The largest percentage change between CH80- and CH80+ was observed in the NH_4^+ water fraction. NH_4^+ in water fraction for CH80- increased by 636%, while NH_4^+ in water fraction for CH80+ increased by 152% (**Fig. 4c**). N recovered as $N0_3^-$ in soil increased by 10% for CH160-, while N recovered as $N0_3^-$ in soil increased by 161% for CH160+ (**Fig. 4c**). N recovered as NH_4^+ in soil for CH160- decreased by 24%, while N recovered as NH_4^+ in soil for CH160+ decreased by 3%. These trends differ from variety CH receiving N rate 80 kg ha^{-1}, where N recovered as NH_4^+ in soil for CH80- decreased by 53% at 5 DAF compared to N recovered as NH_4^+ for CH80+ which decreased by 65% at 5 DAF at MT. N recovered as NH_4^+ in water fraction for CH160- decreased by 78%, while NH_4^+ in water fraction for CH160+ decreased by 82%. These trends are similar for variety CH at nitrogen rate 80 kg ha^{-1}, where a greater percentage of NH_4^+ was observed in treatments not receiving basal N application than for treatments which did receive basal N application. During MT, N recovered as $N0_3^-$ in soil decreased by 23% for IR80-, while $N0_3^-$ in soil increased 116% for IR80+ (**Fig. 4b**). N recovered as NH_4^+ in soil for IR80- and IR80+ decreased by a comparable percentage at MT (48% and 50%, respectively). Similarly, N recovered as NH_4^+ in water increased by comparable percentages for both IR80- and IR80+ (228% and 220%, respectively). N recovered as $N0_3^-$ in soil for IR160- increased by 49%, while N recovered as $N0_3^-$ in soil for IR160+ increased by 86% (**Fig. 4d**). N recovered as NH_4^+ in soil decreased for both IR160- and IR160+ (26% and 39%, respectively). The trend of decreasing N recovered as NH_4^+ in soil at 5 DAF for variety IR-64 was observed at both N rate 160 kg ha^{-1} and N rate 80 kg ha^{-1}. N recovered as NH_4^+ in water increased by 8% for IR160-, while N recovered as NH_4^+ in water for IR160+ decreased by 28% (**Fig. 4d**).

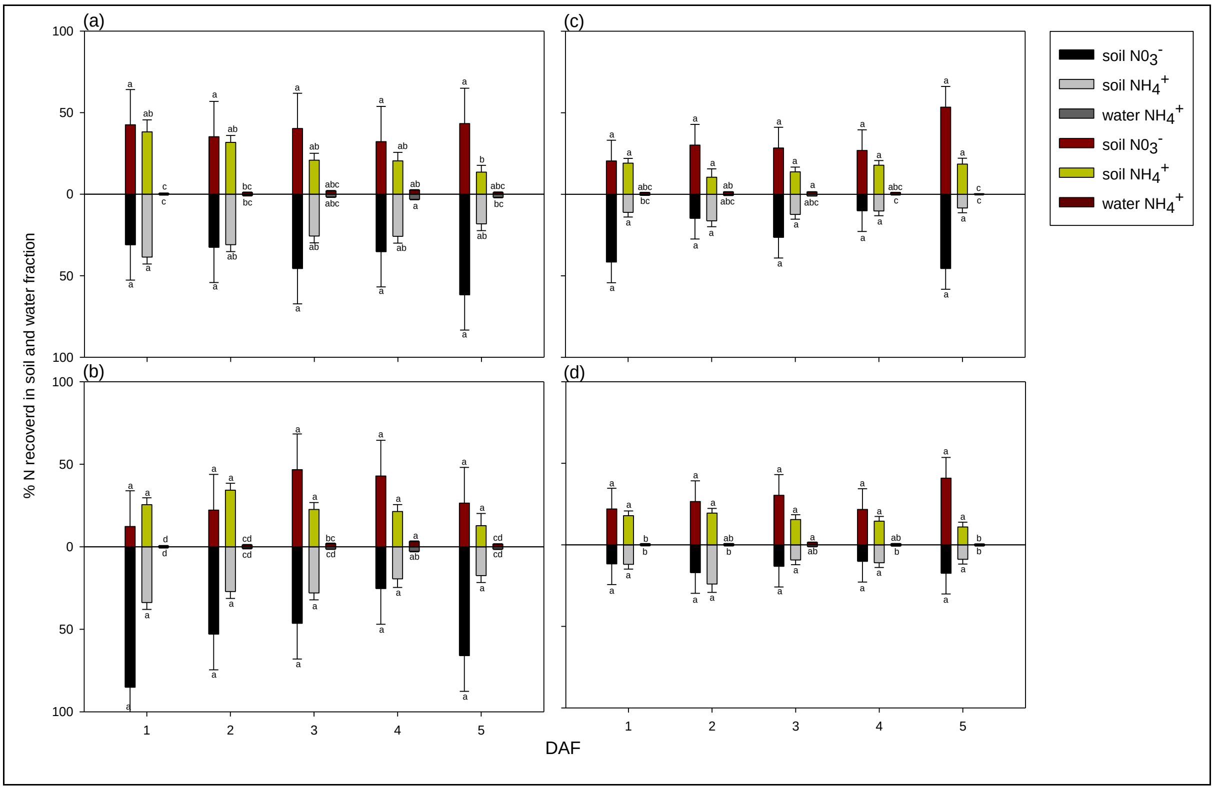

Figure 4. Percent of basal N application (g/L) recovered as $N0_3^-$ and NH_4^+ in soil and water fractions at MT for treatments (a) CH80-/CH80+, (b) IR80-/IR80+, (c) CH160-/CH160+, (d) IR160-/IR160+. Grey scale = treatment not receiving basal N application. Color scale = treatment receiving basal N application. Bars represent standard error. N = 6.

At PI, N recovered as NH_4^+ in water was below detectable limits at 5 DAF. Therefore, comparisons of NH_4^+ in water fraction were analyzed at 4 DAF. N recovered as soil $N0_3^-$ decreased by 71% and 38% for CH80- and CH80+, respectively (**Fig. 5a**). Soil NH_4^+ decreased for both CH80- and CH80+ by 23% and 14%, respectively. N recovered as NH_4^+ in water decreased by 40% for CH80- but increased by 34% for CH80+. N recovered as soil $N0_3^-$ decreased by 68% and 13% for CH160- and CH160+, respectively (**Fig. 5c**). N recovered as NH_4^+ in soil decreased in both CH160- and CH160+ by 21% and 29%, respectively. Unlike variety CH at nitrogen rate 80 kg ha^{-1}, N recovered as NH_4^+ in water increased by 49% and 11% for CH160- and CH160+, respectively. N recovered as $N0_3^-$ in soil decreased by 54% and 58% for IR80- and IR80+, respectively. The decreases in percent were greater than the decreases in percent observed for IR160- and IR160+ at PI (18% and 30%, respectively). N recovered as NH_4^+ in soil decreased by 17%, 22%, and 19% for IR80-, IR80+, and IR160- respectively. N recovered as NH_4^+ in soil increased by 1% for IR160+. No change in N recovered as NH_4^+ in water was observed for IR80- at PI. The greatest change in percent N recovered as NH_4^+ in water was observed in IR80+ (-42%), followed by IR160- (-38%) and IR160+ (-7%) respectively (**Fig. 5b; 5d**).

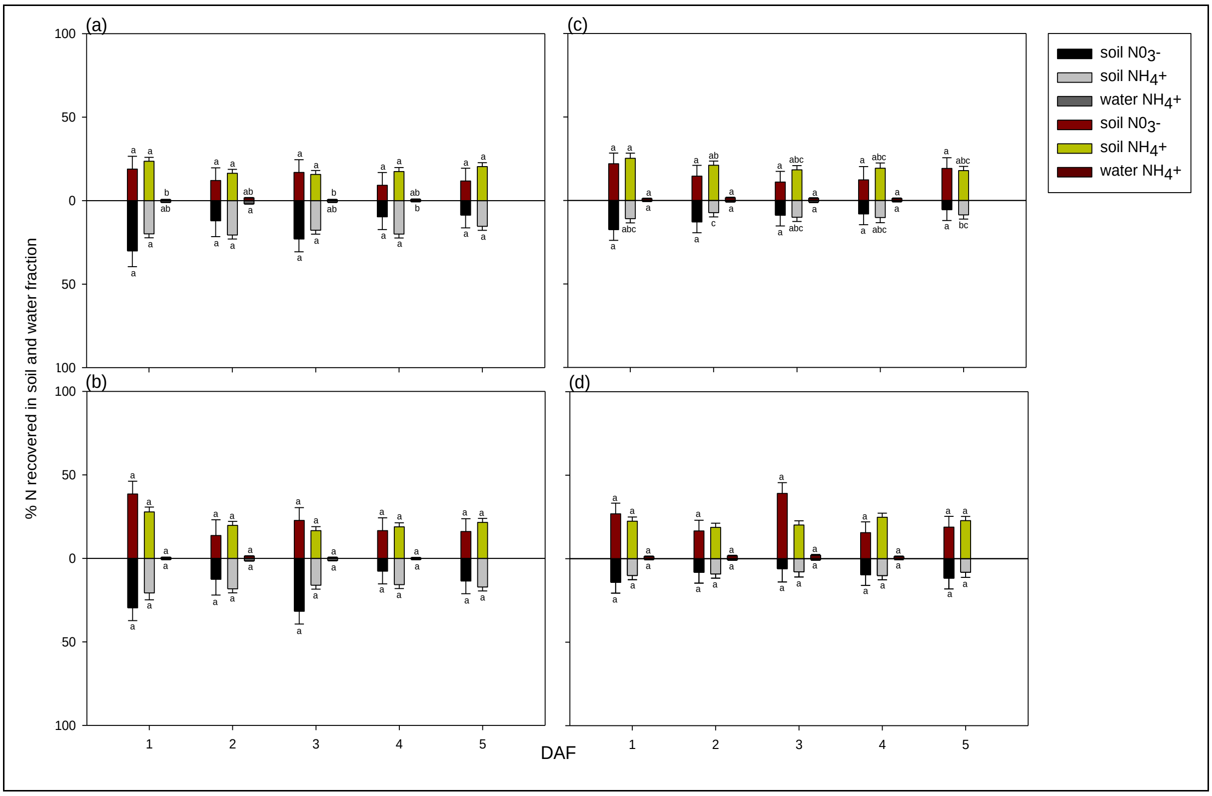

Figure 5. Percent of basal N application (g/L) recovered as $N0_3^-$ and NH_4^+ in soil and water fractions at PI for treatments (a) CH80-/CH80+, (b) IR80-/IR80+, (c) CH160-/CH160+, (d) IR160-/IR160+. Grey scale = treatment not receiving basal N application. Color scale = treatment receiving basal N application. Bars represent standard error. N = 6.

At HD, N recovered as NO_3^- in soil increased by 24% and 28% for CH80- and CH80+, respectively. At N rate 160 kg ha^{-1}, N recovered as NO_3^- in soil decreased for CH160- by 45% and increased for CH160+ 2% (**Fig. 6a**). N recovered as NH_4^+ in soil decreased by 22%, 23%, 36%, and 25% for CH80-, CH80+, CH160- and CH160+, respectively. Similarly, N recovered as NH_4^+ in water decreased in treatments CH80-, CH80+, and CH160+ by 33%, 81% and 5%, respectively. An increase in NH_4^+ in water was observed only in treatment CH160- (69%) (**Fig. 6a; 6c**). N recovered as NO_3^- in soil decreased in all IR treatments. The largest decrease in soil NO_3^- was observed in IR80+ (-37%) followed by IR160- (-27%), IR160+ (-22%), and IR80- (-1%). Decreases in soil NH_4^+ were observed for all IR treatments at HD except for IR80-, which showed a 9% increase in soil NH_4^+. The greatest decrease in soil NH_4^+ was 40%, 29%, and 14% for IR160+, IR80+, and IR160-, respectively. At 1 DAF, N recovered as NH_4^+ in water was beyond the detectable limit for IR160- (0.00 mg/L). Therefore, a significant increase was observed at 4 DAF (0.88 mg/L). N recovered as NH_4^+ in water increased by 4% and 7% for IR80- and IR160+, respectively. N recovered as NH_4^+ in water decreased by 83% for IR80+.

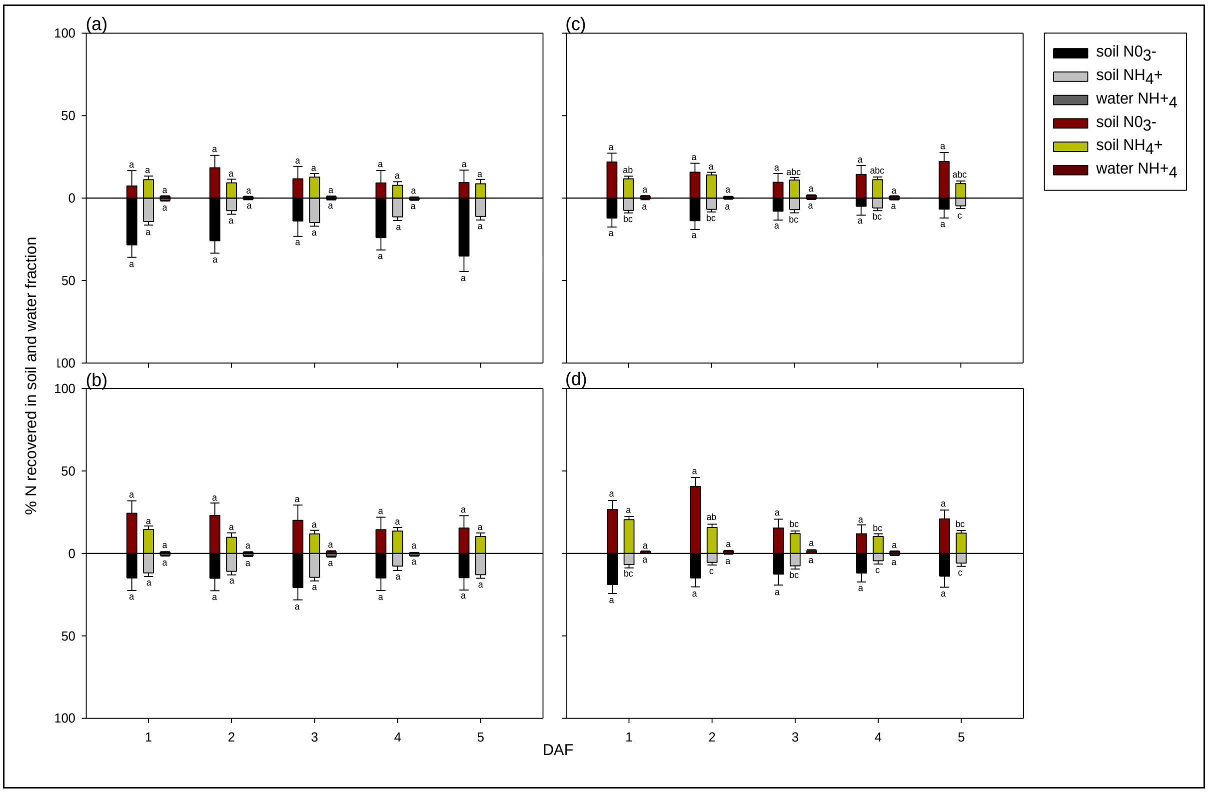

Figure 6. Percent of basal N application (g/L) recovered as NO_3^- and NH_4^+ in soil and water fractions at HD for treatments (a) CH80-/CH80+, (b) IR80-/IR80+, (c) CH160-/CH160+, (d) IR160-/IR160+. Grey scale = treatment not receiving basal N application. Color scale = treatment receiving basal N application. Bars represent standard error. N = 6.

4.3. Relationship between LCC class values and SPAD/SLW in LA and HA

At N rate 80 kg ha^{-1} mean SPAD/SLW values for CH80 in HA were observed within the range of 0.17 to 0.22 at MT (**Fig. 7a**) with minimum and maximum LCC class values of 2 and 3, respectively. In LA, mean SPAD/SLW values for CH80 were between 0.18 to 0.25 with minimum and maximum LCC class values of 4 and 6, respectively (**Fig. 7b**). Mean SPAD/SLW values for IR80 in HA were observed within the range of 0.20 to 0.23 at MT with minimum and maximum LCC class values of 4 and 5, respectively. In LA, mean SPAD/SLW values for IR80 were observed between 0.14 to 0.16 at MT with minimum and maximum LCC class values of 5 and 7, respectively. At N rate 160 kg ha^{-1} mean SPAD/SLW values were greater for IR160 (0.13) than for CH160 (0.08) at MT in HA. The opposite trend was found in LA where mean SPAD/SLW values were greater for CH160 (0.14) than for IR160 (0.12) at MT.

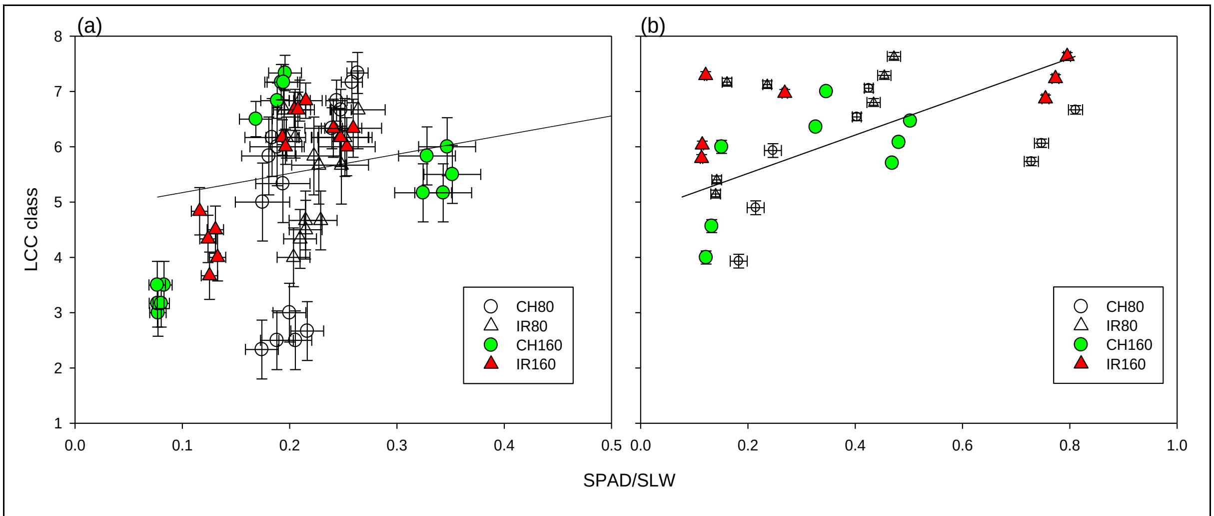

Figure 7. Pooled data for relationship between leaf canopy SPAD/SLW and LCC class values for two varieties across three GS in (a) HA, (b) LA. Regression line and equation represent pooled data across both sites. Bars represent standard error.

Mean SPAD/SLW values decreased by 0.01 between MT and PI for CH80 in HA. In LA, mean SPAD/SLW values increased between MT and PI by 0.55 for CH80. In HA, mean SPAD/SLW values for CH80 at PI were between the range of 0.17 to 0.19 with minimum and maximum LCC class values of 5 and 6, respectively. In LA, mean SPAD/SLW values for CH80 at PI were between the range of 0.73 to 0.81 with minimum and maximum LCC class values of 6 and 7, respectively. Mean SPAD/SLW values for IR80 in HA at PI were observed between 0.22 to 0.26 with minimum and maximum LCC class values of 6 and 7, respectively. In LA, mean SPAD/SLW values for IR80 were observed between 0.43 to 0.47 with minimum and maximum LCC class values of 7 and 8, respectively. In HA, mean SPAD/SLW values for CH160 (0.34) were found to be greater than mean SPAD/SLW values for IR160 (0.25) at PI. In LA, the opposite trend was observed. Mean SPAD/SLW values for IR160 (0.78) were found to be greater than mean SPAD/SLW values for CH160 (0.48) at PI.

Mean SPAD/SLW values decreased between PI and HD for all treatments in HA except for CH80, which increased by 0.07. In LA, mean SPAD/SLW values decreased in all treatments between PI and HD. At HD mean SPAD/SLW values for CH80 were observed between the range of 0.24 to 0.26 in HA (**Fig. 7a**) with minimum and maximum LCC class values of 6 and 7, respectively. In LA, mean SPAD/SLW values for CH80 were observed between the range of 0.40 to 0.43 with minimum and maximum LCC class values of 7 (**Fig. 7b**). In HA, mean SPAD/SLW values for IR80 were observed between the range of 0.19 to 0.21 with minimum and maximum LCC class values of 6 and 7, respectively. In LA, mean SPAD/SLW values for IR80 were observed between the range of 0.24 to 0.25 with minimum and maximum LCC class values of 7 and 8, respectively (**Fig. 7b**). Mean SPAD/SLW values for IR160 (0.20) were found to be greater than mean SPAD/SLW values for CH160 (0.18) in HA at HD. The opposite trend was found in LA where mean SPAD/SLW values for CH160 (0.34) were found to be greater than mean SPAD/SLW values for IR160 (0.28).

4.4. Treatment responses to fertilizer application at three growth stages in LA and HA

At MT, the highest SPAD/SLW values in LA were observed in CH80+ (0.25) which represented an increase of 17.3% between 1 DAF to 4 DAF (**Fig. 8; Table 6**). The lowest SPAD/SLW values were observed in IR80- (0.05) which represented a decrease of 1.3% between 1 DAF to 4 DAF (**Fig. 8a**). Pairwise comparison of treatment means receiving 80 kg N ha^{-1} indicated significant differences among all treatments at 4 DAF in LA. For variety CH, SPAD/SLW values at 4 DAF were significantly greater in CH80+ (0.25) than for CH80- (0.17). For variety IR, SPAD/SLW values at 4 DAF were significantly greater in IR80- (0.23) than for IR80+ (0.05). A similar trend was observed at N rate 160 kg ha^{-1} (**Fig. 8b**) where treatment means for variety CH were significantly greater for CH160+ (0.16) than for CH160- (0.10) and treatment means for variety IR were significantly greater for IR160- (0.13) than for IR160+ (0.09).

At MT, the highest SPAD/SLW values in HA were observed in CH80+ (0.22) and CH80- (0.18) (**Fig. 9a**) which represented increases of 11.2% and 17.8% increases between 1 DAF to 5 DAF, respectively (**Table 6**). The lowest SPAD/SLW values were observed in IR80- (0.10) and

IR80+ (0.11) which represented a decrease between of 5.3% and 0.5%, respectively between 1 DAF to 5 DAF. Pairwise comparison of treatment means receiving N rate 80 kg ha^{-1} indicated significant differences between the two varieties at 5 DAF, but no significant differences were observed within a single variety (**Fig. 9a**). At N rate 160 kg ha^{-1}, the only treatment that increased in SPAD/SLW value between 1 DAF and 5 DAF was observed in CH160- (0.17) which increased by 4.4% (**Table 6**). Treatment means for CH160+ (0.14), IR160- (0.12) and IR160+ (0.11) decreased between 1 DAF and 5 DAF by 5.5%, 11.0%, and 3.2%, respectively (**Table 6**). For all treatments SPAD/SLW values increased between MT to PI in LA (**Fig. 8c; 8d**). Among those treatments receiving N rate 80 kg ha^{-1}, CH80- had the greatest SPAD/SLW value (0.88) at 4 DAF but represented a decrease in SPAD/SLW value between 1 DAF to 4 DAF by 2%.

Table 6. Delta (Δ) SPAD/SLW expressed as a percent value at 4 DAF in LA, 5 DAF in HA at three growth stages.

Treatment	**Growth Stage**					
	MT		**PI**		**HD**	
	LA	HA	LA	HA	LA	HA
CH80-	17.3%	11.2%	-1.6%	-2.2%	-4.4%	10.8%
CH80+	17.2%	17.8%	7.2%	6.3%	-6.0%	9.2%
IR80-	-1.3%	5.3%	9.3%	7.5%	-6.7%	0.8%
IR80+	-2.1%	0.5%	8.3%	29.7%	-5.9%	3.4%
CH160-	11.2%	4.4%	1.4%	-1.7%	-4.5%	17.7%
CH160+	7.1%	-5.5%	4.0%	-8.6%	-6.6%	13.0%
IR160-	0.9%	-11.0%	6.2%	-2.9%	-11.1%	13.0%
IR160+	1.3%	-3.2%	4.7%	7.1%	-10.4%	9.2%

On the contrary, IR80- had the lowest SPAD/SLW value (0.38) (**Fig. 8c**) at 4 DAF but had the greatest percentage increase between 1 DAF to 4 DAF (9.3%) (**Table 6**). At N rate 80 kg ha^{-1}, CH80+ (0.65) and CH80- (0.88) were significantly greater than IR80+ (0.54) and IR80- (0.38), respectively. The opposite trend was observed at N rate 160 kg ha^{-1}, where IR60+ (0.87) and IR60- (0.72) were significantly greater than CH160+ (0.47) and CH160- (0.49). Pairwise comparison of treatment means receiving N rate 80 kg ha^{-1} indicated significant differences among all treatments. Similarly, pairwise comparison of treatment means receiving N rate 160 kg ha^{-1} indicated significant differences among all treatments at 4 DAF (**Fig. 8c; 8d**).

For N rate 80 kg ha^{-1}, SPAD/SLW values increased for CH80-, IR80-, IR80+ and decreased for CH80+ in HA (**Fig. 9c**) between MT and PI. For N rate 80 kg ha^{-1}, the greatest SPAD/SLW value was observed for IR80+ (0.38) while the lowest value was observed for CH80- (0.19). However, pairwise comparison of treatment means for N rate 80 kg ha^{-1} did not indicate significant differences between CH80-, Ch80+, IR80- and IR80+. For N rate 160 kg ha^{-1}, SPAD/SLW values increased for all treatments between MT and PI. The greatest SPAD/SLW value was observed in IR160+ (0.29) which represented the greatest percentage increase between 1 DAF and 5 DAF (7.1%). The lowest SPAD/SLW value was observed for IR160- (0.23) which represented a 3.0% decrease between 1 DAF and 5 DAF. Pairwise comparisons of treatment means for N rate 160 kg ha^{-1} did not indicate significant differences among treatments.

Table 7. Response curves for two varieties at three growth stages 4 DAF in LA.

GS	Treatment	Regression equation	R^2	Treatment	Regression equation	R^2
MT	CH80-	$Y = 0.14 + 9.92e^{-3}x$	1.0	CH160-	$Y = 0.08 + 3.86e^{-3}x$	1.0
	CH80+	$Y = 0.20 + 0.01x$	1.0	CH160+	$Y = 0.15 + 1.68e^{-3}x$	1.0
	IR80-	$Y = 0.24 – 7.85e^{-1}x$	1.0	IR160-	$Y = 0.13 – 6.35e^{-3}x$	1.0
	IR80+	$Y = 0.05 + 2.00e^{-1}x$	1.0	IR160+	$Y = 0.10 – 1.84e^{-3x}$	1.0
PI	CH80-	$Y = 0.91 – 0.02x$	1.0	CH160-	$Y = 0.49 – 8.14e^{-4}x$	1.0
	CH80+	$Y = 0.59 + 0.02x$	1.0	CH160+	$Y = 0.44 + 7.37e^{-3}x$	1.0
	IR80-	$Y = 0.33 + 0.02x$	1.0	IR160-	$Y = 0.65 + 0.03x$	1.0
	IR80+	$Y = 0.50 + 0.03x$	1.0	IR160+	$Y = 0.81 + 0.03x$	1.0
HD	CH80-	$Y = 0.41 – 5.83e^{-3}x$	1.0	CH160-	$Y = 0.30 – 4.43e^{-3}x$	1.0
	CH80+	$Y = 0.46 - 8.97e^{-3}x$	1.0	CH160+	$Y = 0.41 – 8.7e^{-3}x$	1.0
	IR80-	$Y = 0.37 – 8.13e^{-3}x$	1.0	IR160-	$Y = 0.36 – 0.01x$	1.0
	IR80+	$Y = 0.15 – 2.83e^{-3}x$	1.0	IR160+	$Y = 0.27 – 8.96e^{-3}x$	1.0

For all treatments SPAD/SLW values decreased between PI to HD stage in LA (**Fig. 8e; 8f**). SPAD/SLW values from all treatments continued to decrease between 1 DAF to 4 DAF. The largest decrease in SPAD/SLW values between 1 DAF to 4 DAF were observed in IR160- (11.1%) and IR160+ (10.5%), respectively. SPAD/SLW values for treatments CH80- and CH160- decreased by 4.4% and 4.5%, respectively and represent the smallest decrease in SPAD/SLW values at HD in LA (**Fig. 8e; 8f**).

For N rate 80 kg ha^{-1} SPAD/SLW values increased for CH80- and CH80+ and decreased for both IR80- and IR80+ between PI and HD stages in HA (**Fig. 9e**). The greatest SPAD/SLW value was observed in CH80+ (0.27) which represented a 9.2% increase between 1 DAF and 5 DAF. The lowest SPAD/SLW value was observed in IR80- (0.17) which increased slightly by 0.8% between 1 DAF and 5 DAF. Pairwise comparison of treatment means for N rate 80 kg ha^{-1} indicated CH80- and CH80+ were significantly greater than IR80- and IR80+ at 5 DAF. For N rate 160 kg ha^{-1} SPAD/SLW values increased slightly for CH160- and IR160- and decreased slightly for CH160+ and IR160+ between PI and HD stages in HA (**Fig. 9f**). The greatest

SPAD/SLW value was observed in CH160- (0.25) which represented an increase of 17.7% between 1 DAF and 5 DAF. The lowest SPAD/SLW value was observed in IR160+ (0.19) which represented an increase of 9.2% between 1 DAF and 5 DAF. Pairwise comparison of treatment means for N rate 160 kg ha^{-1} did not indicate significant differences between all treatments at 5 DAF in HA during HD stage (**Fig. 9f**).

Table 8. Response curves for two varieties at three growth stages 5 DAF in HA.

GS	Treatment	Regression equation	R^2	Treatment	Regression equation	R^2
MT	CH80-	Y = 0.13 + 0.03x	0.78	CH160-	Y = 0.14 + 0.02x	0.66
	CH80+	Y = 0.15 + 0.05x	0.93	CH160+	Y = 0.14 + 1.88e^{-3}x	0.15
	IR80-	Y = 0.08 + 0.01x	0.63	IR160-	Y = 0.14 – 3.68e^{-3}x	0.49
	IR80+	Y = 0.10 + 0.01x	0.93	IR160+	Y = 0.12 + 0.02x	0.91
PI	CH80-	Y = 0.21 – 0.01x	0.41	CH160-	Y = 0.24 – 6.56e^{-3}x	0.10
	CH80+	Y = 0.13 + 0.04x	0.54	CH160+	Y = 0.29 + 4.94e^{-3}x	0.71
	IR80-	Y = 0.30 + 1.16e^{-3}x	0.63	IR160-	Y = 0.28 – 0.04x	0.86
	IR80+	Y = 0.13 + 0.04x	0.54	IR160+	Y = 0.26 + 7.59e^{-3}x	0.59
HD	CH80-	Y = 0.23 + 1.58e^{-3}x	0.91	CH160-	Y = 0.18 + 0.04x	0.97
	CH80+	Y = 0.25 + 3.58^{-3}x	0.87	CH160+	Y = 0.19 + 0.02x	0.90
	IR80-	Y = 0.18 – 0.01x	0.64	IR160-	Y = 0.19 + 0.02x	0.87
	IR80+	Y = 0.18 – 0.01	0.96	IR160+	Y = 0.18 – 9.12e^{-3}x	0.58

Based on the results of (**Table 8**) CH80+ had a greater y-intercept and steeper slope compared to CH80- at MT in HA which could indicate CH80+ had a quicker response to basal N application. However, at 5 DAF no significant differences were found in mean SPAD/SLW values between CH80- and CH80+. IR80- and IR80+ had parallel slopes at MT in HA, though IR80+ had a greater y-intercept (**Table 8**). Mean SPAD/SLW values for IR80- and IR80+ did not differ significantly at 5 DAF in HA. CH160- and CH160+ had the same y-intercept, though CH160- had a steeper slope during MT in HA. IR160+ had a lower y-intercept but a steeper slope than IR160- which had a negative slope at MT in HA.

Trends observed in HA differ from those observed in LA. Based on the results of (**Table 7**) CH80+ had a greater y-intercept and steeper slope compared to CH80-, and subsequently had significantly greater SPAD/SLW values at 4 DAF. IR80- had a greater y-intercept and steeper slope compared to CH80- and had significantly greater mean SPAD/SLW values at 4 DAF during MT. CH160+ had a greater y-intercept compared to CH160-, but the slope was steeper for CH160-.

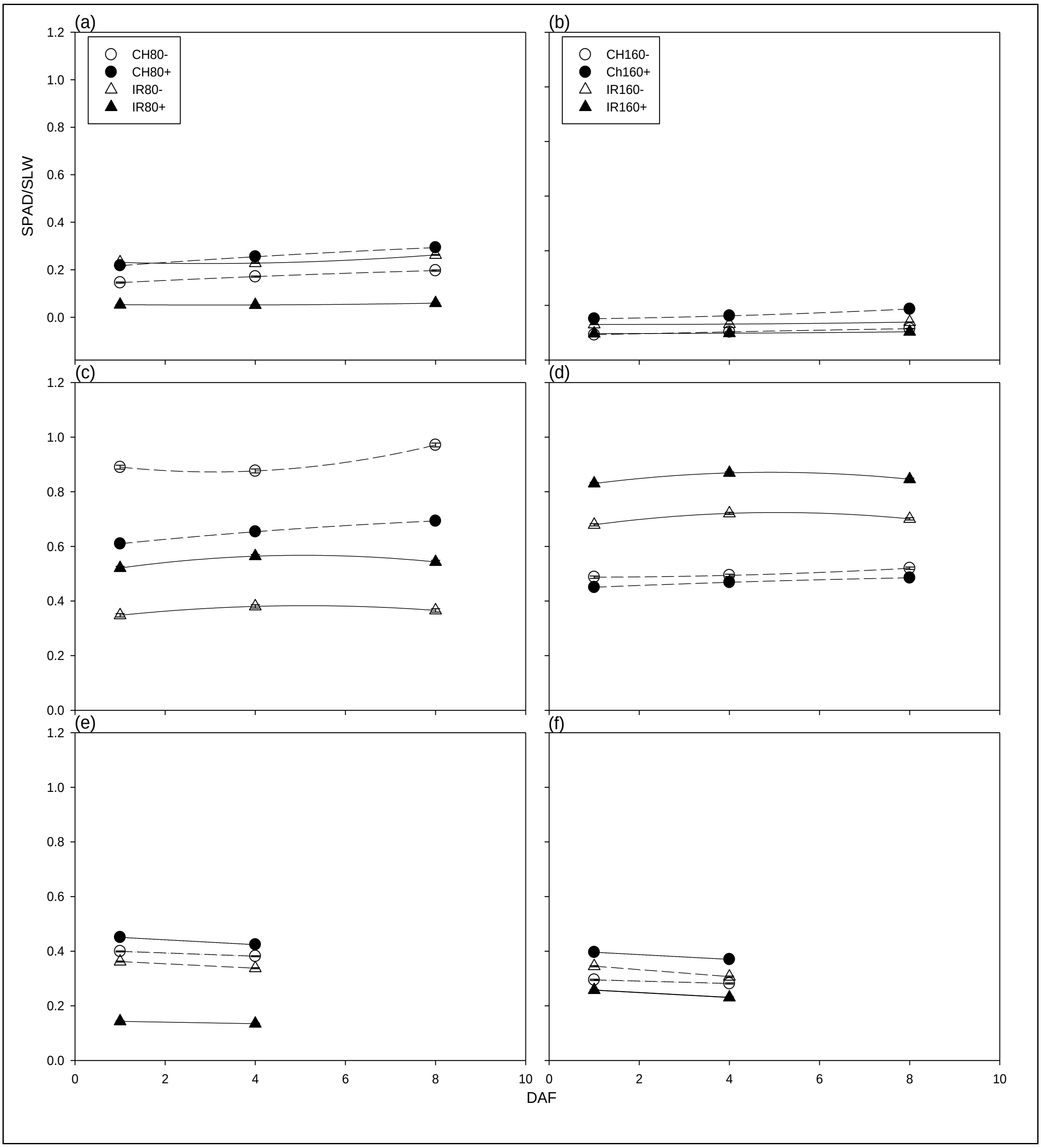

Figure 8. SPAD/SLW values at three GS for variety CH and IR in LA site. (a)-(b) MT, (c)-(d) PI, (e)-(f) HD. Bars represent standard error. N=12.

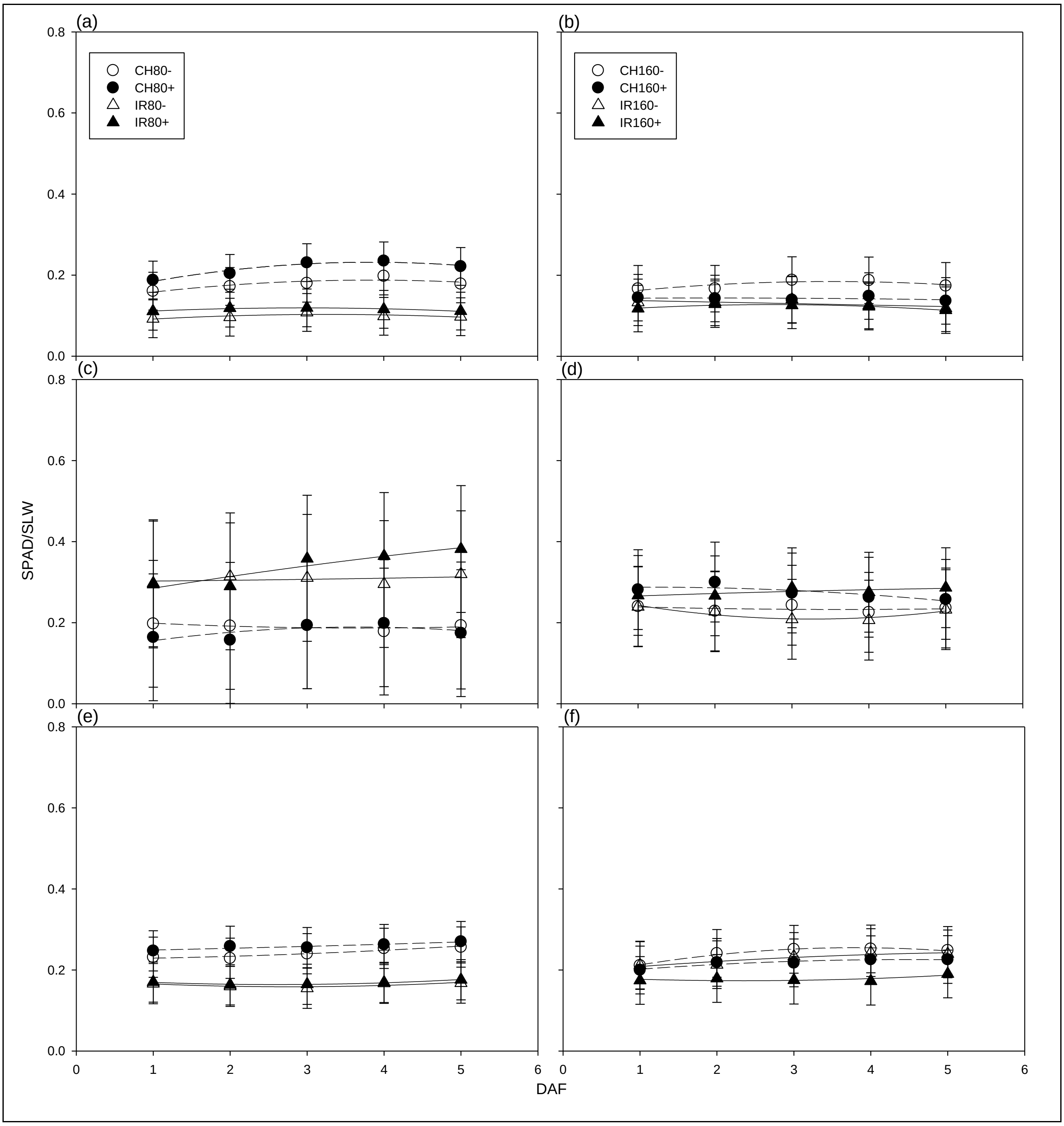

Figure 9. SPAD/SLW values at three GS for variety CH and IR in HA site. (a)-(b) MT, (c)-(d) PI, (e)-(f) HD. Bars represent standard error. N=12.

4.5. SLW, SLA and DW in LA and HA

At MT, variety and the interaction of variety*basal significantly influenced SLW and DW in LA (**Appendix Table B**). SLW Treatment means for variety IR (119.73 mg m^{-2}) were found to be significantly greater than SLW treatment means for variety CH (76.79 mg m^{-2}) in LA. The highest SLW was reported for IR80+ (144.65 mg m^{-2}), while the lowest SLW was reported for CH160+ (48.94 mg m^{-2}) (**Fig. 10c**). DW treatment means for variety IR (0.0449 g) were found to be significantly greater than DW treatment means for variety CH (0.0347 g). The highest DW was reported for IR160+ (0.0571 g). The lowest DW was reported for CH160+ (0.0256 g) (**Fig. 10e**). Rate, variety and the interaction of rate*variety significantly influenced SLA at MT. Treatment means for treatments receiving nitrogen rate 160 kg ha^{-1} (0.0070 m^2 mg^{-1}) were significantly greater than treatment means for those treatments receiving nitrogen rate 80 kg ha^{-1} (0.0040 m^2 mg^{-1}). Treatment means for variety CH (0.0076 m^2 mg^{-1}) were significantly greater than treatment means for variety IR (0.0034 m^2 mg^{-1}). The highest SLA was reported for CH160+ (0.0117 m^2 mg^{-1}). The lowest SLA was reported for IR80+ (0.0025 m^2 mg^{-1}) (**Fig. 10a**). Pairwise comparison of treatment means for SLW, DW and SLA did not indicate significant differences for the interaction of rate*variety*basal at MT.

In HA, variety*basal interaction also affected SLA, SLW, and DW (**Appendix Table C**) at MT. The greatest SLA was reported for CH80+ (0.0102 m^2 mg^{-1}). The lowest SLA was reported for IR80+ (0.0020 m^2 mg^{-1}) (**Fig. 11a**). A similar trend was observed in HA as was observed in LA with the greatest SLW being reported for IR80+ (499.89 mg m^{-2}) at MT. The highest amount of DW at MT was observed in IR160+ (0.1008 g). The lowest amount of DW was observed in IR80- (0.030g). Pairwise comparison of means did not indicate any significant differences in SLW, SLA, DW or the interaction of rate*variety*basal at MT.

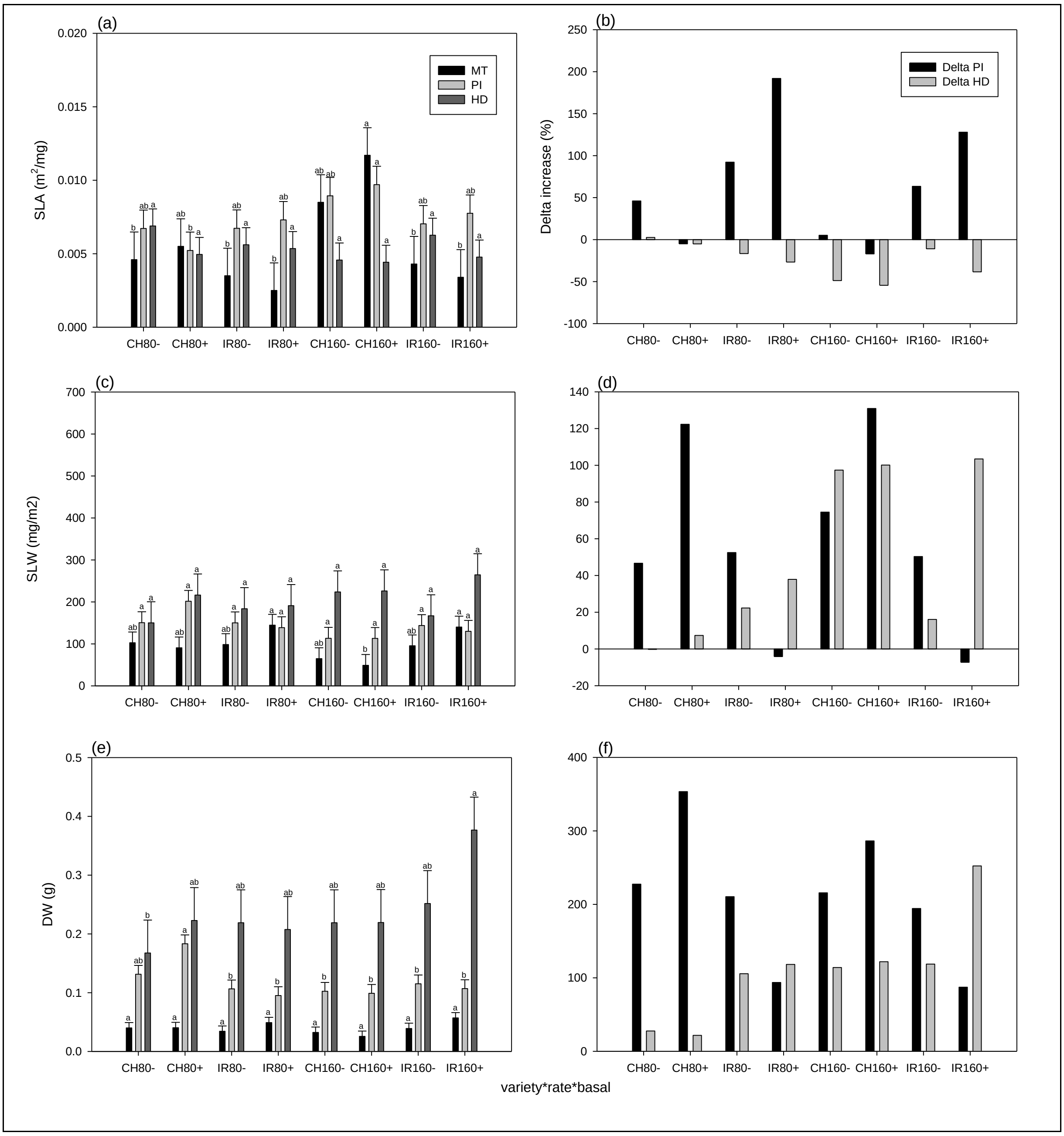

Figure 10. (a) SLA, (b) delta SLA, (c) SLW, (d) delta SLW, (e) leaf canopy DW, (f) delta leaf canopy DW at three GS in LA. Bars represent standard error. N=3.

At PI, rate and the interaction of rate*variety significantly influenced SLA, SLW, and DW in LA (**Appendix Table B**). The greatest SLA was reported for CH160+ (0.0097 m^2 mg^{-1}). The lowest SLA was reported for CH80+ (0.0052 m^2 mg^{-1}) (**Fig. 10a**). The general trend for IR80-, IR80+, IR160- and IR160+ was an increase in delta SLA from MT to PI, with the increase for IR80+ (192%) from MT to PI being greater than the increase for IR80- (92.3%) from MT to PI (**Fig. 10b**).

Similarly, the increase in delta SLA from MT to PI was greater for IR160+ (127.9%) than for IR160- (63.5%) in LA. Pairwise comparison of treatment means for the interaction of rate*variety*basal were not significant. The greatest SLW was reported for CH80+ (201.45 mg

m^{-2}). The lowest SLW was reported for CH160+ (113.01 mg m^{-2}) (**Fig. 10c**). The general trend observed for delta SLW from MT to PI was an increase in CH80- (46.7%), CH80+ (122.3%$^{-}$), CH160- (74.5%), CH160+ (130.9%), with the increase in CH80+ and CH160+ being greater than CH80- and CH160-, respectively (**Fig. 10d**). IR80- (52.5%) and IR160- (50.3%) showed an increase in delta SLW from MT to PI. IR80+ (-4.2%) and IR160+ (-7.3%) showed a decrease in delta SLW from MT to PI. The highest amount of DW at PI was observed in CH80+ (0.1832 g). The least amount of DW was observed in IR80+ (0.0951 g) (**Fig. 10e**). Pairwise comparison test of DW for rate*variety*basal interaction indicated CH80+ (0.1832 g) did not differ significantly from CH80- (0.1313 g) but was significantly greater than CH160- (0.1023 g) and CH160+ (0.0989 g), respectively.

In HA, the interaction of rate*variety had a significant influence on DW (**Appendix Table C**) at PI. DW treatment means for variety Chhomrong receiving nitrogen rate 160 kg ha^{-1} (0.0887 g) were significantly greater than DW treatment means for variety Chhomrong receiving nitrogen rate 80 kg ha^{-1} (0.0436 g). The greatest DW at PI was observed in CH160- (0.0975 g). The least amount of DW at PI was found in CH80- (0.0322 g) (**Fig. 11e**). The greatest SLA in HA was reported for CH80- (0.0273 m^2 mg^{-1}). The lowest SLA was reported for IR80- (0.0093 m^2 mg^{-1}) (**Fig. 11a**). The greatest SLW in HA was reported for IR80- (131.42 mg m^{-2}). The lowest SLW in HA was reported for CH80- (47.22 mg m^{-2}).

At HD, the interaction of rate*variety significantly influenced SLA in LA (**Appendix Table B**). The greatest SLA was reported for CH80- (0.0069 m^2 mg^{-1}) (**Fig. 10a**). The lowest SLA was reported for CH160+ (0.0044 m^2 mg^{-1}). The general trend for IR80- (-16.6%), IR80+ (-26.7%), IR160- (-11%) and IR160+ (-38.5%) was a decrease in SLA from PI to HD, with the decreases in IR80+ being greater than the decrease for IR80- (**Fig. 10b**). Similarly, the decrease in SLA from PI to HD was greater for IR160+ (-38.5%) than for IR160- (-11%). The greatest SLW in LA was reported for IR160+ (264.49 mg m^{-2}). The lowest SLW in LA was reported for CH80- (150.17 mg m^{-2}). The general trend observed for delta SLW from PI to HD in LA was an increase in all treatments except for CH80- which only decreased by -0.2% (**Fig. 10d**).

In HA, the interaction of variety*rate and variety*basal significantly influenced SLA and SLW, respectively at HD. The greatest and lowest SLA was observed in CH80- (0.017 m^2 mg^{-1}) and IR80+ (0.0079 m^2 mg^{-1}) respectively.

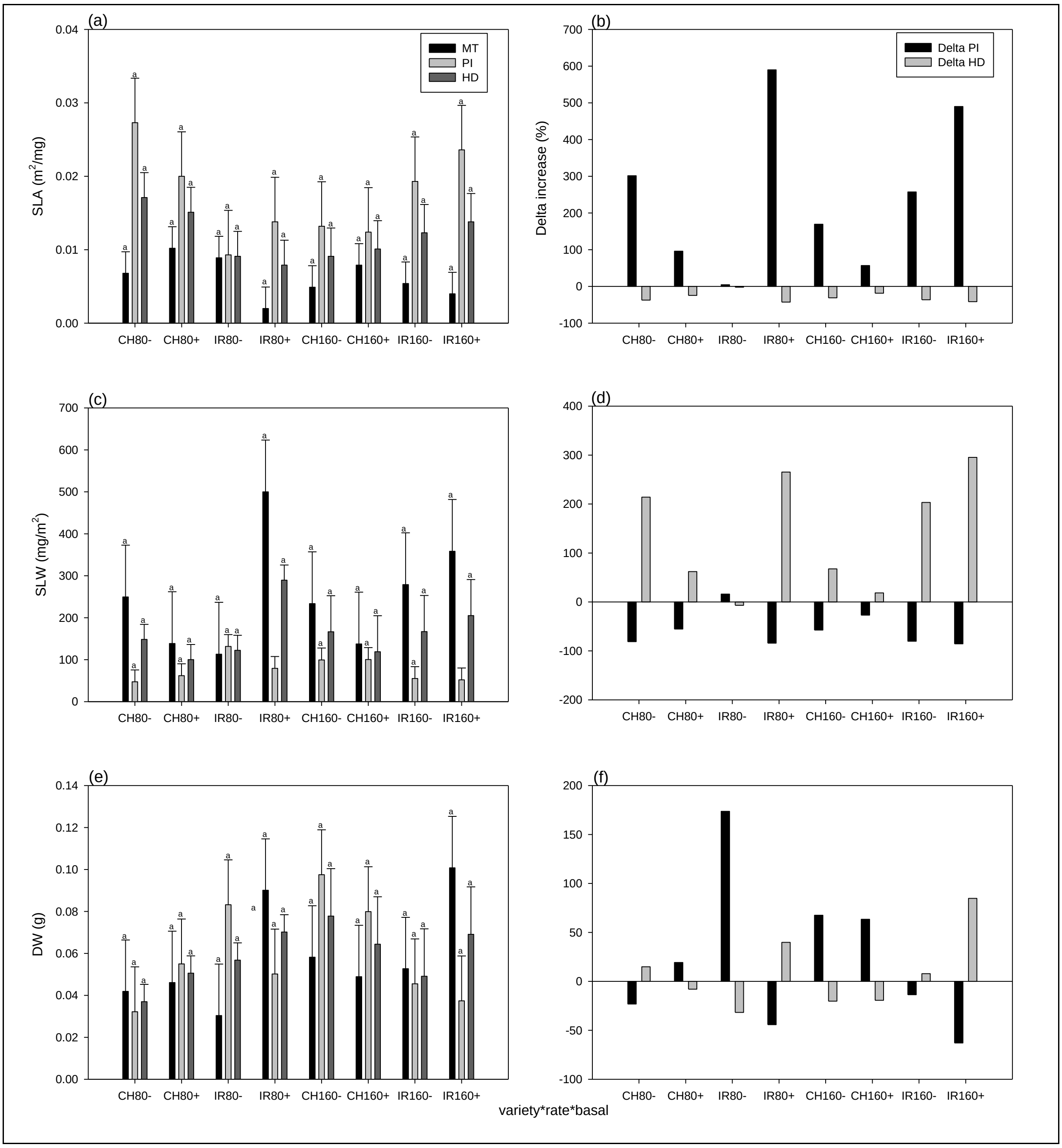

Figure 11. (a) SLA, (b) delta SLA, (c) SLW, (d) delta SLW, (e) leaf canopy DW, (f) delta leaf canopy DW at three GS in HA. Bars represent standard error. N=3.

Pairwise comparison of SLA treatment means did not differ significantly among all treatments at HD. The general trend for delta SLA at HD was a decrease among all treatments (**Fig. 10b**). Pairwise comparison of SLW treatment means did not produce significant differences among all treatments, though IR80+ (289.5 mg m^{-2}) and CH80+ (100.18 mg m^{-2}) had the greatest and least SLW, respectively (**Fig. 10c**). The general trend for delta SLW was an increase among all treatments except for IR80-, which decreased by -6.93% (**Fig. 10d**). The greatest increase in delta SLW at HD was observed in IR160+ which increased by 295.4%.

4.6. Grain yield and yield components in HA and LA

In HA, no significant differences in final grain yield (ton ha^{-1}) were observed at N rate 80 kg ha^{-1} between CH80- and CH80+, or between IR80- and IR80+, respectively (**Table 9**). Similarly, in HA no significant differences were observed at N rate 160 kg ha^{-1} between CH160- and Ch160+, or between IR160- and IR160+, respectively

Table 9. Yield and its components for the interaction of variety*rate*basal in HA and LA. Column means followed by common capitalized letters are not significantly different within a location by the Tukey HSD pairwise comparison test at the 5% level of significance. Column means followed by common lower case letter are not significantly different across locations by the Tukey HSD pairwise comparison test at the 5% level of significance.

Location	Variety	Rate	Basal	Yield (ton ha^{-1})	Panicle Number (m^2)	1000-Grain weight (g)	Filled grains (%)
HA	CH	80	-	3.4 Adefg	319 Abc	32.5 Aabc	77.4 Aab
	CH	80	+	2.7 Afghi	308 Ac	31.2 Abc	71.5 Aab
	IR	80	-	1.5 Bghi	297 Ac	23.3 Bd	33.7 BCcd
	IR	80	+	0.6 Bi	500 Aa	22.7 Bd	13.5 Cd
	CH	160	-	3.3 Adefg	397 Aabc	30.9 Abc	56.4 ABabc
	CH	160	+	3.0 Aefgh	353 Aabc	30.4 Ac	66.1 ABab
	IR	160	-	1.1 Bhi	481 Aab	22.6 Bd	20.6 Cd
	IR	160	+	0.5 Bi	337 Aabc	23.0 Bd	10.2 Cd
LA	CH	80	-	4.0 CDcdef	309 Bc	35.3 ABa	83.6 Aa
	CH	80	+	2.9 Defgh	306 Bc	34.2 ABab	71.9 ABab
	IR	80	-	7.4 ABab	347 Babc	25.0 Cd	66.8 ABab
	IR	80	+	5.8 ABCbc	329 Babc	24.0 CDd	64.4 ABab
	CH	160	-	4.9 BCDcde	339 Babc	35.8 Aa	71.9 ABab
	CH	160	+	5.4 BCDbcd	370 ABabc	34.1 Babc	69.1 ABab
	IR	160	-	8.4 Aa	423 Aabc	24.5 CDd	53.3 Bbc
	IR	160	+	5.8 ABCbc	365 ABabc	23.1 Dd	55.1 Babc

At N rate 80 kg ha^{-1}, grain yield for CH80- and CH80+ were significantly greater than grain yield for IR80- and IR80+, respectively. Similarly, at N rate 160 kg ha^{-1} grain yield for CH160- and CH160+ were significantly greater than IR160- and IR160+.

In all cases, comparisons within N rates revealed that treatments not receiving basal N application obtained greater yield than treatments which did receive basal N application. Grain yield of CH80- was 28% greater than CH80+; grain yield of IR80- was 124% greater than grain yield of IR80+ in HA; grain yield of CH160- had the lowest increase in yield with 8.8%; grain yield of CH160- was 109% greater than grain yield of CH160+. Pairwise comparison of treatments means did not indicated any significant yield increase for treatments receiving N rate 160 kg ha^{-1} compared to treatments receiving N rate 80 kg ha^{-1} in HA at the alpha = 0.05 level (**Table 9**).

In LA, no significant differences in final grain yield were observed at N rate 80 kg ha^{-1} between CH80- and CH80+, or between IR80- and IR80+, respectively (**Table 9**). At N rate 160 kg ha^{-1}, no significant differences were found in grain yield between CH160- and CH160+, or between IR160- and IR160+, respectively. In LA, grain yield for IR80- and IR80+ were significantly greater than grain yield for CH80- and CH80+, respectively. Grain yield for IR160- were significantly greater than grain yield for CH160-. No significant differences were found between grain yield for IR160+ and CH160+.

Figure 12. (a) Sheath rot (Sarocladium oryzae) on developing grains of variety IR-64 in HA. (b) Harvested grains of varieties Chhomrong (left) and IR-64(right).

A similar trend was observed in LA as was observed in HA where in most cases, comparisons within N rates revealed that treatments not receiving basal N application obtained greater yield than treatments which did receive basal N application. Grain yield of CH80- was 35% greater than grain yield of CH80+; grain yield of IR80- was 26% greater than grain yield of IR80+; grain yield of CH160- was 44% greater than grain yield of CH160+. Only in the case of IR160+ did a treatment receiving basal N application obtain a greater yield than a treatment not receiving basal N application. Grain yield for IR160+ was 10% greater than grain yield for IR160-. Pairwise comparison of grain yield across locations indicated no significant differences between CH80-, CH80, CH160- or CH160+, respectively (**Table 9**). However, grain yields were tending be greater in LA for IR80-, IR80+, IR160- and IR160+ than in HA.

Pairwise comparison across locations for percentage of grains filled indicated a higher percentage of filled grains for variety IR-64 in LA than in HA. This trend was observed at both N rate 160 kg ha^{-1} and 80 kg ha^{-1}. In LA, the greatest and least percentage of grains filled was observed in CH80- (83.6%) and IR160- (53.3%), respectively. In HA, the greatest and least percentage of grains filled was observed in CH80- (77.4%) and IR160- (10.2%), respectively.

5. Discussion

5.1. Growing degree-days and temperature differences at three growth stages in HA and LA

In this study, climatic data (**Fig. 2; Table 4**) from two sites differing in altitude along a temperature gradient were recorded to test the hypothesis that differences in the development rate of crop phenological growth stages result from genotype x environment interactions. Results indicate that between MT-PI, and PI-HD a greater number of GDD were accumulated in LA in fewer days (**Fig. 2; Table 4**). These differences can likely be attributed to two reasons: (i) lower mean T_{min} in HA than in LA at MT and PI, respectively; (ii) in HA, mean daily temperature at MT, PI and HD was below the optimum temperature for rice development. These results support the conclusions of Chang and Vergara (1971), Stewart and Langfield (1971) that showed mean daily minimum temperature was a more important factor than maximum temperature for the development from sowing to flowering in field-grown rice.

Based on bilinear models, Summerfield, Collinson, Elllis, Roberts, & De Vries (1992) showed that the optimum temperature for rice development was between 24–26°C in multiple rice cultivars and concluded that within the suboptimal range of temperature, it was the mean daily temperature which largely determined development rate in rice. However, Yin, Kropf and Ellis (1996) reinterpreted the data of Summerfield et al. (1992) and found that the optimum temperature was generally in the range of 27–32°C. These findings were supported by Yin, Kropff and Goudriaan (1996). Further studies proposed an elevated optimum temperature of 30°C–32°C. Based on the findings of these earlier works, it can be concluded that mean daily temperature at MT, PI and HD stages in HA were below the optimum temperature for rice development.

Consideration must also be given to other factors which could contribute to decreased development rate in rice. Attempts to quantitatively assess the effect of temperature on crop development rate assume the effects of day temperature (T_D) and night temperature (T_N) are the same (Yin et al., 1996). However, several studies have indicated a different impact of T_D and T_N on plant growth and development. Roberts (1943) suggested that T_N rather than T_D largely determines the response of plants to temperature. Furthermore, Stuerz et al. (2014) showed that temperature of irrigated floodwater layer affects plant growth via exposure of the rice plant's meristem to temperature extremes.

5.2. Nitrogen content recovered in soil and water fractions in HA

Between 10–75% of the N applied as urea was recovered in the form of NH_4^+ or $N0_3^-$ in soil to a depth of 20 cm at 5 DAF during MT. In the flooded irrigation water, 0.2–3.2% of N applied as urea was recovered as NH_4^+ up to a depth of 5 cm. Results obtained from N recovered in soil are higher than findings by Buresh et al. (1991) who reported values at 10 days after basal fertilizer application between 25.9–43.4% up to a depth of 50 cm in paddy rice with broadcast urea N application. Fillery, Simpson, & De Datta (1986) also reported slightly elevated levels of recovered N of up to 49% of N applied as urea to a depth of 20 cm 21 days after transplanting. Choudhury and Kennedy (2005) also reported levels of apparent N recovered of up to 53%. Buresh et al. (1991) reported mean values of approximately 1.9% of urea applied N recovered in flooded irrigation.

At PI, between 9–39% and 15–25% of N applied as urea was recovered in the form of soil $N0_3^-$ and NH_4^+, respectively at 5 DAF. Between 0.3–1.9% of N applied as N was recovered as water NH_4^+. Similar values were found during HD for soil $N0_3^-$ and NH_4^+. Between 4–40% and 5–20% of N applied as urea was recovered in soil $N0_3^-$ and NH_4^+, respectively. Compared to MT, values for soil $N0_3^-$ and NH_4^+ were considerably lower during early reproductive and heading stages, suggesting that either: (i) a greater amount of N was lost via denitrification, volatilization or leaching during PI and HD, or (ii) the rice plants were better able to utilize the applied N during later GS. In this study, N loss via denitrification, volatilization and leaching were not measured. Therefore, differences in N loss via these mechanisms cannot be determined. In future studies, quantification of such N loss mechanisms could provide a better understanding of N dynamics in cool, HA rice cropping systems. However, based on the increasing values for DW, SLA, SLW and SPAD/SLW between MT to PI, and PI to HD it can be concluded that rice plants utilized applied N to leaf expansion, and increasing thickness.

5.3. Relationship between LCC class values and SPAD/SLW in LA and HA

The LCC method for estimating the N status of rice crop is quick and non-destructive. Numerous studies have been conducted to improve N-use efficiency in farmers' fields by using real-time management methods such as SPAD and LCC (Houshm & Kimaro, 2011). Shukla et al. (2004) determined threshold LCC values for N application in rice-wheat systems in India. Peng et al. (1993) found a greater correlation between N_{DW} when SPAD values were corrected for leaf thickness via SLW. In field trials using three cultivars and three distinct LCC, Yang et al. (2003) concluded that N_{DW} could be better predicted by correcting for LCC/SLW than by LCC alone. However, a limited number of studies have investigated differences in LCC values resulting from leaf thickness due to variety, and N rate.

Results from this study indicated LCC class values between two varieties differed at three growth stages, across two sites when corrected for leaf thickness via SPAD/SLW. These findings contradict results obtained by Islam, Bhuiya, Rahman, and Hussain (2009) who found no significant differences between LCC values in two varieties. In LA, mean SPAD/SLW values for CH80 were higher than mean SPAD/SLW values for IR80 at MT, PI, and HD growth stages but were tending to have lower LCC class values. Similarly, mean SPAD/SLW values for CH160 were higher than mean SPAD/SLW values for IR160 at MT and HD growth stages but were tending to have lower LCC class values in LA (**Fig. 7b**). In HA, mean SPAD/SLW values for CH80 were tending to be higher than mean SPAD/SLW values for IR80 at HD but had similar LCC class values. At PI, all treatments had LCC values of 6, though SPAD/SLW values differed among all treatments. Compared across sites, LCC class values were tending to be 2 class values higher in LA than in HA for CH80 and CH160 at MT, though SPAD/SLW values were not significantly different. Similarly, LCC class values were tending to be 2–3 class values higher in LA than in HA for IR80 and IR160 at MT, though SPAD/SLW values were not significantly different. Results from the present study indicate that differences in LCC class values differ among rice varieties as a function of genotype and leaf thickness. To validate these findings, further research should consider increasing the number of contrasting varieties across multiple growth environments.

5.4. Treatment responses to fertilizer application at three growth stages in LA and HA

In the present study, it was hypothesized that nutrient uptake dynamics are driven by temperature and genotype across altitudinal gradients. Fertilizer response data indicated significant differences in the mean SPAD/SLW values between the two varieties at two N application rates, with or without basal N application, and at different phenological GS at two separate locations differing in altitude (**Fig. 8; Fig. 9**). In HA, variety Chhomrong had higher SPAD/SLW values at N rate 80 kg ha^{-1} than variety IR-64 up to 5 DAF during MT and HD. In LA, variety Chhomrong had higher mean SPAD/SLW values at N rate 80 kg ha^{-1} than variety IR-64 up to 4 DAF during MT, PI and HD. Significant differences were also found within varieties at both locations. In LA, CH80+ had higher mean SPAD/SLW values than CH80- at MT and HD. IR80- had higher mean SPAD/SLW values than IR80+ at MT and HD. CH160+ had higher SPAD/SLW values than CH160- up to 4 DAF during MT and HD. The present findings also supported the results obtained by Islam et al. (2009) who reported an increase in SPAD values from MT to PI, and a transient decrease from PI to flowering.

5.5. SLW, SLA and DW in LA and HA

SLW is the product of leaf density and thickness (Witkowski & Lamont, 1991). Peng et al. (1993) demonstrated that differences in SLW are responsible for the variations in the relationship between rice leaf N_{DW} and SPAD values. Therefore, differences in leaf thickness formed in different genotypes, physiological ages or growth environments will have a direct influence on the estimation of chlorophyll readings provided by SPAD (Jinwen et al., 2011)

Results from the present study support the findings of Jinwen et al. (2011) who also reported an increase in SLW from MT to ripening period. In LA, SLW increased in all treatments from MT to PI except for IR80+ and IR160+, which decreased by 4.3% and 7.3%, respectively. Mean SLA also increased for all treatments between MT and PI in LA except for CH80+ and CH160+. For treatments CH80-, CH80+, IR80-, CH160-, CH160+ and IR160- the partitioning of DW can be explained by the increasing SLW rather than SLA. This would indicate these treatments allocate more photosynthates into leaf thickness, rather than leaf area. Conversely, the decrease in SLW in IR80+ and IR160+ between MT and PI can likely be explained by the increase in SLA and DW during the respective GS. Since SLA is defined as the projected leaf area per unit leaf dry mass (Evans & Poorter, 2001) an increase in either DW or a decrease in leaf area would result in an increased SLA. These results would indicate that IR80+ and IR160+ allocated more photosynthates into expanding SLA, rather than increasing SLW. Between MT and PI, SLA for IR80+ and IR160+ increased by 192% and 127%, respectively. In LA, mean DW increased for all treatments between PI and HD. SLW also increased for all treatments between PI and HD except for CH80-, which decreased slightly by 0.2%. SLA decreased in all treatments between PI and HD except for CH80, which increased by 2.5%. These results indicate that between PI and HD, all treatments except CH80- allocated more photosynthates into increasing leaf thickness rather than expanding SLA. Shimono et al. (2012) also reported a decrease in SLA between vegetative and reproductive phase when rice plants were subject to lower root zone temperatures.

In HA, DW increased for CH80+, IR80-, CH160- and CH160+ and decreased for CH80, IR80+, IR160- and IR160+ between MT and PI. Unlike in LA where SLW increased in all treatments except IR80+ and IR160+, SLW decreased in all treatments between MT to PI except for IR80- in HA, which increased by 16%. SLA increased for all treatments in HA between MT and PI. These results indicate that treatments CH80+, IR80-, CH160- and CH160+ utilized the increased DW accumulation to expand SLA, rather than increase leaf thickness. Of the treatments which increased DW, only IR80- simultaneously increased SLW indicating DW was allocated to increase leaf thickness rather than to expand SLA.

Between PI and HD, mean DW increased for CH80-, IR80+, IR160-, and IR160+ and decreased for CH80+, IR80-, CH160-, and CH160+ in HA. SLA decreased in all treatments between PI and HD. SLW increased in all treatments except for IR80-. These results show the opposite trend as was observed between MT and PI in HA, where the increase in DW was utilized to expand SLA. In this case, the increased DW was utilized to increase leaf thickness rather than expanding SLA.

5.6. Grain yield and yield components in HA and LA

An underlying objective of the present study was to identify the optimum N application rate and split for different rice cultivars in two different agro-ecological zones. To achieve this objective, two fertilizer N rates were applied in splits at basal, MT, PI and HD growth stages. Based on data obtained in **Table 9**, results indicate that final grain yield of two contrasting varieties were not significantly reduced at N rate 80 kg ha^{-1} compared to N rate 160 kg ha^{-1}. These results support the conclusion of Zhang et al. (2009) who also found no significant differences in grain yield between N rate 70 kg ha^{-1} and N rate 150 kg ha^{-1} when fertilizer was applied in three splits of 30%, 40% and 30% of total N at basal, MT and PI, respectively. Further, at both HA and LA grain yield of contrasting varieties were not significantly reduced when the application of basal N was shifted to later phenological growth stages, compared to grain yield obtained when N was applied at basal. In most cases treatments not receiving basal N application obtained slightly greater yield than treatments which did receive basal N application, though the differences were not significantly different. In field trials using three N rates and four splits, Mahajan, Chauhan, and Gill (2011) also reported no significant differences in final grain yield between treatments not receiving basal N and those which did receive basal N at rates of 120, 150, 180 kg ha^{-1}, respectively.

In LA, final grain yield and filled grain percentage were significantly greater than in HA for variety IR-64 at both N rate 160 kg ha^{-1} and N rate 80 kg ha^{-1} due to sheath rot in HA caused by *Saracladium oryzae* (**Fig. 12a**). Sheath rot reduces grain yield by retarding or aborting panicle emergence and producing unfilled seeds and sterile panicles. Sheath rot also reduces grain quality by causing panicles to rot and grains to become discolored. Various studies have reported yield losses between 20−85% in Taiwan, and 30−80% in Vietnam, the Philippines, and India (International Rice Research Institute, 2018).

6. Conclusion and Outlook

This study was conducted to evaluate soil temperature related N dynamics in HA rice cropping systems. The objectives were to (i) identify the optimum rate and split for two different cultivars in two different agro-ecological zones; (ii) determine the evolution of soil nitrate-N and ammonium-N; (iii) determine N uptake within the plant. Soil and water samples, along with SPAD and LCC were assessed 1 day before and up to 5 DAF in HA and 8 DAF in LA for two contrasting varieties at MT, PI and HD.

The omission of basal N did not significantly reduce final yield for two contrasting rice varieties compared to the application of basal N at both locations, respectively. These results provide justification that during early growth stages in cool, HA rice cropping systems the application of basal N could be shifted to later phenological growth stages to more appropriately match crop N demand without reducing final grain yield or specific yield parameters.

In the present study, NH_4^+ and $N0_3^-$ in the soil and water fractions were analyzed using Reflectoquant® reflectometric methods using quick-test strips. This method is suitable for obtaining range values in field settings. However, accuracy and precision could be improved by carrying out lab analysis of NH_4^+ and $N0_3^-$ for soil and water. In HA, the percent of applied urea recovered as NO_3^- and NH_4^+ in the soil and water fractions were found to be greatest in the soil NO_3^- fraction at MT, and lowest in the water NH_4^+ fraction at HD. The change in percentage values of applied urea recovered as NO_3^- and NH_4^+ at 5 DAF varied between variety, rate and basal N application at all three growth stages in HA. To gain a better understanding of the N use efficiency during early vegetative growth stages in cool environments, further studies should consider investigating longer time periods after each fertilizer application. For example, soil and water samples could be taken every second day for up to 15 DAF to capture N dynamics in soil and water.

To evaluate C_{leaf}, SPAD and LCC measurements of the top three uppermost fully expanded leaves were recorded 1 day before each fertilizer application and up to 5 DAF in HA and up to 8 DAF in LA. Results from this study indicated significant differences in the mean SPAD/SLW values between two contrasting rice varieties at two N application rates, with or without basal N application, at different GS and two locations differing in altitude. Additionally, differences in LCC class values were found to differ among rice varieties as a function of genotype and leaf thickness at both locations. To validate these findings, further research should investigate a larger number of contrasting varieties across multiple growth environments.

7. References

Africa Rice Center (AfricaRice) (2018). http://www.africarice.org/. Accessed 6/20/18.

Alam, M. M., Ladha, J. K., Khan, S. R., Khan, A. H., & Buresh, R. J. (2005). Leaf color chart for managing nitrogen fertilizer in lowland rice in Bangladesh. *Agronomy Journal*, *97*(3), 949–959. https://doi.org/10.2134/agronj2004.0206

Ali, A.M., Thind, H.S., Sharma, S., & Singh, Y. (2015). Site-specific nitrogen management in dry direct-seeded rice using chlorophyll meter and leaf colour chart. *Pedosphere*, *25*(1), 72–81. https://doi.org/10.1016/S1002-0160(14)60077-1

Basnayake, J., Sihathep, V., Sipaseuth, Sonekham, P., & Senthonghae, M. (Ed.) (2003). Effects of time of planting on agronomic and yield performance of several rice cultivars under various temperature conditions in Lao PDR. Retrieved from www. regional. org. au/au/asa/2003/

Bobade, P., Chandrawans, S.K., Patel, S.R., & Kausik, D. (2018). Thermal responses on different wheat cultivars under different thermal environments for Chhattisgarh plain. *MAUSAM*, *69*(1), 81–96.

Buresh, R. J., De Datta, S.K., Samson, M.I., Phongpan, S., Snitwongse, P., Fagi, A.M., & Tejasarwana, R. (1991). Dinitrogen and nitrous oxide flux from urea basally applied to puddled rice soils. *Soil Science Society of America Journal*, *55*(1), 268–273. https://doi.org/10.2136/sssaj1991.03615995005500010046x

Chang, T. T., & Vergara, B. (1971). Ecological and genetic aspects of photoperiod-sensitivity and thermo-sensitivity in relation to the regional adaptability of rice varieties. *International Rice Community News Letter*. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302258017

Choudhury, A. T. M. A., & Kennedy, I. R. (2005). Nitrogen fertilizer losses from rice soils and control of environmental pollution problems. *Communications in Soil Science & Plant Analysis*, *36*(11-12), 1625–1639. https://doi.org/10.1081/CSS-200059104

Counce, P. A., Keisling, T. C., & Mitchell, A. J. (2000). A uniform, objective, and adaptive system for expressing rice development. *Crop Science*, *40*(2), 436. https://doi.org/10.2135/cropsci2000.402436x

Debaeke, P., Rouet, P., & Justes, E. (2006). Relationship between the normalized SPAD index and the nitrogen nutrition index: Application to durum wheat. *Journal of Plant Nutrition*, *29*(1), 75–92. https://doi.org/10.1080/01904160500416471

Dingkuhn, M., Luquet, D., Kim, H., Tambour, L., & Clement-Vidal, A. (2006). EcoMeristem, a model of morphogenesis and competition among sinks in rice. 2: Simulating genotype responses to phosphorus deficiency. *Plant Biology*, *33*, 325–337. https://doi.org/10.1071/FP05266

Dingkuhn, M., Sow, A., Sambs, A., Diack, S., & Asch, F. (1995). Climatic determinants of irrigated rice performance in the Sahel-I: Photothermal and microclimatic responses of flowering. *Agricultural Systems*, *48*(4), 411–433. https://doi.org/10.1016/0308-521X(94)00027-I

Dobermann, A., & Fairhurst, T. (2000). *Rice: Nutrient disorders & nutrient management*. Singapore: Potash & Phosphate Institute, Potash & Phosphate Institute Canada, International Rice Research Institute.

Engels, C. (1994). Effect of root and shoot meristem temperature on shoot to root dry matter partitioning and the interanal concentrations of nitrogen and carbohydrates in maize and wheat. *Annals of botany*, *73*(2), 211–219. Retrieved from https://watermark.silverchair.com/730211

Evans, J., & Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, cell & environment*, *24*(8), 755–767. https://doi.org/10.1046/j.1365-3040.2001.00724.x

Fageria, N. (2007). Yield physiology of rice. *Journal of Plant Nutrition*, *30*(6), 843–879. https://doi.org/10.1080/15226510701374831

Fageria, N., dos Santos, A. B., & Cutrim, V. d. A. (2009). Nitrogen uptake and its association with grain yield in lowland rice genotypes. *Journal of Plant Nutrition*, *32*(11), 1965–1974. https://doi.org/10.1080/01904160903245121

Fageria, N., & Knupp, A. (2013). Upland rice phenology and nutrient uptake in tropical climate. *Journal of Plant Nutrition*, *36*(1), 1–14. https://doi.org/10.1080/01904167.2012.724136

Fageria, N., Moreira, A., & Coelho, A. M. (2011). Yield and yield components of upland rice. *Journal of Plant Nutrition*, *34*(3), 361–370. https://doi.org/10.1080/01904167.2011.536878

Fageria, N. K., Baligar, V. C., & Jones, C. A. (1997). *Growth and mineral nutrition of field crops* (2nd ed.). New York City, New York: Marcel Dekker Inc.

Fageria, N.K., & Baligar, V.C. (2005). Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy*, *88*, 97–185. https://doi.org/10.1016/S0065-2113(05)88004-6

Farrell, T., Fox, K. M., Williams, R. L., Fukai, S., Reinke, R. F., & Lewin, L. G. (Ed.) (2001). Temperature constraints to rice production in Australia and Laos: A shared problem. Canberra, ACT: Australian Centre for International Agricultural Research. Retrieved from https://espace.library.uq.edu.au/view/UQ:95820

Fillery, I.R.P., Simpson, J.R., & De Datta, S.K. (1986). Contribution of ammonia volatilization to total nitrogen loss after applications of urea to wetland rice fields. *Fertilizer Research*, *8*(3), 193–202. https://doi.org/10.1007/BF01048620

Fukai, S. (1999). Phenology in rainfed lowland rice. *Field Crops Research*, *64*(1-2), 51–60. https://doi.org/10.1016/S0378-4290(99)00050-7

Garg, A., Sangwan, V., He, C., Dey, M., Duan, F., Li, F., & Wu, R. (Ed.) (2002). *Rice Science: Innovations and Impact for Livelihood: Applying agricultural biotechnology produce salt- and drought-tolerant rice plants*. Bejing, China: Science Publishers Inc.

Gholizadeh, A., Saberioon, M., Borůvka, L., Wayayok, A., & Mohd Soom, M. (2017). Leaf chlorophyll and nitrogen dynamics and their relationship to lowland rice yield for site-specific paddy management. *Information Processing in Agriculture*. (4), 259–268. https://doi.org/10.1016/j.inpa.2017.08.002

Guindo, D., Wells, B. R., & Norman, R. J. (1994). Cultivar and nitrogen rate influence on nitrogen uptake and partitioning in rice. *Soil Science Society of America Journal*, *58*(3), 840–845. https://doi.org/10.2136/sssaj1994.03615995005800030030x

Gunawardena, T. A., Fukai, S., & Blamey, F. P. C. (2003). Low temperature induced spikelet sterility in rice. I: Nitrogen fertilisation and sensitive reproductive period. *Australian Journal of Agricultural Research*, *54*(10), 937–946. https://doi.org/10.1071/AR03075

Ha, L. V., Mitchell, J. H., & Fukai, S. (2017). Genotypic consistency for low temperature tolerance at the booting stage in rice grown under flooded and non-flooded conditions. *Field Crops Research*, *213*, (in progress). https://doi.org/10.1016/j.fcr.2017.06.027

Houshm, A., & Kimaro, A. (2011). Calibrating the leaf color chart for rice nitrogen management in Northern Iran. *African Journal of Agricultural Research*, *6*(11), 2627–2633. https://doi.org/10.5897/AJAR10.980

Huang, J., He, F., Cui, K., Buresh, R. J., Xu, B., Gong, W., & Peng, S. (2008). Determination of optimal nitrogen rate for rice varieties using a chlorophyll meter. *Field Crops Research*, *105*(1-2), 70–80. https://doi.org/10.1016/j.fcr.2007.07.006

International Rice Research Institute (IRRI) (2018). www.knowledgebank.irri.org/training/fact-sheets/pest.../sheathrot. Accessed 7/12/18.

Islam, M. S., Bhuiya, M. S. U., Rahman, S., & Hussain, M. M. (2009). Evaluation of SPAD and LCC based nitrogen management in rice (Oryza sativa L.). *Bangadesh Journal of Agricultural Research*, *34*(4), 661–672. https://doi.org/10.3329/bjar.v34i4.5841

Jinwen, L., Jingping, Y., Dongsheng, L., Pinpin, F., Tiantai, G., Changshui, G., & Wenyue, C. (2011). Chlorophyll meter's estimate of weight-based nitrogen concentration in rice leaf is influenced by leaf thickness. *Plant Production Science*, *14*(2), 177–183. https://doi.org/10.1626/pps.14.177

Julia, C., Wissuwa, M., Kretzschmar, T., Jeong, K., & Rose, T. (2016). Phosphorus uptake, partitioning and redistribution during grain filling in rice. *Annals of botany*, *118*(6), 1151–1162. https://doi.org/10.1093/aob/mcw164

Juskiw, P. E., Jame, Y. W., & Kryzanowski, L. (2001). Phenological development of spring barley in a short-season growing area. *Agronomy Journal*, *93*(2), 370–379. https://doi.org/10.2134/agronj2001.932370x

Khush, G.S., & Virk, P.S. (2005). *IR varieties and their impact*. Los Baños, Philippines: International Rice Research Institution.

King, D. C. (2007). *Rwanda (Cultures of the World)*. New York City, New York: Benchmark Books.

Krishnan, P., Ramakrishnan, B., Raja Reddy, K., & Reddy, V. R. (2011). High-temperature effects on rice growth, yield, and grain quality. *Advances in Agronomy*, *111*, 87–206. https://doi.org/10.1016/B978-0-12-387689-8.00004-7

Kumar, A., Vashistha, M., Thind, H. S., & Gupta, R. K. (2016). Fertilizer nitrogen management in irrigated transplanted rice using dynamic threshold greenness of leaves. *Agricultural Research*, *5*(2), 174–181. https://doi.org/10.1007/s40003-016-0213-y

Lu, J. J., & Chang, T. T. (Ed.). (1980). *Rice in its temporal and spatial perspectives*. Westport, CT: AVI Publishing CO., Inc.

Mae, T. (1997). Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant Soil*, *196*, 201–210.

Mahajan, G., Chauhan, B. S., & Gill, M. S. (2011). Optimal nitrogen fertilization timing and rate in dry-seeded rice in northwest India. *Agronomy Journal*, *103*(6), 1676–1682. Retrieved from https://dl.sciencesocieties.org/publications/aj/abstracts/103/6/1676

Matsushima, S., Tanaka, T., & Hoshino, T. (1964). Analysis of yield-determining process and its application to yield-prediction and culture improvement of lowland rice: LXX. Combined effects of air-temperatures and water-temperatures at different stages of growth on the grain yield and its components of lowland rice. *Japanese Journal of Crop Science*, *33*(1), 53–58. Retrieved from https://www.jstage.jst.go.jp/article/jcs1927/33/1/33_1_53

Mikkelsen, D. S. (1970). Recent advances in rice plant tissue analysis. *Rice Journal*, *73*(6), 2–5.

Nagasuga, K., Murai-Hatano, M., & Kuwagata, T. (2011). Effects of low root temperature on dry matter production and root water uptake in rice plants. *Plant Production Science*, *14*(1), 22–29. https://doi.org/10.1626/pps.14.22

Peng., S, Garcìa, F.V., Laza, R.C., & Cassman, K.G. (1993). Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. *Agronomy Journal*, *85*, 987–990.

Raun, W. R., & Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, *91*(3), 357–363. https://doi.org/10.2134/agronj1999.00021962009100030001x

Richards, R. (1991). Crop improvement for temperate Australia: Future opportunities. *Field Crops Research*, *26*(2), 141–169. https://doi.org/10.1016/0378-4290(91)90033-R

Roberts, R. H. (1943). The role of night temperatures in plant performance. *Science*, *98*(2542), 265. https://doi.org/10.1126/science.98.2542.265

Sarwar, N., Ali, H., Maqsood, M., Ullah, E., Shahzad, M., Mubeen, K., & Ahmad, S. (2013). Phenological response of rice plants to different micronutrients application under water saving paddy fields on calcareous soil. *Turkish Journal of Field Crops*, *18*(1), 52–57. Retrieved from http://dergipark.gov.tr/tjfc/issue/17122/179042

Sasaki, T. (1979). The Relationship between Germination Rate of rice seeds at low temperature and the subsequent early growth of seedlings: V: Germination and emergence of radicle at low temperature. *Japanese Journal of Crop Science*, *48*(1), 39–45. https://doi.org/10.1626/jcs.48.39

Shimazaki, Y., Satake, T., & Watanabe, K. (1963). Studies on the growth and nutrient absorption of the rice plant reared with cool water irrigation during its early growth stage. *Res. Bull. Hokkaido Natl. Agric. Exp. Stn.*, *80*, 1–12.

Shimono, H., Okada, M., Kanda, E., & Arakawa, I. (2007). Low temperature-induced sterility in rice: Evidence for the effects of temperature before panicle initiation. *Field Crops Research*, *101*(2), 221–231. https://doi.org/10.1016/j.fcr.2006.11.010

Shimono, H., Fujimura, S., Nishimura, T., & Hasegawa, T. (2012). Nitrogen uptake by rice (Oryza sativa L.) exposed to low water temperatures at different growth stages. *Journal of Agronomy and Crop Science*, *198*(2), 145–151. https://doi.org/10.1111/j.1439-037X.2011.00503.x

Shimono, H., Hasegawa, T., Moriyama, M., Fujimura, S., & Nagata, T. (2005). Modeling spikelet sterility induced by low temperature in rice. *Agronomy Journal*, *97*(6), 1524. https://doi.org/10.2134/agronj2005.0043

Shrestha, S., Asch, F., Dusserre, J., Ramanantsoanirina, A., & Brueck, H. (2012). Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes. *Field Crops Research*, *134*, 216–228. https://doi.org/10.1016/j.fcr.2012.06.011

Shukla, A. K., Ladha, J. K., Singh, V. K., Dwivedi, B. S., Balasubramanian, V., Gupta, R. K., & Padre, A. T. (2004). Calibrating the leaf color chart for nitrogen management in different genotypes of rice and wheat in a systems perspective. *Agronomy Journal*, *96*(6), 1606–1621. Retrieved from https://www.researchgate.net/profile/Arvind_Shukla11/publication/242244253_Calibrati ng_the_Leaf_Color_Chart_for_Nitrogen_Management_in_Different_Genotypes_of_Rice_and_Wheat_in_a_Systems_Perspective/links/551a30b30cf244e9a4585624.pdf

Soil Science Society of America. (1997). *Glossary of soil science terms*. Madison, Wisconsin: SSSA.

Stewart, G.A., & Langfeld, E.C.B. (1971). Temperature responses of IR8 rice. *Australian Institute Agricultural Science Journal*.

Stuerz, S., Sow, A., Manneh, B., & Asch, F. (2014). Leaf area develoment in response to meristem temperature and irrigation system in lowland rice. *Field Crops Research*, *163*, 74–80. https://doi.org/10.1016/j.fcr.2014.04.001

Summerfield, R.J., Collinson, S.T., Elllis, R.H., Roberts, E.H., & De Vries, F.P. (1992). Photothermal responses of flowering in rice (Oryza sativa). *Annals of botany*, *69*(2), 101–112. Retrieved from https://academic.oup.com/aob/article-abstract/69/2/101/161675

Swan, J.B., Schneider, E.C., Moncrief, J.E., Paulson, W.H., & Peterson, A.E. (1987). Estimating crop growth yields and grain moisture from air growing degree-days and residue cover. *Journal of Agronomy*, *79*, 53–60.

Thind, H. S., Kumar, A., Gupta, R. K., Kaul, A., & Vashistha, M. (2012). Fixed-time adjustable dose site-specific fertilizer nitrogen management in transplanted irrigated rice (Oryza sativa L.) in South Asia. *Field Crops Research*, *126*, 63–69. https://doi.org/10.1016/j.fcr.2011.09.007

Toriyama, T. (1994). Studies on estimation of nitrogen mineralization pattern of lowland rice field and nitrogen fertilizing model for rice plant. *Res. Bull. Hokuriku Natl. Agric. Exp.*, *36*, 147–198. Retrieved from https://ci.nii.ac.jp/naid/10012145261/

Traore, A., & Maranville, J.W. (1999). Nitrate reductase activity and diverse grain sorghum genotypes and its relationship to nitrogen use efficiency. *Agronomy Journal*, *91*, 863–869. https://doi.org/10.2134/agronj1999.915863x

Van Wambeke, A. (1974). *Management Properties of Ferralsols*. Rome, Italy: Food and Agriculture Organization of the United Nations. Retrieved from www.fao.org/docrep/x5867e/x5867e00.htm#contents

Witkowski, E. T. F., & Lamont, B. B. (1991). Leaf specific mass confounds leaf density and thickness. *Oecologia*, *88*(4), 486–493. https://doi.org/10.1007/BF00317710

Yang, W. H., Peng, S., Huang, J., Sanico, A. L., Buresh, R. J., & Witt, C. (2003). Using leaf color charts to estimate leaf nitrogen status of rice. *Agronomy Journal*, *95*(1), 212–217. https://doi.org/10.2134/agronj2003.2120

Yin, X., Kropf, M.J., & Ellis, R.H. (1996). Rice flowering in response to diurnal temperature amplitude. *Field Crops Research*, *48*(1), 1–9. https://doi.org/10.1016/0378-4290(96)00033-0

Yin, X., Kropf, M.J., & Goudriaan, J.A.N. (1996). Differential effects of day and night temperature on development to flowering in rice. *Annals of botany*, *77*(3), 203–213.

Yoneyama, T., Fukuda, M., & Kouchi, H. (1989). Partitioning of carbon, nitrogen, phosphorus, potassium, calcium, and magnesium in a semidwarf high-yielding rice variety: Comparison with a conventional japonica variety. *Soil Science and Plant Nutrition*, *35*(1), 43–54. https://doi.org/10.1080/00380768.1989.10434735

Yoshida, S. (1981). *Fundamentals of rice crop science*: International Rice Research Institution.

Zamski, E., & Schaffer, A. A. (1996). *Photoassimilate distribution in plants and crops: source-sink relationships.*: Books in soils.

Zhang, L., Lin, S., Bouman, B.A.M., Xue, C., Wei, F., Tao, H., Yang, X., Wang, H., Zhao, D., & Dittert, K. (2009). Response of aerobic rice growth and grain yield to N fertilizer at two contrasting sites near Beijing, China. *Field Crops Research*, *114*(1), 45–53. https://doi.org/10.1016/j.fcr.2009.07.001

Zhang, Q. C., & Wang, G. H. (2005). Studies on nutrient uptake of rice and characteristics of soil microorganisms in a long-term fertilization experiments for irrigated rice. *Journal of Zhejiang University. Science. B*, *6*(2), 147–154. https://doi.org/10.1631/jzus.2005.B0147