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Disaggregating the Effect of Drought and Heat Stress During Flowering on Spikelet Fertility in Rice

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Disaggregating the Effect of Drought and Heat Stress During Flowering on Spikelet Fertility in
Rice

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by

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Abstract

Due to rice's wide geographic distribution, extending from 50°N to 35°S, rice is forecasted to be the most vulnerable crop to warming global climates. Previous studies have predicted lower rice yields and increasing rice yield variability due to higher frequencies of heat stress events, and a higher variability in precipitation patterns due to global warming. As such, understanding the effects of drought and heat stress intensity and frequency on rice yields is of upmost importance to feeding the growing global population.

Given that drought and high-temperature stress often occur together, it is essential to disaggregate the two individual stressors and examine possible interactions by modeling them simultaneously. A reliable and robust temperature and drought threshold inducing rice spikelet sterility under field conditions involving cultivars with highly varying phenology has been a major limitation for devising adaptation strategies for rice breeders and to estimate heat stress and drought impacts by the climate and crop modeling communities. It is in this spirit that this study was designed. This study examines and quantifies the individual marginal effects of drought and heat stress occurring simultaneously under field conditions by means of a regression analysis. Moreover, a cardinal threshold is found for drought as well as for heat in relation to spikelet fertility. This study utilizes canopy temperature threshold instead of the more commonly found ambient temperature thresholds in the literature. The canopy temperature threshold is more precise since canopy temperature has a more direct connection to spikelet temperature and therefore spikelet fertility than ambient temperature. Another contribution of this thesis is from modeling the relationship between ambient temperature, drought and canopy temperature as a recursive system which will allow future research to estimate the effects of changes in global ambient temperature to spikelet fertility. The results of this study found that exposure to a

canopy temperature over a threshold of 33°C causes a severe increase in rice spikelet sterility. An estimated drought threshold of 12 kPa (kilopascal) was found to be the most detrimental to spikelet fertility. This is important given two of the largest pressures facing future rice production are heat and drought stress. Another related finding is that in the presence of heat stress the availability of adequate water during flowering can decrease sterility by 14.16 %. The results of this study, which are variety specific, can allow for understanding the properties of combined heat and drought stress, which can provide information to rice breeders on how to promote reproductive-stage drought tolerance through improved germplasm and attempt to help mitigate the effects of a global climate change.

Contents

Chapter 1

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the global population, with over 200 million households in the low-income world alone depending on it as their primary staple source of income and calories (Muthayya et al., 2014; Khush, 2005). However, future rice production will have to take place in warmer and drier environments (Kadam et al., 2014). Due to its wide geographic distribution, extending from 50°N to 35°S, rice is forecasted to be the most vulnerable crop to warming global climates (Jagadish et al., 2012, Jagadish et al., 2011, FAO). Recent studies (IPCC, 2013, Jagadish et al., 2012) have predicted lower rice yields and increasing rice yield variability due to higher frequencies of heat stress events, and a higher variability in precipitation patterns due to global warming. As such, understanding the effects of drought and heat stress intensity and frequency on rice yields is of upmost importance to feeding the growing global population.

1.1 Heat Stress in Rice

In tropical environments, heat stress is emerging as a major environmental constraint in rice production. Heat stress during the rice flowering period, a temperature sensitive time for fertilization, has been documented to cause extensive yield damage throughout the rice growing world (Ishimaru et al., 2010, Rezaei et al., 2015). A heat wave of daytime ambient temperatures above 38℃ for more than 20 straight days resulted in 5.18 million tons paddy rice loss in 2003 in the Yangtze River Region in China (Li et al., 2004). Gourdji et al. (2013) predicted the percentage of cultivated rice that will suffer from at least 5 days of heat stress (>36℃) during flowering to be 16% by 2013 and increase to 27% by 2050.

The Intergovernmental Panel on Climate Change (IPCC) (2013) predicts that mean global temperatures will rise 2°C from 2046 to 2065 and as such the mean global temperature increase could increase the probability and frequency of heat stress events during rice flowering. Furthermore, it is probable that heat stress events will occur not only with a higher frequency but also with a higher duration. In terms of rice spikelet fertility, and subsequent yield, the reproductive stage includes a sequence of sensitive developmental events that have little flexibility or compensatory capacity against heat stress (O'Toole and Chang, 1979). Among these processes are anther dehiscence (the release of the pollen from the anthers by natural bursting open of the anthers for the discharge of the pollen on the stigma which enables fertilization), pollination – the deposit of the pollen on the stigma -, pollen germination and pollen tube growth (Jagadish et al., 2010; Matsui, 2002). Spikelet fertility is directly related to spikelet temperature and the tolerance of a rice variety to high air-temperature induced spikelet sterility depends directly on the temperature inside the spikelet itself (Weerakoon et al., 2008). Shi et al. (2015) found a significant negative relationship between spikelet tissue temperature and spikelet sterility. There is a difference between air temperature (recorded 2 m above the canopy) - temperature around the panicle, tissue temperature (measured inside the rice panicle) and ambient air temperature and each effects sterility at different temperature thresholds. Canopy temperature is typically lower than ambient temperature due to stomatal conductance which is referred to as canopy temperature depression (CTD) below air temperature as canopy temperature is cooler than the air, under well-watered, dry conditions (Amani et al., 1996). CTD varies significantly for different genotypes (Shi et al., 2015) indicating that some genotypes are more efficient at cooling themselves under high temperatures. The driver for spikelet sterility induced by heat stress at flowering can be attributed to anther indehiscence,

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because the swelling of the pollen grains, the driving force behind anther dehiscence in rice, is inhibited, whereas the female reproductive organ is not damaged when exposed to heat stress (Jagadish et al., 2014, Matsui et al., 1999, Yoshida 1981). In the light of the susceptibility of rice fertility to heat stress (Downton & Slatyer, 1972) and global warming predictions (IPCC, 2013) future heat stress events could provide a challenge to global food security.

1.2 Drought Stress in Rice

Apart from extreme heat events, climate change, for instance in terms of possible precipitation decrease in some regions of the world has already caused warning impacts on water resources. Global warming is one of the greatest pressures on water availability together with pollution, population growth, land use changes and others (Kang et al., 2009). Similar to heat stress events, drought stress during critical developmental stages in rice is among the major challenges to sustained rice production, in terms of yield, its stability and quality (S. V. K. Jagadish et al., 2012, Porter & Semenov, 2005). In the wake of increasing mean global surface temperature, extreme variability in precipitation events over most of the mid-latitude land masses and over wet tropical rice growing regions will likely increase by the end of this century (IPCC, 2013). Burke et al. (2006) predicts that the percentage of land under severe drought will increase from 10% at the beginning of the 21st century to about 40% by the end. Mean duration of the droughts is forecasted to increase by the factor five under the same time period (Burke et al., 2006). Pandey et al. (2008) claim that at least 23 million ha of rice area (20% of the total rice area) in Asia are prone to drought of varying intensities, using a deficit from the long-term rainfall average of 20% or more as a definition for drought stress. Water scarcity during critical rice development periods such as flowering, as a consequence of climate change are currently unfolding in large parts of the rice growing world (Pandey et al., 2008; Raman et al., 2012) .

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Like heat stress, flowering is considered the most sensitive development stage to drought (Krishna Jagadish et al., 2011, Jagadish et al., 2010, Liu et al., 2006, Cruz & Toole, 1984, Matsui, 2002). Peduncles, the panicle stalks, in drought stressed rice plants were shown to be shorter than in the controls, trapping the basal part of the panicle in the sheath flag leaf and causing lower fertility in unexserted (trapped) spikelets (Jagadish et al., 2011; Ji et al., 2005).

Currently, mild and severe droughts frequently take place in predominantly rain- fed rice areas, like north-east Thailand, Laos, central Myanmar and east and north- east India (Wassmann et al., 2009). O'Toole (2004) stated that rice is the most vulnerable crop to drought stress as it currently relies heavily on water supply through irrigation. He asserts that Asia cannot continue depending on both the quantity and quality of freshwater resources the way it currently does, because competition for the resource increases. By 2025, 15–20 million ha of irrigated rice are expected to undergo some form of water scarcity; an estimated 2.5 million ha of wet-season irrigated rice areas in north China, 2.1 million ha in Pakistan and 8.4 million ha in north and central India will experience 'physical water scarcity' by 2025 (Bouman et al., 2007; Tuong and Bouman, 2003). Dropping groundwater tables in major groundwater-depletion arenas in the North China Plain, in the Indian states of Punjab, Haryana, Rajasthan, Maharashtra, Karnataka and northern Gujarat in Tamil Nadu and hard-rock southern India has led to increasing water scarcity affecting rice production in northern India, Pakistan and China (Bouman et al., 2007). Given previous studies (Cruz & Toole, 1984; Liu et al., 2006; Jagadish et al., 2011) have shown that drought stress during flowering can lead to large increases in sterility and thus decreases in yield, increased frequency and severity of drought in these rice production areas could pose a threat to global food security in a warming climate.

1.3 Combined Heat and Drought Stress in the Rice Literature

Given the high correlation of heat and drought stress simultaneously occurring many studies have modeled the total effect of both (Ayeneh et al., 2002; Baker et al., 1997; Boonjung & Fukai, 1996; Challinor et al., 2007; Coast et al., 2014; Eyshi Rezaei et al., 2015; Ferris et al., 1998; Gourdji et al., 2013; Heinemann et al., 2008; Jagadish et al., 2010; Jagadish, Craufurd, & Wheeler, 2007). But to our knowledge none have modeled them simultaneously disentangling the marginal effect of each. Often times heat stress induces drought stress and it is important to take into account that plant tissue temperatures can be significantly warmer than ambient air under dry conditions or cooler than air under suitable soil water conditions and, as such, eliciting the effect of each individually can be problematic (Hatfield et al., 2011).

Heat stress with regard to its effects on spikelet fertility is well documented by Jagadish et al. (2007, 2008, 2010) and Matsui (2002) and many attempts to find or establish critical thresholds have been made (Laborte et al., 2012, Satake and Yoshida, 1978, Prasad et al., 2006, Gourdji et al., 2013, Ishimaru et al., 2010, Rang et al. , 2011, Sánchez et al., 2014, Welch et al., 2010, Nakagawa, 2002, Yoshida 1981, Zou et al., 2009). Yet, Challinor et al. (2007) claim that the quantification of heat impacts on future crop yields as regards climate predictions is still in its infancy, since quantitative studies on spikelet fertility are mostly based on controlled conditions experiments with determined thresholds and there is no consensus on a heat threshold in the literature.

Other studies have concentrated exclusively on drought and its effects on spikelet fertility during flowering concluding that, similar to heat stress drought affects the reproductive organs and therefore anther dehiscence, pollen shedding and germination (Wassmann et al., 2009). These and other studies on drought stress in rice failed, however, to appropriately express their

findings on plant damage in relation to appropriate actual drought measurements or establish definitive thresholds, e.g. Bouman et al. (2007); Tuong & Bouman (2003), Pandey et al. (2004), Heinemann et al. (2008), Bates et al. (2008). In contrast to these past studies, this research attempts to find critical heat and drought thresholds simultaneously.

Spatial analysis has shown that heat stress during the susceptible reproductive stage is likely to coincide with periods of water scarcity in rice growing regions of Bangladesh, the eastern part of India, southern Myanmar and the north of Thailand (Wassmann et al., 2009). Jagadish et al. (2011), Liu et al. (2006) and Rang et al. (2011) conducted studies on combined heat and drought stress in rice at International Rice Research Institute (IRRI). In general, however, literature and in-depth understanding of combined heat and drought stress is currently lacking (Rizhsky et al., 2004; Rizhsky, Liang, & Mittler, 2002), but are important where crop losses are a product of multiple stressors (Jagadish et al., 2012). Combined heat and drought stress can lead to additive detrimental effects on growth physiology during different phenological stages of the rice plant, leading to significantly lower productivity (Kadam et al., 2014; Porter & Semenov, 2005).

In terms of key physiological processes determining spikelet fertility during abiotic stress, in their experiment, imposing 38ºC for five consecutive days during anthesis and drought stress causing 50-60% decrease in flag leaf water content, Rang et al. (2011) detected that heat stress caused an 8% reduction in peduncle length, i.e. the length of the panicle stalk, water stress 24% reduction in peduncle length and combined heat and water stress a 27% reduction in peduncle length. The number of germinated pollen on the stigma was reduced when exposed to heat (81%), drought (59%) and concomitant stress (84%). Spikelet fertility declined more under

heat stress alone (77%) and combined stress (71%) than due to water stress conditions (21%) (Rang et al., 2011).

Given that drought and high-temperature stress often occur together, it is essential to disaggregate the impacts of the two individual stressors and examine possible interactions by modeling their impacts simultaneously. A reliable and robust temperature and drought threshold inducing rice spikelet sterility under field conditions involving cultivars with highly varying phenology has been a major limitation on rice breeders to devise adaptation strategies and on the climate and crop modeling communities to estimate heat stress and drought impacts. This study is designed to overcome these limitations. Using field data from experiments conducted at Los Baños, Philippines, in 2012, 2013 and 2014, this study estimates spikelet fertility under field conditions which is crucial because the majority of existing studies, e.g. Abeysiriwardena et al. (2002), Matsui et al. (1997), Shi et al. (2015), Weerakoon et al. (2008), Rang et al., 2011 on combinations of abiotic stress, were performed under controlled, laboratory conditions, featuring a considerable gap between their findings and the actual situation in the field (Merquiol et al., 2002; Mittler, 2006; Wassmann et al., 2009). Furthermore, this study explores the important relationship of canopy temperature to ambient temperature and drought because they are all interrelated. Ambient temperature is often used by climate modelers because of readily available data. However; canopy temperature is a larger driver of sterility temperature events than ambient air temperature. Thus by using regression analysis on spikelet fertility under abiotic stressful (drought and heat) growing conditions, this study provides estimates on how each abiotic stress affects sterility. Given the assumption that canopy temperature is affected by atmospheric temperature as well as water stress conditions in rice cultivars, the findings of this study can help

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to advance a holistic predictive model of global warming in a differentiated manner, i.e. adjusting for drought occurrence.

In addition to estimating the marginal effects of the two abiotic stress effects, a further contribution of this research is the simultaneous estimation of thresholds for both heat and drought stress on sterility. A lack of consensus on an appropriate threshold under field conditions has resulted in a wide range of critical temperature thresholds (35 to 41 $^{\circ}$C) used for experimental and modeling exercises (Rezaei et al., 2015, Sánchez et al., 2014). One objective of the study is to find a canopy heat threshold in terms of spikelet fertility rather than more commonly used ambient temperature threshold. Typically, ambient temperature data are mostly obtained from controlled chambers or weather stations not located on the actual experimental side. However, there can be large variations in ambient and rice plant tissue temperatures, e.g. depending on relative humidity. In the underlying experiment, canopy temperatures were measured for each replicate under field conditions. Because canopy temperature is more closely linked to the tissue or panicle temperature than ambient temperature - it represents the surrounding micro climate of the spikelets. Thus, revised thresholds for canopy temperature can assist climate modelers to better predict the critical ambient temperatures that could jeopardize food security (Abeysiriwardena et al., 2002; Porter & Semenov, 2005; Prasad et al., 2006, Rezaei et al., 2015, Siebert et al., 2014). In the same manner, this study estimates a drought threshold beyond which the severity in sterility increases, which would be a first in the literature. Our study provides a robust threshold that can be effectively used by breeders, physiologists, and climate and crop modelers. The objectives of this study are to: 1) estimate the individual effects of heat and drought stress simultaneously on sterility 2) calculate a canopy heat stress threshold where significant sterility occurs and 3) calculate a drought stress threshold where significant sterility

occurs. Our objectives address the lack of a reliable and robust critical canopy temperature and drought thresholds that triggers damage under field conditions, which could be used for large scale phenotyping to help rice adapt to a rapidly changing climate.

Chapter 2

Methodology
1.4 Combined Drought and Heat Stress - Experiment Setup

The underlying database comes from a field experiment conducted at the International

Rice Research Institute (IRRI), Los Baños, (14º11'N, 121º15'E, 21m), Philippines in April and

May of 2012, 2013 and 2014. Three indica rice cultivars – Nagina 22 (N22), Anjali and Dular –

were used to test the effects of combined heat and drought stress during flowering on spikelet

fertility in rice and examine the relationship between canopy temperature, ambient temperature

and drought. The three varieties have varying degrees of tolerance to drought and heat stress, as

recommended for this type of investigation by Torres et al. (2012). Anjali, a variety released by

CRURRS (Central Rainfed and Upland Rice Research Station), India, in 2002, is a semi-tall (85-

90 cm) and early maturing (95-95 days) variety (Kumar et al., 2014), developed for drought

prone upland regions of Odisha, Jharkhand, Bihar, Assom and Chhattigarh states of eastern

India. Anjali is moderately tolerant to drought and its ecosystem is rainfed upland, direct seeded,

yield expectations are 2-3 t/ha (Diwakar & Kumar, 2012). Dular is a landrace cultivar from

India (Wang et al., 1998) that belongs to group O. sativa type aus genotypes that is characterized

by a greater ability to exploit soil moisture when water stressed which is attributed to a favorable

root distribution along the soil profile, i.e. advantageous deep root length (Gowda et al., 2012).

N22, an upland variety from India, is recognized to be tolerant to both, water and heat stress at

the flowering stage (Selote & Khanna-Chopra, 2004, Prasad et al., 2006, Satake & Yoshida,

1978, Rang et al., 2011, Ishimaru, 2010, Jagadish et al., 2010, 2012, Bahuguna et al., 2014). An

important factor making N22 tolerant to heat is resilience of the germinating pollen over a very

large temperature range from 5.6-45.4℃ (Coast et al., 2014). Although N22 is favorable in heat

prone areas of rice production it is criticized for its poor agronomic performance and poor combining attributes (Bahuguna et al., 2014).

The experimental data were obtained during the end of April until the beginning of May of 2012, 2013 and 2014. Each of the three varieties had three replications per year, organized as randomized complete block design (RCBD), shown for the three years of the experiment Figure A1, A2, A3. Dormancy of the seeds was broken by an exposure to $50\,°C$ for a period of three days, followed by pre-germination and sowing in seeding trays. Fourteen days after germination, the seedlings were transplanted at a spacing of 20*15 cm with two seedlings per hill.

Table 1 shows the seeding and transplanting dates and other important phenological events for each year and variety. Potassium (40kg K ha^{-1} as KCl), Phosphorus (30kg P ha^{-1} as single superphosphate), Zinc (5kg Zn ha^{-1} as zinc sulfate heptahydrate) were applied on the plots one day before transplanting. Nitrogen fertilizer in the form of urea was applied in three steps (60kg ha^{-1} as basal, 45kg ha^{-1} at mid tillering, and 45kg ha^{-1} three days before panicle initiation). Pre-emergence herbicide was used to control weeds in the plots and manual weeding was employed when needed.

Table 1: Summary of Phenological Events

Variable	2012	2013	2014
Location	International Rice Research Institute (IRRI), Los Baños, (14º11'N, 121º15'E, 21m), Philippines		
Cultivars	Dular, Anjali, N22	Dular, Anjali, N22	Dular, Anjali, N22
Seeding Date			
Dular	23 Feb	21 Feb	21 Feb
Anjali	28 Feb	21 Feb	21 Feb
N22	4 March	26 Feb	26 Feb
Transplanting			
Dular	8 March	7 March	7 March
Anjali	13 March	7 March	7 March
N22	18 Mar	12 Mar	12 Mar
Draining	16 April	10 & 23 April	21 April
Re-water	5 May	4 May	3 May
50% Flowering			
Dular	24/25 Apr	23-25 Apr	29 Apr
Anjali	30Apr/1 May	23-27 Apr	26-27 Apr
N22	4-6/9-11 May	25-28 Apr	26-27 Apr
Panicle Maturity/Harvest			
Dular	21/24/29 May 2/8 June	15/21 May	19/22 May
Anjali	21/24/29 May 2/8 June	11/15/21 May	15/19 May
N22	21/24/29 May 2/8 June	21/27 May	15 May
Number of Observations	18	18	18

2.2 Environmental Conditions

IRRI is located in a humid, tropical, lowland environment which allows for two to three rice crops per year. Approximately 45% of the rice area in Southeast Asia is under irrigation, thus, under similar conditions as the test plots of this experiment; the largest areas being found in Indonesia, Vietnam, the Philippines and Thailand (Mutert & Fairhurst, 2002; Redfern et al., 2012). Worldwide, about 93 million hectares of rice are represented by irrigated lowland systems, providing 75% of the world's rice production (IRRI., 2013)

Heat stress was induced by scheduling the transplanting and thus flowering so that it would coincide with naturally high temperatures during late April and early May which represent the hottest period of the year at IRRI. To ensure the occurrence of the anthesis of the three varieties simultaneously, Dular, which had the longest vegetative stage, was transplanted ahead of the shorter duration varieties N22 and Anjali (Table 1).

In order to impose drought and heat stress simultaneously, the experimental format included a drought stress treatment (where water was drained from the paddy during flowering) and an irrigated control treatment. Before starting the drought treatment, all experimental plots were uniformly and completely irrigated, followed by opening the bunds and water outlets around the stress plots to drain them. The drainage for the drought stress plots started at the booting stage, about 10 days before flowering, to ensure that all of anthesis stage for each variety took place under drought stress (Table 1). Daily visual inspections served for determination of the dates when each phenological stage was achieved; the stages were panicle initiation, heading, 50% flowering, 100% flowering and panicle maturity. To ensure the exclusion of rainfall, rainout shelters were used on the drought stress plots when stress was induced. The drought stressed and the control replications were established in separate field blocks to avoid problems with seepage, following Torres et al. (2012).

Throughout the experiment, no incidences of pest or disease were observed. After 19, 11 and 12 days of stress in 2012, 2013 and 2014 respectively, the stressed plots were re-flooded in order to guarantee that drought stress occurred only during the flowering period and subsequently the crop grew under optimum conditions until it was harvested. The experimental design was aimed at quantifying sterility, which is determined during flowering, drought stress was only induced then. At the termination of the drought period for stressed plots, the soil

moisture contents were similar in the three years, 50 – 60 kPa, and the plants had to have passed the target stage to reinstate post-flowering optimal water conditions.

Cardinally, an extreme heat threshold is determined by exposure-level above which substantial yield losses start to accumulate, which are strongly driven by spikelet sterility induced during the flowering stage (Bheemanahalli et al., 2015). Spikelet fertility was examined at harvest where empty spikelets were counted post-harvest and regarded as sterile. The panicles were hand-threshed, filled and unfilled grains (including half-filled grains and empty grains) were separated by submerging them in tap water, a seed blower was used to divide half-filled and empty grains. Sub-samples were taken to count the total number of filled, half-filled and empty grains to determine seed-set manually (percentage of number of filled and half-filled grains over the total number of spikelets on the panicle).

*1.5 Experimental Measurements and Statistical Analysis*

An important aspect for modeling abiotic stress effects on crops, is to examine the effects at particular developmental stages, since temperature thresholds can be more detrimental at specific periods of development (Ferris et al., 1998; Porter & Semenov, 2005). As such the regression format used here examines spikelet fertility caused by abiotic stress during flowering, cumulating the number of hours spent above a specific canopy temperature threshold, since it is assumed that temperature effects are cumulative over time. Whereas numerous studies impose a specific level of temperature stress to quantify heat effects on fertility (Abeysiriwardena et al., 2002; Matsui et al., 1997; Weerakoon et al., 200), here, a threshold is estimated from the field experimental data themselves. Canopy temperature measured by plot, variety, and year is used for measuring the effects of heat stress. Canopy temperature observations are a better proxy of panicle temperature than the ambient temperature recorded at weather stations that are often

distant from the experimental sites and as such provide a practical alternative to ambient temperatures (Fukuoka et al., 2012). Canopy temperature measurements were recorded every 10 minutes but only temperatures from 8:30 am to 2:00 p.m. were utilized in this study, since they represent the time of the day in which the rice spikelets complete flowering within a particular day and the sensitive reproductive organs are exposed to heat stress (Bahuguna et al., 2014). Canopy temperature was averaged over 10 minutes using MINCER (Micrometeorological Instrument for the Near-Canopy Environment of Rice) (Fukuoka et al., 2012). Ambient temperature data are not plot specific and were derived from the weather station at IRRI, recorded every 15 minutes.

Plot specific soil moisture was measured by a tensiometer which does not directly sense soil water content, but rather soil water/moisture tension which has the advantage of being independent from the soil type and reflecting the actual water requirements of the crop. Tensiometer measurements were recorded daily between 11am and 12pm on the stressed plots with the porous cup at 30 cm soil depth, the unit of measurement is kilopascals (kPa). Soil moisture tension measures suction, so it is correct to be reported as negative numbers. Henceforth in this study, the absolute value of the tensiometer readings are used in the statistical analysis which is a common procedure (MEA, 2015). Table A1 shows adjustments (primarily for missing observations) made to the data before it could be used in the regression analyses.

Figure 1 illustrates the daily tensiometer measurements for all three years of the study after the stress period was initiated.

Figure 1: Tensiometer Readings after Stress Initiation Averaged over Variety for each Year in the Study.
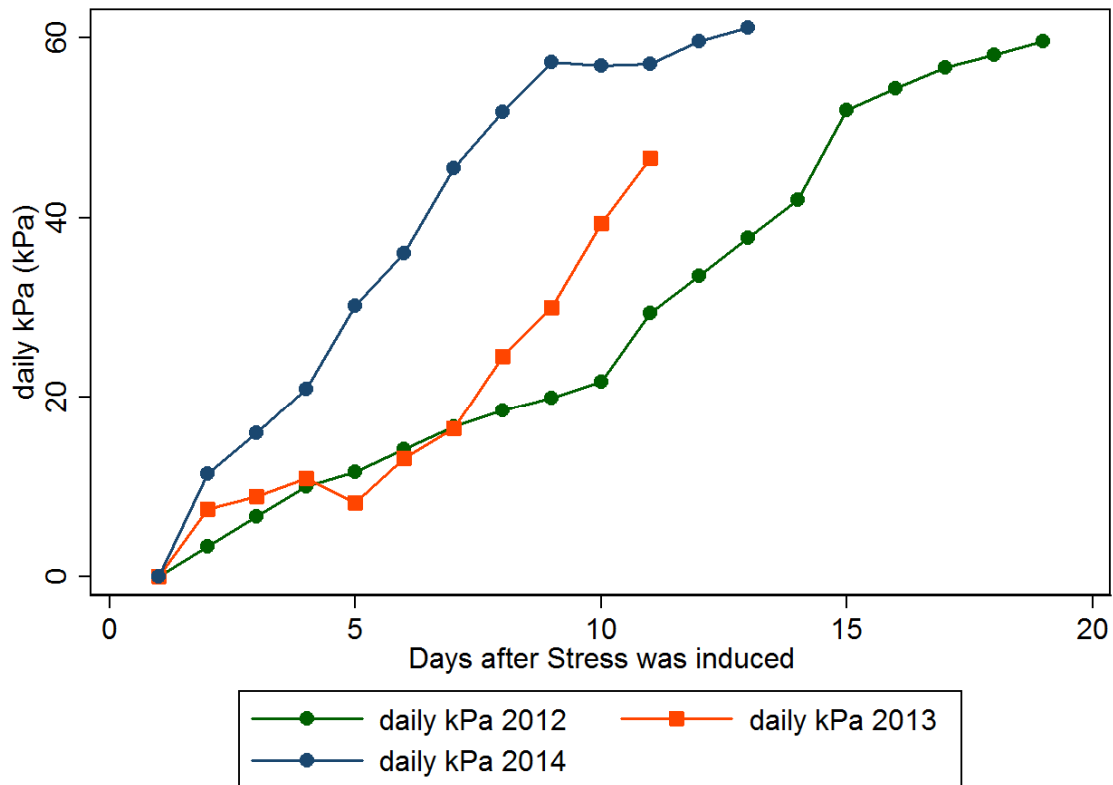
The thresholds for drought as well as for canopy temperature are found through regression analyses using varying threshold levels and choosing the thresholds that give the highest explanatory power. The drought stress variable is constructed as the sum of the number of days spent under drier conditions than a given kPa threshold value. Likewise, the canopy temperature variable is the sum of the number of hours spent above a given canopy temperature threshold during flowering over stress period i. The given thresholds are varied from 12 to 52 for kPa and from 26°C to 40°C for canopy temperature. The two optimal thresholds for drought and heat stress are first identified in separate grid search analyses – one for drought and one for heat stress. For draught a series of regression models are estimated where the draught variable differs

by the assumed threshold.   The optimal draught stress threshold is identified by that model in the grid search that has the highest R^2. A similar search is done to identify the optimal canopy temperature threshold. Once ranges for the local optima are determined, a grid search is employed in two dimensions (drought and heat stress) to get the optimal threshold pair for explaining spikelet variability. In the one-dimensional as well as later in the two-dimensional grid search, the optimal canopy temperature exposure thresholds range from 26°C to 40°C – in increments of 1°C - to find the best fit with regards to sterility. The range chosen for possible canopy temperature thresholds relied on existing literature on temperature – mostly ambient temperature – thresholds (Coast et al., 2014; Gourdji et al., 2013). To estimate a drought threshold above which losses in spikelet fertility become severe, thresholds between 12 and 52 kPa were analyzed in the separate drought model and the two-dimensional grid search. This range of possible thresholds was considered as reasonable because 10 to 25 kPa reflects a soil at field capacity (Schwankl et al., 1992) and plants should be re-watered when soil tensiometers installed at 30 cm depth register soil water tension of about -50 to -70 kPa to help plants survive as these levels are extremely severe and can lead to 100 % sterility and if extended can result in plant death (Torres et al., 2012).

The model for measuring the direct impact of canopy temperature and draught stress on spikelet fertility and that was used in the grid search for the optimal canopy temperature and drought thresholds is a multivariate regression model specified as:

$$y_{spik} = \alpha_c + \alpha_y + \beta_c c + \beta_k kPa + u_{spik} \tag{1}$$

where y_{spik} is spikelet fertility at harvest in percent for observation i, α_c is a vector (two components) of fixed effects for cultivar type, α_y a vector (two components) of fixed effects for year, β_c is the coefficient of c, the number of hours during the stress period the crop spent above

the specified canopy temperature threshold d, and β_k is the coefficient of the drought variable kPa, the number of days the crop spent above the specified kPa threshold. The term u_{spik} is a random error term representing the impacts of unobserved factors on spikelet fertility. Specifying spikelet fertility as a function of canopy temperature (c) and drought (kPa) allows for estimating and testing for the impact of amount of time spent under more severe conditions beyond certain thresholds. In the experiment data are observed in each year for each variety under both draught and non-draught conditions.

According to Eyshi Rezaei et al. (2015) it remains unclear if heat stress only arises above some threshold or is exclusively the accumulation of heat that affects in spikelet fertility. Several crop modeling studies, like Schlenker & Roberts (2009), use growing degree days (GDD) in combination with cardinal temperature thresholds. So, we modeled a piece-wise linear regression model to detect breakpoints and nonlinearities in the effect of drought and temperature on spikelet fertility. It was applied with one single threshold, implying two temperature variables. Night time temperature, from 6pm to 6am, and the Vapor Pressure Deficit (VPD), an environmental factor composed of Relative Humidity (RH) and temperature, were also part of possible alternative multivariate regression models. To ensure a comprehensive approach, different models were estimated, e.g. amount of hours above the threshold, a weighted sum of hours by how much the threshold was exceeded, nonlinear functions of drought and canopy temperature exposures (Table 3).

Given the IPCC estimates that mean global temperatures are expected to increase by up to 2°C from 2046 to 2065, results from equation 1 could be used to predict the impact of warming scenarios on spikelet fertility in rice. Hence, apart from establishing temperature and drought thresholds, this research can help to predict the effects of a warming rice growing world.

Because most warming scenarios (IPCC, 2013) look solely at changes in ambient temperature and canopy and panicle temperature have been established as the drivers of sterility, a relationship between the two temperatures (ambient and canopy) must be estimated. This is confounded by the fact that ambient temperature also affects the soil moisture condition which in turn can also affect canopy temperature. This structure implies a recursive model with three equations. The first equation in the system specifies kPa as a function of ambient temperature, the second equation specifies canopy temperature as a function of ambient temperature and kPa, and the third equation specifies spikelet fertility as a function of canopy temperature (c) and kPa as given in equation (1).

Assuming ambient temperature has a statistically significant impact on kPa, soil water potential is regressed on ambient temperature in the following manner:

$$y_{kpai} = \eta_1 + \eta_2 ambient + u_{kpa} \quad (2)$$

Where y_{kpa} is daily average soil water tension in *kPa* for observation i in year t, η_a is the coefficient of *ambient,* daily average of mean ambient temperature and u_{kpa} is a random error term. Equation 2 accounts for the indirect effect of ambient temperature on draught, since drought is intensified by an increase in ambient air temperature and Equation 3, specified below, shows how drought and ambient temperature directly influence canopy temperatures.

As such, we model canopy temperature as a function of ambient temperature (c) and draught (kPa) 1) *direct* effect on canopy temperature and 2) an *indirect* effect via increased kPa. We model this by

$$y_{ci} = \alpha_v + \mu_c c + \mu_k kpa + u_i \quad (3)$$

where y_{ci} is the ten-minutes-mean canopy temperature in ℃ (Celsius) for observation i, recorded every 10 minutes, $\boldsymbol{\alpha}_v$ is a fixed effect for cultivar v, μ_c is the coefficient of c, the ten-minutes-mean ambient temperature in ℃ Celsius and μ_k is the coefficient of kpa, the average tensiometer measurement in *kPa* every 10 minutes. u_i is a random error term that represents other impacts on 10-minutes canopy temperature that were unobserved. Equation 3 accounts for the direct effect of ambient temperature on canopy temperature. The coefficients in $\boldsymbol{\alpha}_v$ reflect the differences in genotype in cooling canopy temperature at given drought and ambient temperature stress levels. Equation 1 represents the direct effect of drought and canopy temperature on spikelet fertility.

Chapter 3

Results
3.1 Regression Results

      Table 2 shows the summary statistics of the experiment by stress/non-stress, variety and

year. Table 3 contains the results of alternative specifications of Equation (1) that were estimated

but not used in the subsequent analysis. Table 4 shows the results of the preferred model. The

preferred model for spikelet fertility (equation 1) resulted in an adjusted R-square of 67.34%.

This model and all subsequent models were estimated using robust standard errors. The preferred

model includes a linear functional form of hours above the canopy temperature threshold, which

was determined to be 33˚C and a logarithmic function of days above the drought threshold,

which was determined to be 12 kPa.

Table 2: Summary Statistics of Spikelet Fertility, Heat Stress Exposure and Drought Stress Exposure

	Spikelet Fertility (%)	Count of Hours exceeding Temperature Threshold 33 °C	Count of Days exceeding Drought Threshold 12 kPa
All Data			
Stress	41.91	29.80	9.96
	(13.19)	(6.48)	(3.78)
Control	64.99	18.02	0.00
	(10.66)	(20.24)	(0.00)
Dular			
Stress	40.77	29.11	10.56
	(11.73)	(7.57)	(3.40)
Control	64.64	18.06	0.00
	(11.83)	(21.21)	(0.00)
Anjali			
Stress	40.86	30.67	9.44
	(13.77)	(6.66)	(4.59)
Control	60.51	18.00	0.00
	(7.60)	(21.26)	(0.00)
N22			
Stress	44.09	29.61	9.89
	(15.18)	(5.81)	(3.62)
Control	69.83	18.00	0.00
	(11.06)	(20.72)	(0.00)
2012			
Stress	35.59	24.056	13.67
	(12.38)	(3.63)	(1.32)
Control	66.096	0.44	0.00
	(12.32)	(0.30)	(0.00)
2013			
Stress	36.27	29.17	5.22
	(11.13)	(5.38)	(1.30)
Control	57.67	7.83	0.00
	(5.84)	(0.00)	(0.00)
2014			
Stress	53.86	36.17	11.00
	(6.85)	(3.40)	(1.00)
Control	71.21	45.78	0.00
	(8.84)	(0.33)	(0.00)

Table 3: Alternative Multivariate Regression Specifications of Equation (1)

Variable	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Threshold kPa	12		12	12	14		14	12	12	12
C	-	32	32	32		32	32		32	33
Intercept	68.80**	67.74**	75.61**	75.72**	65.98**	78.10**	80.05**	78.28**	78.11**	69.78**
	(3.56)	(3.40)	(4.72)	(4.83)	(3.43)	(12.09)	(13.66)	(7.49)	(7.54)	(3.31)
Year 2013	-12.85**	-2.03	-9.26**	-9.11**	-5.60	-0.88	-0.139	-4.31	-6.85	-6.009*
	(3.56)	(3.40)	(3.84)	(4.00)	(3.79)	(4.42)	(6.29)	(6.92)	(8.01)	(3.45)
Year 2014	8.86**	20.19**	17.77**	18.36**	12.98**	18.91**	21.77**	21.48**	17.64	20.52**
	(3.41)	(5.71)	(5.66)	(6.58)	(3.40)	(5.24)	(5.06)	(9.43)	(11.21)	(4.91)
Anjali	6.74*	-6.11*	-6.75**	-6.69*	-6.88**	-6.60**	-7.11**	-5.53*	-6.17*	-6.631**
	(3.39)	(3.24)	(3.28)	(3.34)	(3.31)	(3.28)	(3.20)	(3.46)	(3.63)	(2.69)
Dular	-3.54	-4.32	-4.08	-4.23	-3.86	-4.38	-4.21	-2.79	-3.18	-3.987
	(3.39)	(3.24)	(3.29)	(3.45)	(3.31)	(3.29)	(3.21)	(3.40)	(3.47)	(3.03)
kPa	-2.13**		-1.69**	-1.21	-5.48**		-4.84**	-1.86**	-2.61**	
	(0.26)		(0.33)	(2.99)	(0.97)		(1.06)	(0.32)	(1.21)	
kPa^2					0.29**		0.261**			
					(0.081)		(0.078)			

Table 3: Alternative Multivariate Regression Specifications of Equation (1) – Continued

Variable	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Klog										-8.61** (1.06)
Drought Stress (binary)		-19.60** (3.27)				-17.24** (4.60)				
c		-0.30* (0.16)	-0.35** (0.17)	-0.36* (0.18)		0.80 (1.02)	-0.96 (1.23)			-0.34** (0.13)
VPD								-14.46** (10.08)	-12.34 (10.66)	
kPa*c				-0.010 (0.065)						
K* VPD									0.553 (0.86)	
R^2	0.6612	0.7020	0.6903	0.6905	0.6846	0.7035	0.7036	0.6754	0.6730	0.7104
Adjusted R^2	0.6259	0.6640	0.6508	0.6367	0.6444	0.6584	0.6743	0.6340	0.6223	0.6734

*, ** level of significance at the 10 and 5% levels respectively

Table 4: Results of Preferred Estimates of Equation (1)

Variable	Parameter Estimate
Intercept	69.781** (3.312)
Year2013	-6.009* (3.446)
Year2014	20.519** (4.915)
Anjali	-6.631** (2.692)
Dular	-3.987 (3.030)
Canopy (hours)	-0.3398** (0.126)
Kpalog (days)	-8.608** (1.055)
Number of observations	54
R-Square	0.7104
Dependent Mean	53.44864
Adj R-Sq	0.6734

3.2 Drought Effects under Preferred Model

The preferred logarithmic function for the drought variable and a threshold of 12 kPa result in the highest explanatory power in line with the physiological characteristics of rice and is therefore included into the preferred combined model with the heat variable (Tables 3 and 4).[1] Alternative specifications for estimating drought were also considered: a reciprocal function of the tensiometer-based data, a spline or piece-wise linear function similar to Schlenker & Roberts (2009)– including a linear function representing the number of days above the threshold and a second linear variable representing the days spent under stress conditions (kPa > 0), but less severe than the more harmful threshold. All resulted in a lower R^2 and in some cases resulted in non-sensical results and thus the logarithmic specification was chosen (Table 3).

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[1] The decision for the preferred model in terms of specification and appropriate threshold for kPa was made based on r-squares, however, shows that the combined model with squared terms of the number of days above the drought threshold and the number of hours spent above the canopy temperature threshold lead to a slightly higher adjusted R-square than the preferred specification. Yet, the squared term of kPa would mean at first a decreasing fertility and, after an accumulation of more dry days, again increasing fertility happening within the the experiment observed range of days above the drought threshold which is physiologically not consistent and was not observed in the experiment.

Figure 2: Estimated Fertility under the Logarithmic and Linear Functional Foms of the Drought: Using Days over 12 kPa Threshold under the Average Growing Year for N22
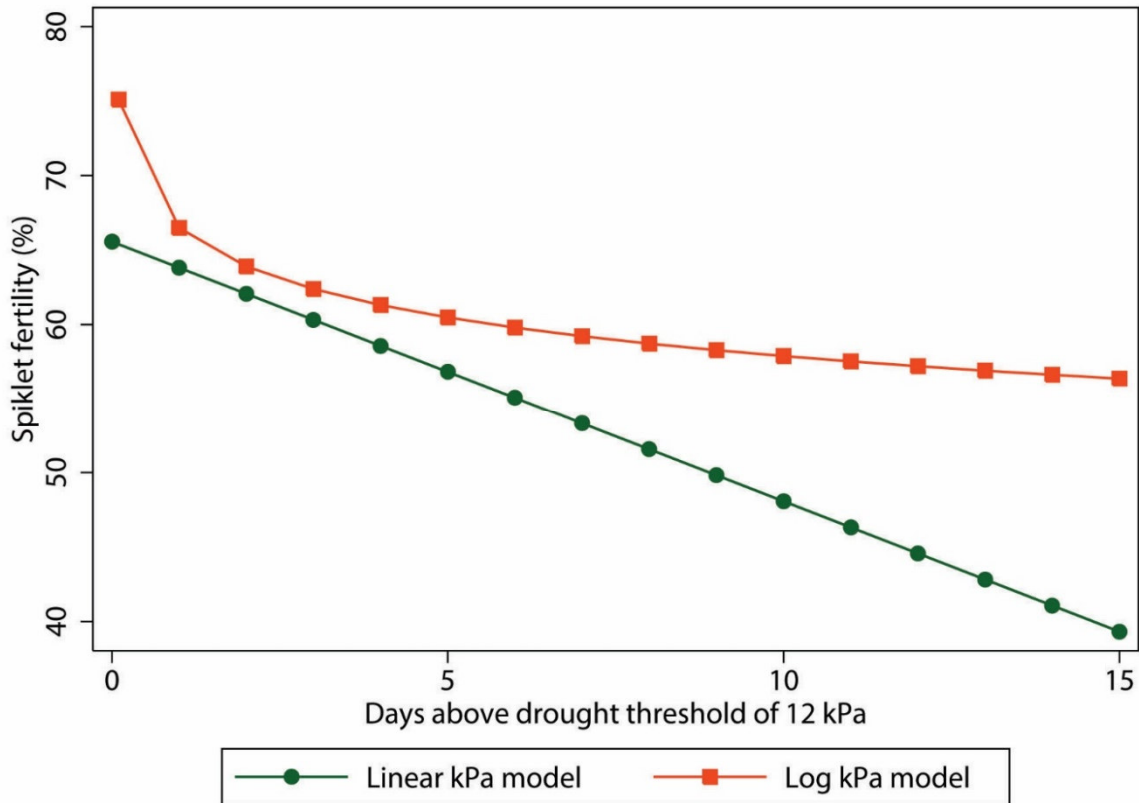
Previous studies have investigated rice's responses to drought stress in terms of a control-stress comparison or linear functions (Henry et al., 2011; Liu et al., 2006). Alternative specifications for estimating drought were also considered: a reciprocal function of the tensiometer-based data, a spline or piece-wise linear function similar to Schlenker & Roberts (2009)– including a linear function representing the number of days above the threshold and a second linear variable representing the days spent under stress conditions (kPa > 0), but less severe than the more harmful threshold. All resulted in a lower R2 and in some cases resulted in non-sensical results and thus the logarithmic specification was chosen (Table 3).

Figure 2 shows the differences in estimated spikelet fertility when using a linear

functional form of the days spent above the estimated optimal 12 kPa threshold and using the

preferred logarithmic form, using an average year and the variety N22.[2]

Figure 2 illustrates that the linear function gives very different impacts of drought on

spikelet fertility, throughout. Since the logarithmic function gives a better fit than the linear, we

conclude that results relying on a linear model might give misleading predictions. This is an

important finding as a linear function, like used by Boonjung & Fukai (1996), Cruz & Toole

(1984) and Kumar & Panu (1997) could overestimate the effects of drought on fertility during a

growing season.

The statistically significant ($p < 0.01$) drought coefficient (*kpa*) of -8.608 (Table 4),

measured in days above the 12kPa threshold, has to be interpreted taking into account the

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[2] The average year is computed by multiplying the intercept (Table 4) by three - for the three trial years - and adding
the two respective year dummy variable coefficients for 2013 and 2014, before dividing by three again.

logarithmic functional form. The coefficient (-8.608) can be interpreted as the relative change of spikelet fertility with increasingly severe drought conditions depending on the level of drought that a variety has already experienced. To find the change in spikelet fertility for a change in number of days under drought conditions, $\frac{\partial y_{fi}}{\partial kpa} = \frac{\beta_2}{kpa}$ is employed. On average the drought stressed plots spend 13.67 days above the threshold of 12kPa in 2012. At this level, fertility decreases by 0.635% for an additional day spent above the 12kPa threshold. In 2013, at its sample mean for varieties under drought stressed conditions of 5.22 days, an additional day above 12kPa caused a 1.66% decrease in fertility, and in 2014, by 0.783% as an additional day of drought is faced by the crop at the sample mean of 11 days of drought stress on the stressed plots. The estimates indicate a decreasing marginal effect due to the logarithmic drought specification. These findings are important because it appears that droughts do not need to be prolonged to have large negative effects on fertility. That is, the majority of fertility damage happens quickly after reaching the 12kPa threshold as depicted in Figure 2.

3.3 Heat Effects on Spikelet Fertility

While some studies (Ayeneh et al., 2002; Baker et al., 1997; Boonjung & Fukai, 1996; Challinor et al., 2007; Coast et al., 2014; Eyshi Rezaei et al., 2015; Ferris et al., 1998; Gourdji et al., 2013; Heinemann et al., 2008; Jagadish et al., 2010; Jagadish, Craufurd, & Wheeler, 2007) look at heat and drought stress in isolation from each other, this study estimates their impact letting the two forces interact with each other simultaneously. Previously in this study, when estimating the thresholds independently the drought threshold was estimated to be 12kPa and the canopy to be 32℃ (Table A2 and Table A3). While the essence of this study is to estimate both parameters simultaneously, it is important to estimate them independently first and compare the

results to previous literature. Wassmann et al. (2009) use 33℃ as a critical ambient temperature threshold to identify rice growing areas that are vulnerable to heat stress. Likewise, Tack et al. (2015) used an ambient temperature threshold of 33℃ for their yield modeling approach in terms of salinity and high temperatures in rice. Matsui et al. (1997) established heat thresholds for spikelet sterility in two japonica rice cultivars by means of high air temperature treatments on rice plants at middle heading stage as flowering time temperatures causing 50% of decrease in spikelet fertility. They report 36℃ and 38.0-38.5℃ respectively for two japonica cultivars. Sánchez et al. (2014) conclude from their literature review that 37℃ would be an appropriate heat threshold for spikelet fertility during anthesis in rice. The threshold found in our research is consistent with the range of previously established thresholds. That being said, the previous heat thresholds were determined for ambient, not canopy temperature. When we do our two-dimensional grid search over the threholds of canopy heat and drought simultaneously, we arrive at the same kPa threshold as for the individual approach (12 kPa) and a slightly higher canopy threshold of 33℃ (Table A4). Thus, in our data set there appear to be no large differences between estimating the thresholds independently or simultaneously. This robustness of this result needs to be explored further in other data sets.

The preferred specification of canopy temperature in equation (1) was a linear form, and as such the coefficient of -0.340 (p < 0.01) represents the marginal effect, indicating that every additional hour spent above 33 °C during the flowering window from 8:30am – 2:00pm, results in a decrease in spikelet fertility of 0.340% (Table 4). Unlike the estimated effects of drought, which were found to be the most detrimental in the first few days, canopy temperature stress, given its preferred linear form, has a constant negative effect on spikelet fertility throughout the flowering period. Thus, the length of the heat stress appears to be the driving factor in sterility.

3.4 Fixed Effects

The interpretation of each of the cultivar fixed effects is the cultivar's resilience to both heat and drought is important in terms of its possible implications for rice breeding and screening for heat and drought stress tolerance. Anjali was estimated to have lower average spikelet fertility than N22 by 6.63% ($p < 0.05$), including stressed and control plots, whereas Dular is not statistically different ($P > 0.1$) than N22 (Table 4). This is consistent with existing literature as N22 is recognized to be drought as well as heat tolerant whereas drought tolerance is expressed in Dular and Anjali is susceptible to both heat and drought stress (Gowda et al., 2012, Selote & Khanna-Chopra, 2004, Prasad et al., 2006, Satake & Yoshida, 1978, Rang et al., 2011, Ishimaru, 2010, Jagadish et al., 2010, 2012, Bahuguna et al., 2014). Not surprisingly when heat and drought stress are imposed Anjali is estimated to have the largest reduction from its non-stressed fertility. The results in Table 5 provide evidence that N22 is the most resilient, in terms of fertility, of the three lines to both heat and drought stress.

Table 5: Average Spikelet Fertility under Non-Stressed and Stress (Heat and Drought) Stressed Conditions for all Three Trial Years

	Anjali	Dular	N22
Non-Stressed	61.87	64.50	68.50
Stressed	49.17	51.93	55.99
% Change*	21%	19%	18%

* % Change compared to baseline Sterility (non-stressed)

The fixed effects for years were also statistically significant for all years in the study. Spikelet fertility was 20.52% higher in 2014 when compared to 2012 ($P<0.01$) and 6.01% lower in 2013 than in 2012 ($P<0.1$) (Table 4). A possible explanation for the heightened fertility in

2014 was the length of the imposed drought stress period. In 2012, the stressed plots were exposed to 19 days of stress whereas only 13 days in 2014. As stated above, the drought treatment was ended when the kPa reached a range of 50 – 60 kPa and when flowering was completed. Re-watering occurred later in 2012, as ambient temperatures were lower 2012 and as such the flowering period took longer to complete (Table 1).

3.5 Warming scenarios

The parameter estimates of the two models (Equation 2, 3) that are used in the calculation of the impacts of climate warming on sterility are presented on Table 9. The *total* effect of a *marginal* increase in ambient temperature on the spikelet fertility is composed of several steps consisting of a *direct* and *indirect* effect. First, the ambient temperature has a direct effect on kpa (Equation 2). Second, ambient temperature and kpa both have a significant effect on canopy temperature (Equation 3). Therefore, ambient temperature has a direct and an indirect effect on canopy temperature: the indirect is through the increase kpa and then the direct effect of ambient temperature on canopy temperature. As such, across the varieties increasing ambient temperature by 1℃ was found to have a direct effect in raising canopy temperature by 0.969℃ ($p < 0.01$), all else equal (Table 6). This makes intuitive sense as the rice plant has the capability of cooling itself and as such the increase should be under 1˚C. Because kPa (drought) is known to effect canopy temperature as well it was estimated (Equation 2) that an increase in soil water potential by 1 kPa, increased average canopy temperature by 0.0230℃ ($p < 0.01$), for all three varieties (Table 6).

Previous literature has shown that there are differences amongst genotypes' ability to lower canopy temperature during stress (Selote & Khanna-Chopra, 2004; Prasad et al., 2006;

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Satake & Yoshida, 1978; Rang et al., 2011; Jagadish et al., 2010; Jagadish et al., 2012). Similar results are apparent in our estimates as Anjali, for stressed and non-stressed plots, was estimated to have an average canopy temperature 0.137̊ C higher than N22 (P < 0.01), *ceteris paribus* (Table 6). Dular was not estimated to have a different canopy temperature (P>0.1), under the assumption of the same level of drought stress and ambient temperature. This most likely reflects function of Anjali's lower ability to keep its tissue temperature low as ambient temperature and drought increase, since it is susceptible to both abiotic stresses. The model specifications with logarithmic and reciprocal specifications for average ambient temperature were not used because they led to slightly lower R-squares than in the preferred linear model (Table A5 and Table A6).

In terms of the indirect effect of ambient temperature on canopy temperature (equation 2) it was found that an increase of daily ambient temperature by 1̊C was statistically significant (p < 0.05) in increasing daily kPa by 2.98 for all three varieties (Table 7).

Table 6: Results of Preferred Regression Model on Canopy Temperature

Variable	Parameter Estimate
Intercept	0.2115
	(0.2604)
ambient10	0.9689**
	(0.0082)
kpa	0.02958**
	(0.00080)
Anjali	0.13693**
	(0.04029)
Dular	-0.04332
	(0.0415)
Number of observations	8767
Dependent Mean	31.88226

R-Square	0.5334
Adj R-Sq	0.5332

Table 7: Results of Preferred Regression Model on Drought (kPa)

Variable	Parameter Estimate
Intercept	-66.72790*
	(33.965)
ambient	2.98038**
	(1.0548)
Number of observations	387
Dependent Mean	29.19889
R-Square	0.0203
Adj R-Sq	0.0178

To calculate the effects (direct and indirect) of an ambient temperature increase we averaged the year fixed effects. Increases of ambient temperature from 0.25°C to 2.0°C, given IPCC estimates indicate a 2.0°C in ambient temperature is plausible, in steps of 0.25°C were estimated. To each observed tensiometer reading in each year, we add the respective ambient temperature increase (depending on the warming scenario) and derive the marginal effect of ambient temperature estimated in equation 2, as follows.

$$gw_{kpa_{value i}} = observed\ kpa\ value + \partial ambient * 2.98038 \quad (4)$$

Then new canopy temperature input datasets for Equation 1, the regression on spikelet fertility, are computed for the different warming scenarios as hours above the threshold 33 ℃ are re-estimated via Equation 2:

$$gw_{canopy_{value i}} = observed\ canopy\ values + \partial ambient * (.96885 + 2.98038 * 0.02958) \quad (5)$$

The dependent variable $gw_{canopy_{value_i}}$ is the estimated ten minutes average for canopy temperature for variety i. The ambient temperature increase $\partial ambient$ is according to the respective scenarios analyzed. For the combined direct and indirect effect (.969+2.980*.0296) of ambient temperature on canopy temperature, the coefficients can be found on Table 6 and Table 7. As ambient temperature is increased by 1 ℃, canopy temperature is estimated to increase by 0.969 ℃. The indirect effect is expressed as the increase in kPa as ambient temperature is increased by 1 ℃ (2.980 kPa) if combined with the increase in canopy temperature (0.0296 ℃) for a 1kPa increase in soil water potential. Table 8 represents the effect of a 1 ℃ increase in ambient temperature on the canopy temperature increase for the rice variety N22.

Table 8: Direct and Indirect Effects of a 1℃ Increase in Ambient Temperature on Canopy Temperature for the Rice Variety N22

Effect	Increase in kPa (kPa)	Increase in Canopy (℃)	Marginal Effect on Canopy (℃)
Direct		0.97	0.97
Indirect (via kPa increase)	2.98	0.029*	0.030*2.98
Total (Direct + Indirect)			(0.97+0.030*2.98) = 1.06

* As derived from Equations 2 and 3
* Given that canopy temperature is modeled as variety specific, the fixed effect coefficients for Anjali and Dular are 0.13693 and - 0.04332 respectively

The results of Equation 5 lead to the updated canopy temperature values which are then plugged into Equation 1. Likewise, a new kPa input data set, updated by the global warming scenarios, is created following Equation 4, due to the fact that kPa changes as ambient temperature increases. Tables A7 and A8 show the new canopy temperature and kPa inputs for

Equation 1 under each of climate change scenarios. The effects of warming scenarios on fertility

are illustrated on Figures 3 and 4 and can be found more in detail in Table 9.

Figure 3: The Effects of Ambient Temperature Warming on Spikelet Fertility - under Flooded
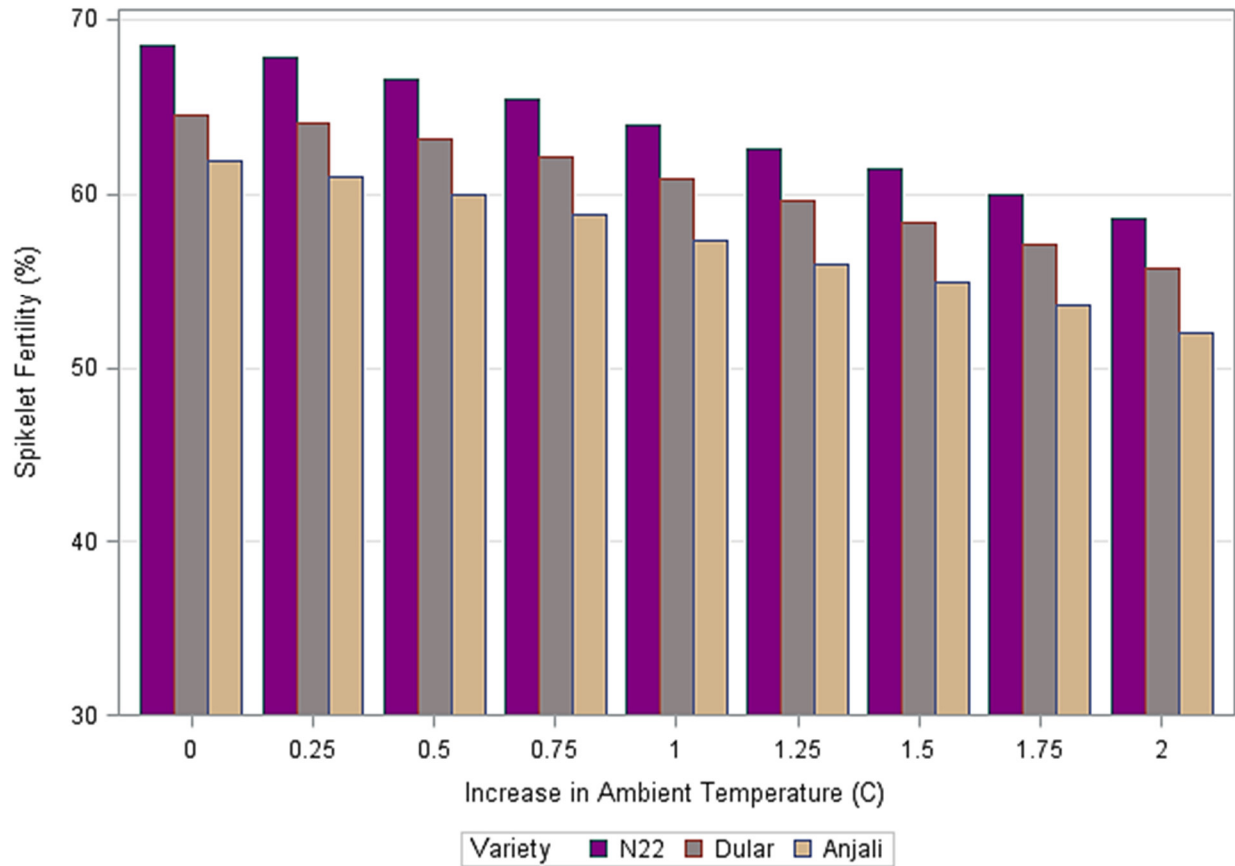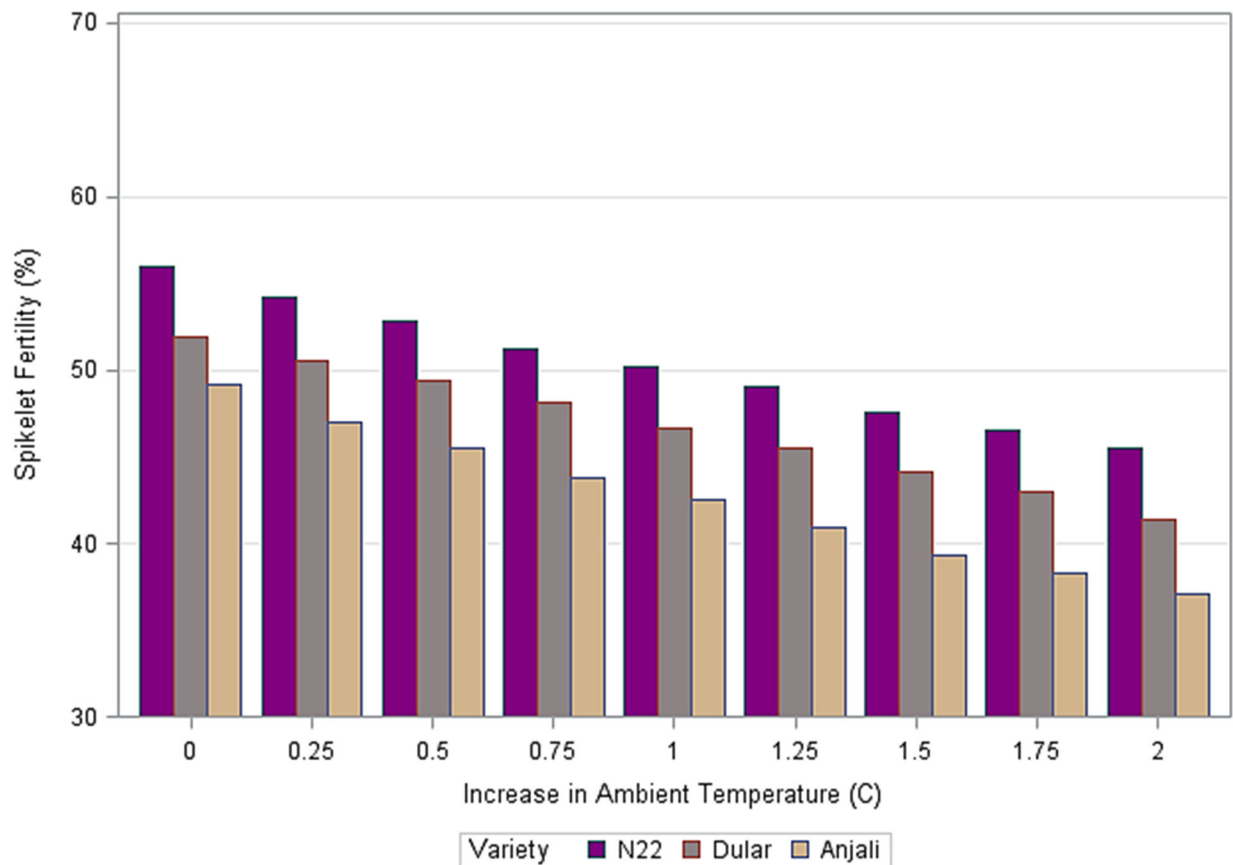Non-Drought Stress Condition

Figure 4: The Effects of Ambient Temperature Warming on Spikelet Fertility - under Drought Stress Conditions

Figures 3 and 4 and Table 9 show the decrease, by variety, under warming scenarios for both drought and fully flooded conditions. When combining the system of equations (equations 1-5) the connection between drought and heat stress becomes clear. The decrease in percent spikelet fertility – as an average across the varieties - with an associated ambient temperature increase of 0.25 °C is 14.39 % in the presence of drought stress – compared to 0.67 under flooded conditions. For an increase of 1.25 °C, average fertility decreases by 19.77% under stress and only 5.55% in the flooded control, for an increase of 2.0 °C, average fertility decreases by 23.65% under stress and only 9.49% in the flooded control. This would seem to indicate that drought stress is more detrimental to spikelet fertility than heat stress, or at the very least heat stress can be partially mitigated through the presence of a flooded paddy. Figures 3 and 4 also

indicate large genotype differences in fertility under both types of abiotic stresses as indicated by the statistical significance of the varietal fixed effects discussed before.

The three varieties show - in terms of spikelet fertility - a very similar response to the warming scenarios. For the most extreme warming scenario in our study (2.0 °C) we find that without the presence of water fertility drops 23.02 % for N22 (from 68.50% to 45.48%), 23.13% for Dular (from 64.49% to 41.36%) and 24.79 % for Anjali (from 61.87% to 37.08) – compared to the observed fertility under flooded conditions. Under that same warming scenario if water is available (no drought stress) fertility only drops by 9.89, 8.76 and 9.82%, respectively.

Table 9: Absolute and Relative Cultivar Effects of Warming Scenarios on Average Spikelet Sterility

		Spikelet Fertility Estimated	Absolute % Fertility and Absolute Change in % Fertility under different Scenarios				
		(%)	observed	0.25	0.5	0.75	1.0
Control	N22	68.50[A]	68.50 0.00*	67.86 -0.64	66.59 -1.91	65.44 -3.06	64.01 -4.49
	Dular	64.50[A]	64.50 0.00	64.03 -0.47	63.19 -1.30	62.17 -2.32	60.89 -3.61
	Anjali	61.87[B]	61.87 0.00	60.97 -0.91	60.00 -1.87	58.83 -3.04	57.36 -4.51
	Average	64.96	64.96 0.00	64.28 -0.67	63.26 -1.69	62.15 -2.81	60.75 -4.20
Stress	N22	55.99[A]	55.99 -12.51**	54.14 -14.36	52.84 -15.67	51.19 -17.31	50.13 -18.37
	Dular	51.93[A]	51.93 -12.57	50.57 -13.93	49.34 -15.16	48.12 -16.38	46.70 -17.79
	Anjali	49.17[B]	49.17 -12.70	46.99 -14.88	45.49 -16.38	43.82 -18.05	42.52 -19.36
	Average	52.36	52.36 -12.59	50.57 -14.39	49.22 -15.73	47.71 -17.25	46.45 -18.51
			1.25	1.5	1.75	2.0	
Control	N22		62.59 -5.91	61.50 -7.00	59.97 -8.53	58.61 -9.89	
	Dular		59.61 -4.89	58.32 -6.17	57.10 -7.40	55.74 -8.76	
	Anjali		56.02 -5.85	54.59 -6.93	53.56 -8.31	52.05 -9.82	
	Average		59.41 -5.55	58.25 -6.70	56.88 -8.08	55.47 -9.49	
Stress	N22		49.04 -19.46	47.57 -20.93	46.54 -21.97	45.48 -23.02	
	Dular		45.53 -18.96	44.19 -20.30	42.95 -21.55	41.36 -23.13	
	Anjali		40.99 -20.88	39.36 -22.51	38.36 -23.51	37.08 -24.79	
	Average		45.19 -19.77	43.71 -21.25	42.61 -22.34	41.31 -23.65	

Note: Means followed by the same letter are not different at P <0.05 using adjusted Tukey mean comparison.* difference between spikelet fertility (in %) resulting from respective non-stress (in terms of water) climate change scenario and the observed non-stress spikelet fertility (in %) in the experiment. ** Difference between spikelet fertility (in %) resulting from respective stress (drought) climate change scenario and the observed non-stress spikelet fertility (in %) in the experiment

Chapter 4
Discussion

The results of this study found that exposure to a canopy temperature over a threshold of 33°C causes a statistically significant increase in rice spikelet sterility. This is unique in that most previous research has focused on ambient temperature, not the more relevant canopy temperature for impacts on sterility. Results indicate that the canopy threshold of 33°C would be equivalent to an ambient temperature threshold of 33.97°C which is in the range of the previously reported threshold of 33 ˚C (Wassmann et al., 2009; Tack et al., 2015).

An estimated drought threshold of 12 kPa was found to be the most detrimental to spikelet fertility. While robust in this study, given the lack of drought thresholds in the literature, this threshold level should be explored further in future research. A large contribution of this study is estimating the threshold and marginal effects of canopy temperature on rice sterility. While most existing climate models forecast ambient temperature, existing physiology literature suggests that it is canopy—not ambient—temperatures that affect sterility. That being said, it is important to also include ambient temperature because it affects canopy temperature, but since canopy temperature is a function of genotypes and drought, canopy temperature is not all encompassing. As such we specify an ambient-canopy temperature relationship and use it to estimate how increased ambient temperatures affects sterility via canopy temperature. Secondly, for the first time, we estimate a drought stress threshold where severe sterility is induced. This is important given two of the largest pressures facing future rice production are heat and drought stress. Historically, it has been difficult to disentangle heat and drought stress because they often occur simultaneously and are endogenous. In this sense, this study was able to model each effect separately, given the experimental set up, but then able to use the two effects; direct-

ambient/canopy and indirect-ambient/kPa/canopy effects to estimate the effects of warming ambient temperatures on rice sterility.

The relationship between spikelet fertility and drought defined by a logarithmic kPa specification shows that most of the drought damage to fertility occurs quickly, after only few days over 12kPa. We conclude that not the length, but rather the frequency and severity of drought may be the important factors influencing rice yields.

It seems apparent from the ambient temperature warming scenarios simulated here that the presence of water greatly reduces sterility in the face of heat stress. Besides, the differences in spikelet fertility in terms of varieties found in the underlying experiment go along with the findings from previous studies, e.g. on drought resistance by Gowda et al. (2012) showing the highest drought tolerance for N22 and the lowest for Anjali. Especially with regard to the warming scenarios, and when drought stress is present, the greater ability of N22 to cope with combined stress becomes clear, followed by Dular and the consistently less drought and heat tolerant Anjali.

Under a warming scenario of 2.0 ˚C, average sterility was found to decrease by -9.49% with the presence of flooded conditions and -23.65% under drought stress. This suggests that heat stress can partially be mitigated by the presence of water in the field. Future research should therefore be especially focused on the aforementioned important rice-growing regions of Bangladesh, the eastern part of India, southern Myanmar and the north of Thailand (Wassmann et al., 2009, Jagadish et al., 2011, Liu et al., 2006, Rang et al., 2011) that are prone to experience combined heat and water stress during the susceptible reproductive stage. Large breeding efforts should be oriented towards adapting varieties to better cope with drought stress.

Generally, crop modeling efforts can be improved by higher spatial resolution (Kang et al., 2009) and future research could be improved by large field studies, using hourly instead of daily kPa measurements and spikelet temperature instead of canopy temperature, as done by Maruyama et al. (2013). Furthermore, more varieties should be included across a wide range of drought and heat susceptibility ratings. Adaptation strategies, e.g. in terms of agronomical management, can offset some of the negative effects of climate change in the near future, but in the longer run, the development of suitable varieties will become essential (Soora et al., 2013; Wassmann et al., 2009). Tolerance to individual stresses as well as multiple stress tolerance have to be a goal of breeding effects (Wassmann et al., 2009). In this sense, understanding the properties of combined heat and drought stress can provide information to breeders on how to promote reproductive-stage drought tolerance through improved germplasm (Liu et al., 2006).

Chapter 5

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Chapter 6

Appendix

Figure A1: Design of Experiment on the Effect of Heat and Drought Stress on Spikelet Fertility during Flowering in Rice: IRRI, 2012

Stressed   Flooded

Rep3	18 V3		Rep3	36 V1
	17 V2	Rep3	Rep3	35 V3
	16 V1			34 V2
	15 V2			33 V2
	14 V3		Rep2	32 V1
	13 V1	Rep2	Rep2	31 V3
JK	12 V1			30 V3
	11 V3			29 V2
	10 V2			28 V1

Rep1   Rep1

Road

V1: Dular   V2: Anjali   V3: N22
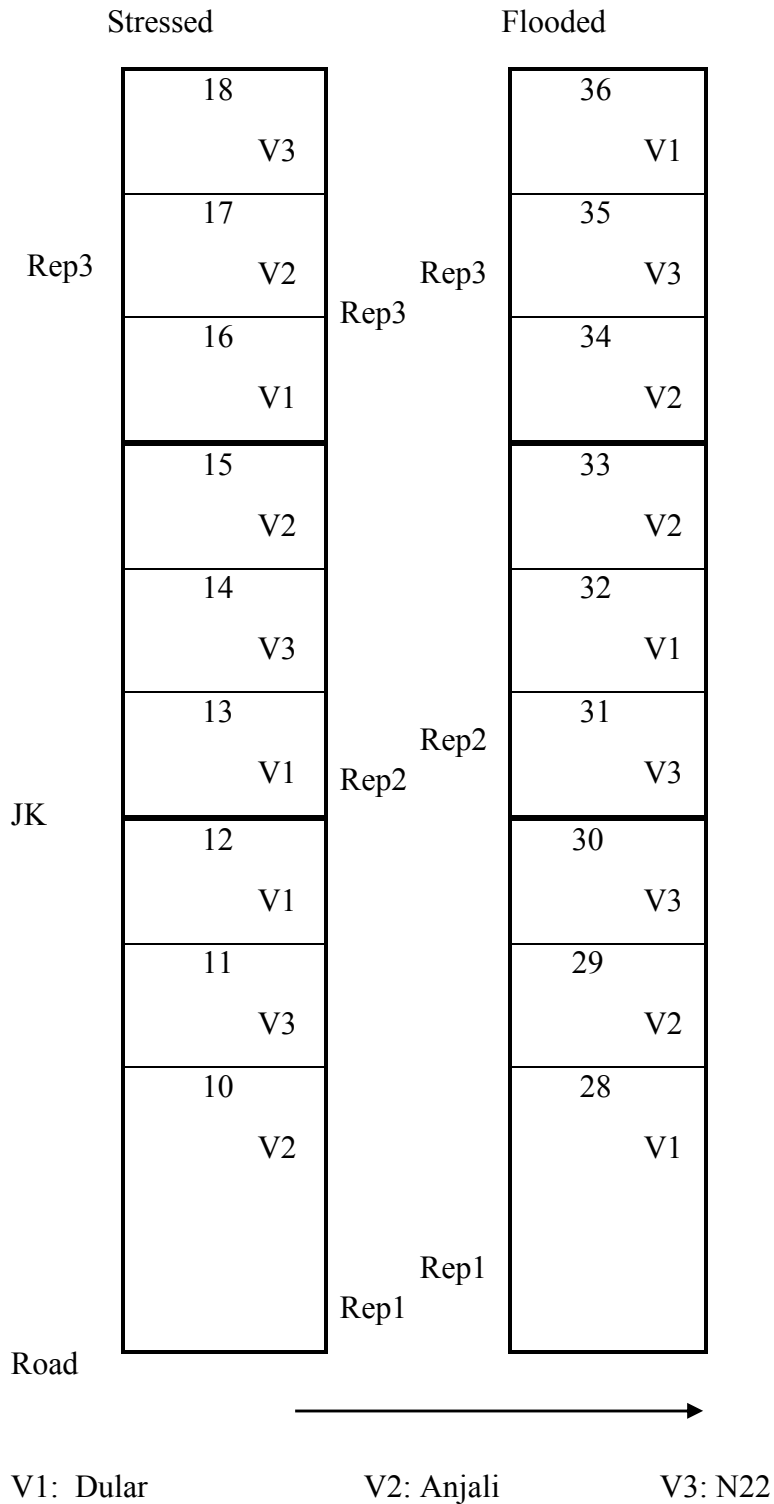
Figure A2: Design of Experiment on the Effect of Heat and Drought Stress on Spikelet Fertility during Flowering in Rice: IRRI, 2013

Stressed   Flooded

36	18
V3	V3
35	17
V2 Rep3	V1
Rep3	
34	16
V1	V2
33	15
V2	V2
32	14
V1	V3
31 Rep2	13
V3 Rep2	V1
30	12
V3	V1
29	11
V2	V2
28	10
V1	V3

Rep2
Rep2

Rep1
Rep1

Road

V1: Dular   V2: Anjali   V3: N22

51
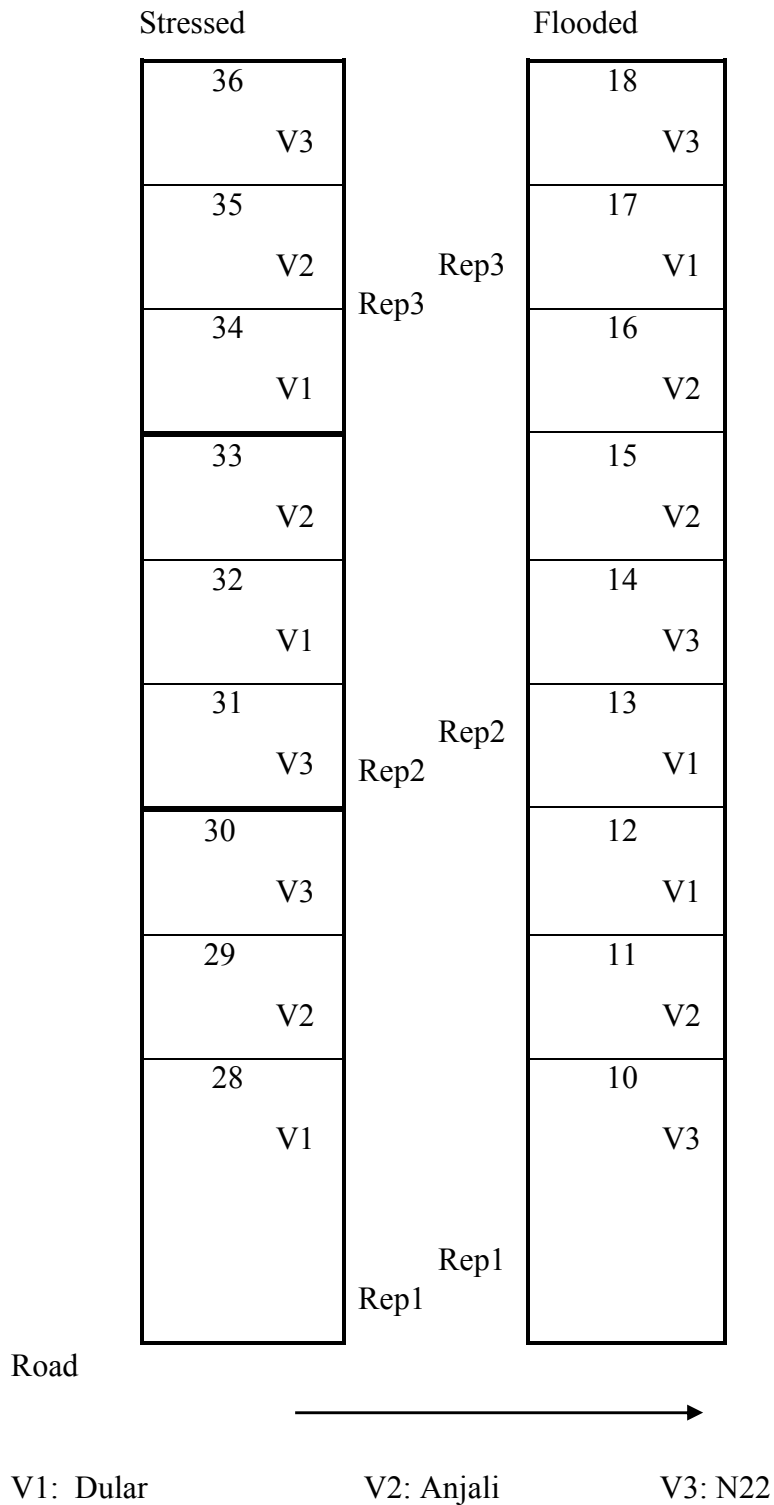
Figure A3: Design of Experiment on the Effect of Heat and Drought Stress on Spikelet Fertility during Flowering in Rice: IRRI, 2014

Stressed   Flooded

36 V3	18 V3
35 V1	17 V2
34 V2	16 V1
33 V2	15 V2
32 V3	14 V1
31 V1	13 V3
30 V1	12 V3
29 V2	11 V2
28 V3	10 V1

Rep3   Rep3

Rep3

Rep2   Rep2

Rep2

Rep1

Rep1

JK

Road

V1: Dular   V2: Anjali   V3: N22

52
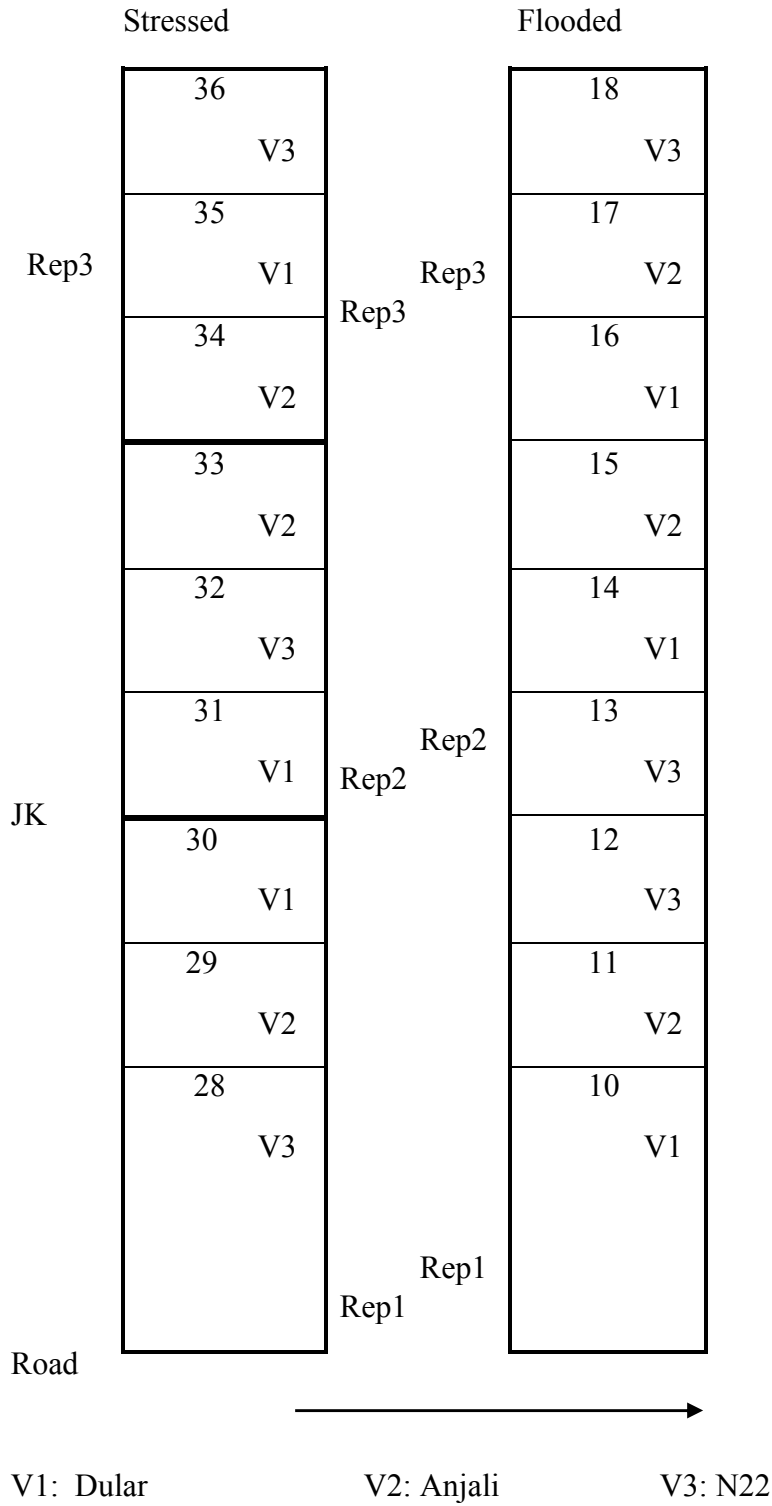
Table A1: Adjustments made to the Experiment Data prior to the Regression Analysis

Data Set	Computation /Measurement	Data Gap	Adjustment
Canopy Temperature	Measured with MINCER	In 2013, only control measurement for N22	Used 2013, N22 control canopy temperature data for all three varieties
		Anjali Stress and Dular Stress 2013, 4/24/2013 only data from 10 am	Used data from N22 stress 2013 for 4/24/2013, until 10 am
		No control data for Dular Control 2012	Used average of Anjali & N22
		No control data for Anjali Control 2014	Used average of Dular & N22
Soil Water Potential	Measured with Tensiometer	In 2012, only one measurement per variety (stress)	Used data from Plot with measurement in the same replication
		No measurements for flooded plots	Assume they always have 0 kPa, because they are saturated
		No tensiometer readings for the 4/17/2012, 4/18/2012, 4/19/2012, 4/22/2012, 4/24/2012, 4/25/2012, 4/28/2012, 4/29/2012, 5/2/2012, 5/4/2012, 4/24/2013	Interpolated between the measurement of previous and the following day of the missing day

Table A2: Ranking of R-squares with Drought Thresholds (Spikelet Fertility regressed on log of Number of Days over Threshold)

Obs.	R^2	Threshold (kPa)	R^2	Threshold (kPa)
1	0.681	12	0.562	35
2	0.667	13	0.562	30
3	0.660	14	0.562	31
4	0.659	15	0.557	32
5	0.649	16	0.557	33
6	0.649	17	0.551	36
7	0.637	18	0.551	37
8	0.637	19	0.544	41
9	0.626	20	0.540	40
10	0.626	21	0.538	38
11	0.618	22	0.538	39
12	0.618	23	0.538	43
13	0.613	26	0.533	42
14	0.612	25	0.516	44
15	0.611	24	0.516	45
16	0.609	27	0.516	46
17	0.571	28	0.516	47
18	0.571	29	0.496	48
19	0.562	34	0.466	49
20			0.422	50
21			0.339	52
22			0.336	53

Table A3: Ranking of R-squares with Canopy Temperature Thresholds (Spikelet Fertility regressed on Number of Hours over Threshold)

Obs	R^2	Threshold (ºC)
1	0.702	32
2	0.698	33
3	0.692	31
4	0.687	34
5	0.685	29
6	0.683	30
7	0.682	39
8	0.682	40
9	0.681	38
10	0.679	35
11	0.678	28
12	0.678	27
13	0.677	37
14	0.677	36
15	0.677	26

Table A4: Top Ten Highest R-squares with Canopy Temperature and Drought Thresholds (Spikelet Fertility regressed on Number of Hours over Temperature Threshold and Log of Number of Days over kPa Threshold)

R^2	ºC	kPa
0.7105	33	12
0.7082	32	12
0.7066	32	51
0.7050	32	43
0.7048	32	52
0.7047	32	44
0.7047	32	45
0.7047	32	46
0.7047	32	47
0.7045	33	44

Table A5: Results of Regression Model on Canopy Temperature - Logarithmic Ambient Temperature

Variable	Parameter Estimate
Intercept	-73.52**
	(1.125)
ambientlog	30.23**
	(0.324)
kpaabs	0.030**
	(0.00086)
Anjali	0.137**
	(0.041)
Dular	-0.043
	(0.041)
Number of observations	8767
Dependent Mean	31.88
R-Square	0.5334
Adj R-Sq	0.5332

Table A6: Results of Regression Model on Canopy Temperature - Reciprocal Ambient Temperature

Variable	Parameter Estimate
Intercept	60.44**
	(0.315)
ambientrecip	-932.04**
	(10.06)
kpaabs	0.0294**
	(0.00086)
Anjali	0.13680**
	(0.041)
Dular	-0.04315
	(0.0416)
Number of observations	8767
Dependent Mean	31.88226
R-Square	0.5303
Adj R-Sq	0.5301

Table A7: Global warming Scenarios and their Impact on Hours spent above 33˚C Canopy Temperature

Var.	Stress	Scenario	Hours above 33℃	Var.	Stress	Scenario	Hours above 33℃
Anjali	0	Estimated	18.00	N22	0	Estimated	18.00
		0.25℃ increase	20.67			0.25℃ increase	19.89
		0.50 ℃ increase	23.50			0.50 ℃ increase	23.61
		0.75 ℃ increase	26.94			0.75 ℃ increase	27.00
		1.0 ℃ increase	31.28			1.0 ℃ increase	31.22
		1.25 ℃ increase	35.22			1.25 ℃ increase	35.39
		1.50 ℃ increase	38.39			1.50 ℃ increase	38.61
		1.75 ℃ increase	42.44			1.75 ℃ increase	43.11
		2.00 ℃ increase	46.89			2.00 ℃ increase	47.11
	1	Estimated	30.67		1	Estimated	29.61
		0.25℃ increase	36.33			0.25℃ increase	34.22
		0.50 ℃ increase	40.50			0.50 ℃ increase	37.94
		0.75 ℃ increase	43.89			0.75 ℃ increase	41.50
		1.0 ℃ increase	47.72			1.0 ℃ increase	44.61
		1.25 ℃ increase	52.22			1.25 ℃ increase	47.72
		1.50 ℃ increase	56.50			1.50 ℃ increase	51.56
		1.75 ℃ increase	59.44			1.75 ℃ increase	54.61
		2.00 ℃ increase	63.11			2.00 ℃ increase	57.61
Dular	0	Estimated	18.06				
		0.25℃ increase	19.44				
		0.50 ℃ increase	21.89				
		0.75 ℃ increase	24.89				
		1.0 ℃ increase	28.67				
		1.25 ℃ increase	32.44				
		1.50 ℃ increase	36.22				
		1.75 ℃ increase	39.83				
		2.00 ℃ increase	43.83				
	1	Estimated	29.11				
		0.25℃ increase	32.67				
		0.50 ℃ increase	36.06				
		0.75 ℃ increase	39.22				
		1.0 ℃ increase	43.39				
		1.25 ℃ increase	46.72				
		1.50 ℃ increase	50.17				
		1.75 ℃ increase	53.83				
		2.00 ℃ increase	58.39				

Table A8: Global Warming Scenarios and their Impact on Hours spent above 12 kPa

Var.	Variable	Days above 12 kPa	Var.	Variable	Days above 12 kPa
Anjali	Estimated	9.44	N22	Estimated	9.89
	0.25℃ increase	10.11		0.25℃ increase	10.67
	0.50℃ increase	10.33		0.50℃ increase	10.78
	0.75℃ increase	11.89		0.75℃ increase	12.11
	1.00℃ increase	11.89		1.00℃ increase	12.11
	1.25°C increase	11.89		1.25℃ increase	12.22
	1.50℃ increase	12.44		1.50℃ increase	12.78
	1.75℃ increase	12.44		1.75℃ increase	12.78
	2.00℃ increase	12.56		2.00℃ increase	12.89
Dular	Estimated	10.56			
	0.25℃ increase	11			
	0.50℃ increase	11.22			
	0.75℃ increase	11.67			
	1.00℃ increase	11.67			
	1.25℃ increase	11.78			
	1.50℃ increase	12.33			
	1.75℃ increase	12.33			
	2.00℃ increase	12.44			