Evaluating the impact of different thermal growing conditions and ozone on PBW550 wheat (*Triticum Aestivum)* cultivar

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Certificate of Examination

This is to certify that the dissertation titled "Evaluating the impact of different thermal growing conditions and ozone on PBW550 wheat (Triticum Aestivum) cultivar" submitted by Ms. Aakanksha Meena (Reg. No. MS15161) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Baerbel. Sinha **Dr. Anoop Ambili** **Dr. Raju Attada**

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Dated: 4, May, 2020

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Baerbel Sinha at the Indian Institute of Science Education and Research Mohali.
This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Aakanksha Meena
(Candidate)

Dated: 4-5-2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Baerbel Sinha
(Supervisor)

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- ***Aakanksha Meena***

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Notation (abbreviations)

OTCs Open Top Chamber studies

FACE Free air enrichment setup

AOT40 accumulated hourly Ozone exposure above a cut-off threshold concentration of 40 ppb during daylight hours (7 am to 7 pm or 8 am to 8 pm)

M7 7 hour average ozone from 9 am to 4 pm

CONTENTS

Abstract

In this study we have explored the phenology of the PWD-550 cultivars of *triticum aestivum* during wheat growing season from November 2018 to April 2019. Seeds of the cultivar PWD-550 were acquired from breeders.

To study our objective, wheat was grown in three different plots with variations in growth conditions and time periods. Sowing was done on November 1st for plot 1, November 15th and December 1st for plot 2 and 3 respectively. Different parameters such as grain yield per plant, 1000 grain weight, number of effective tillers, head length, number of shrivelled grains, temperature, plant phenology, and time period taken for reaching specific growth stages were analysed *triticum aestivum* cultivar PBW550. These analyses were performed to observe their effects on the yield of wheat cultivars.

The PBW550 cultivars show a similar thermal sum to reach flag leaf stage for plot 1 and plot 2 but a significantly lower thermal sum for plot 3. This is despite the fact that the temperature at which the thermal sum is conventionally capped was not reached before this growth stage. Plants on plot 3 did not reach tillering growth stage before temperatures, dropped as winter started early.

We observe, that there is decrease in active number of tiller in plot 3 in compare to plot-1 and plot-2. The decrease in the number of active tillers correlates strongest (R=0.99) with the number of days with optimum temperatures for photosynthesis before the plant reaches the flag leaf stage. Plot 3 also shows shorter heads compared to plot-1 and plot-2. This head length correlates best with the first day of heat stress experience by the plant expressed in days after sowing (R=1) and the number of days with optimum temperatures for photosynthesis before the plant reaches the flag leaf stage (r=0.99). Head length is strongly anti-correlated with heat stress between anthesis and maturity (R=-0.98) and the ozone exposure (M7) from anthesis to maturity (R=-0.99). Anti-correlation means the stronger the stressor the shorter head length. The number of normalized to the head length is perfectly anticorrelated with the ozone exposure from flag leaf stage to anthesis (R=-1) indicating that ozone exposure just before the anthlers become visibly could most significantly reduce grain number. The 1000 grain weight is weakly correlated with the number of days with optimum temperatures for photosynthesis before the plant reaches

the flag leaf stage (R=0.67). This data seems to suggest that ozone exposure and heat stress after anthesis were not the main drivers of yield loss in late sown wheat.

Chapter 1

Introduction

1.1 Literature review:

Wheat is an important food crop that comes third after corn (popular in Africa and South America) and rice (the main stable of Asia) in terms of providing calories to the global population. Annually over 765 million tons of wheat are produced (FAO 2019).

Globally, heat stress (Asseng et al. 2015, Zampieri et al. 2017) and elevated tropospheric ozone level (Mills et al. 2018a, b) are the largest challenges in increasing wheat yields losses.

It has been estimated that in Punjab and Haryana elevated tropospheric ozone mixing ratios alone cause relative yield losses of 27-41% (Sinha et al. 2015).

1.2 Effects on the wheat yield losses from the tropospheric ozone over India:

Among the essential food crops in the world wheat and potato are considered to be very sensitive to O3, while rice and corn are somewhat more resilient to ozone stress. (G. Mills et al. 2007)(Feng and Kobayashi 2009) Numerous experimental studies most of them conducted in OTCs or FACE setups, where a wheat cultivar is grown on the same field, with the same inputs in terms of fertilizer and water and weather but exposed to different ozone levels in different treatment, have quantified by how much elevated [O3] reduces the yield of wheat.

Table 1: Wheat production losses over India reported by different sources for ozone in ppb (M7) and ppb h (AOT40)

Source	Year of estimate	RYL [%]	CPL [10^6 t]	ECL [10^9 \$]	O_3 data	metrics	Exposure yield equation	Source of equation
Avnery et al. 2011	2000	8.2	6	0.75	Model MOZART v2.4 IPCC SRES emissions	M7	$RY = \frac{e^{-\left(\frac{M7}{137}\right)^{2.34}}}{e^{-\left(\frac{25}{137}\right)^{2.34}}}$	Lesser et al. 1990 Winter wheat
		26.7	26	3.75		AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007
Ghude et al.2014	2005	5	3.5	0.62	WRF-Chem v3.2.2 with MOSCART & average of 6 inventories	AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007
R.Van Dingenen et al.2009	2000	13.2	11.6-29.1	1.7-4.3	TM5 with EDGAR3.2 inventory	M7	$RY = 1 - \frac{e^{-\left(\frac{M7}{137}\right)^{2.34}}}{e^{-\left(\frac{25}{137}\right)^{2.34}}}$	Lesser et al. 1990
		27.6				AOT-40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al.2007
Shyam Lal et al.2017	2011-14	4.2	4		Observations	M7	$RY = \frac{e^{-\left(\frac{M7}{137}\right)^{2.34}}}{e^{-\left(\frac{25}{137}\right)^{2.34}}}$	Lesser et al. 1990
		15	14.17			AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007
Mills et al.2018		12.2	13		EMEP MSC-W v4.8	POD_3IAM		
		21.5	21			AOT40		
S.B Debaje et al. 2014	2002-07	5-11	8.6	2.6	Observations	M7	$RY = \frac{e^{-\left(\frac{M7}{137}\right)^{2.34}}}{e^{-\left(\frac{25}{137}\right)^{2.34}}}$	Lesser et al. 1990
			28.8		Observations	AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007

Long-term exposure to elevated O_3 levels damages not only natural vegetation, it also results in considerable reduction in crop yields and economic damages (Greskowiak

2014). In developing countries like India air pollution continues to rise due to the rapid economic growth. Increases in ozone-precursor NOx are mainly driven by power generation and the transport sector, while inefficient combustion in the form of crop residue burning, open waste burning and domestic fuel usage drives anthropogenic VOC emissions (Greskowiak 2014)(Horowitz 2006) (Horowitz et al. 2003). However, one must not forget that the biosphere contributes by far more reactive VOCs to the troposphere than any anthropogenic activity. In India trees have been part of both the urban and rural landscape even in intensely farmed regions and hence increases in NOx emissions typically result in elevated ozone mixing ratios.

Table 2 shows the estimates of wheat production losses over India reported by different sources in the literature in the past decade. Different studies report relative yield losses ranging from 5 to 28% resulting in wheat production losses ranging from 3.5 to 29 million tonnes of wheat and economic damages ranging from 0.5 to 4 billion dollar. It is interesting to note that all but one study (Gina Mills et al. 2018; Sharps et al. n.d.,)(Pleijel et al. 1991) use the same crop exposure to yield loss relationships for M7 (Lesser et al. 1990) and AOT40 (G. Mills et al. 2007). In all studies the M7 based relationship leads to lower crop production loss estimates, while the AOT40 based relationship results in larger loss estimates when the same ozone data is used as input into the calculation. The POD3 based relationship lies somewhere in between the two extremes, however, this global study of crop damage estimates with POD3 used one singular set of environmental response functions to predict the stomatal opening of wheat plants grown all around the globe. Such a uniform approach assumes all different wheat cultivars in the world are equally sensitive to e.g. high temperatures, low temperatures, lack of soil moisture and dry air, irrespective of whether the breeding happened in Rajasthan, India one of the hottest places to grow wheat or in Sweden, one of the coldest countries where wheat is grown. This would be highly unlikely in the real world; hence more research characterizing the environmental response functions of different cultivars are clearly required and global modelling estimates will likely have to use different environmental response functions for cultivars grown in different climate zones to yield robust results.

Table 2: Wheat production losses for individual states of India reported by different sources for ozone in ppb (M7) and ppb h (AOT40)

Source	Year of estimate	Location	RYL [%]	CPL [10^6 t]	ECL [10^6usd]	O_3 data	metrics	Exposure yield equation	Source of equation
B.Sinha et al.2015	2012-14	Punjab	18% to 27%			Observations IISER Mohali station January to March	AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007
		Haryana							
		Punjab	27% to 41%	8.97	1514			$RY = -0.000026 \times AOT40 + 1.01$	B. Sinha et al.2015
		Haryana		6.58	1111				
Debaje 2009	2002-07	Punjab	10.6	2.04	290	Observations from literature review for the months January to March	M7	$RY = 1 - \frac{e^{-\left(\frac{M7}{137}\right)^{2.34}}}{e^{-\left(\frac{25}{137}\right)^{2.34}}}$	Lesser et al. 1990
		Haryana	10.6	1.24	176				
		Rajasthan	10.6	.85	121				
		Uttar Pradesh	8.8	2.6	375				
		Uttarakhand	9.2	.09	13				
		Punjab	29.8	7.3	1037		AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007
		Haryana	29.8	3.6	511				
		Rajasthan	29.8	3.06	435				
		Uttar Pradesh	25.6	9.4	1337				
		Uttarakhand	27.2	.31	44				
Ghude et al. 2014	2005	Punjab	0.8%	0.15		Modelled ozone for December to February	AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007
		Haryana	1%	0.1					
		Rajasthan	4%	0.25					
		Uttar Pradesh	3%	0.6					
		Uttarakhand	5%	0.05					
Shyam Lal et al.2017	2011-14	North India (Uttar Pradesh, Punjab, Haryana, Rajasthan and Uttarakhand)	4%	2.76		Observations , ISRO ATCTM sites January to March	M7	$RY = 1 - \frac{e^{-\left(\frac{M7}{137}\right)^{2.34}}}{e^{-\left(\frac{25}{137}\right)^{2.34}}}$	Lesser et al. 1990
			13%	8.97			AOT40	$RY = -0.0000161 \times AOT40 + 0.99$	Mills et al. 2007

Overall, therefore, the data in Table 1 and 2 suggest that the largest range of uncertainty is caused by lack of studies evaluating how sensitive wheat is to crop production losses caused by ozone. This knowledge gap is one that I seek to address in my current work.

Another thing worth noting is that estimates of the ozone exposure can vary significantly depending on the Model and emission inventory used to come up with the estimate. Estimates using the MOZART v2.4 model with an emission inventory by the IPCC SRES. (Avnery et al. 2011) and estimates with the TM5 model initialized with EDGAR3.2 inventory (Van Dingenen et al. 2009) almost agree with observation based estimates which evaluated CPCB data state wise. (Debaje 2014) A much lower estimated ozone exposure comes from a study using the WRF-CHEMv3.2.2 initialized with an average of six different emission inventories(Greskowiak 2014) and an observational study averaging the ozone exposure data zone wise before applying it to the crop production instead of processing the data state-wide (Lal et al. 2017).

Table 2 shows wheat production losses for individual states of North India reported by different sources. Since our observations are from the state of Punjab, I will focus primarily on the values reported by different authors for the North Indian states in my literature analysis. It can be seen from the table that studies by different authors report very different relative yield losses for the same start. For example, for Punjab the RYL values range from ~0.8% of the potential yield estimated by Ghude et al. (2014) to 41% by Sinha et al. (2015) with resulting in wheat production losses ranging from 0.15 to 8.97 million tonnes of wheat in the state of Punjab. The literature review reveals that the lowest estimate originates from model studies (Greskowiak 2014) while the observation based studies reveal higher estimates. This can partially be explained by the fact that the modelling studies looked at the ozone exposure during the Months of December to February and did not include the month of March, which in Punjab is most crucial to the grain filling of the wheat. A modelling analysis with the right ozone accumulation window may thus result in a more robust estimate. It is interesting to note that all but one study (Sinha et al. 2015). use the same crop exposure to yield loss relationships for M7 (Lesser et al. 1990) and AOT40 (G. Mills et al. 2007). In all except the Sinha et al. (2015) study the M7 based relationship leads to lower crop production loss estimates, while the AOT40 based relationship results in larger loss estimates when the same ozone data is used as input into the calculation. Sinha et al. (2015) developed new equations by fitting open top and plant chamber studies conducted with South Asian cultivars at Banares Hindu University in India, in Lahore, Pakistan and in Japan to generate a new exposure yield relationship that reflects how sensitive South Asian wheat cultivars are to ozone. That new relationship doubles the estimated yield loss when the same ozone data is used as input in comparison to the old relationship which assumes all wheat cultivars to be equally sensitive to high ozone exposure. For the two other observation based studies.(Debaje 2014) (Lal et al. 2017) The exposure yield relationship used is identical and so is the ozone sensitive window (January to March), however, the two studies used different stations to estimate ozone exposure. Debaje et al. (2014) used observations from Delhi to estimate exposure in entire Punjab, Haryana and Rajasthan, while Nainital, a mountain top station was taken as representative of Uttarakhand and Agra as representative for Uttar Pradesh. Lal et al. (2017) averaged the data of Kullu, Mohali, Pantnagar, Delhi, Agra and Kanpur to estimate the exposure for the entire Northern Zone. Sinha et al. (2015) on the other hand treated the observations at Mohali as representative of the states of Punjab and Haryana and estimated the highest ozone exposure for these states. Overall, therefore, the data in Table 2 suggest that the largest range of uncertainty is caused by very limited published ozone observational data resulting in large uncertainties of the yield loss estimate. In addition, many agricultural experts in India are highly critical of any work indicating that ozone might be responsible for yield losses as they hold high temperatures, not ozone responsible for the large yield suppression seen in late sown wheat. Therefore, in my current work, I intend to look at both ozone and temperature stress experienced by the same cultivar PBW550 and attempt to better understand the impact of both factors on wheat yield.

1.3 Effect of heat stress on Indian wheat yield losses:

Table 3: Estimated wheat production losses due to heat stress in India reported by different sources

Source	Year of estimate	Location	RYL	Source of Temperature data	Additional attributes
Lobell et al. 2012	2000-2009	IGP	15-20% for 2°C Temperature increase	GSOD http://www.ncdc.noaa.gov/cgi-bin/res40.pl?page=gsod.html	8-9 days of shortening of growing seasons
Lesk et al. 2016	1964-2007	Global	5%	EM-DAT, http:// www.emdat.be/database,	Additionally ,5% reduction in harvested area
Texteira et al. 2013	1971-2000	Global	No impact in India	IPPC historical data	
Zaveri & Lobell 2019	1970-2010v	India	No impact on irrigated wheat, 5%-12% loss due to heat waves under rain fed conditions	Indian Meteorological Department, gridded data	
Rao et al. 2015	1980-2013	India	7% for a 1 °C Temperature increase	CRU monthly surface temperature data, University of East Anglia, UK	

GSOD stands for Global Summary of the Day dataset

EM-DAT stands for Emergency Events Database

CRU stands for Climate Research Unit

Table 3 shows estimates of wheat production losses due to heat stress by different authors. A large variety in estimates can be seen that can be broadly divided into two schools of thought. One approach is to rely on historical yield variations and weather data and use extreme events such as the 2010 heat wave to estimate the yield loss due to heat stress (Lesk et al. 2016, Rao et al. 2015). Along somewhat similar line the difference in yield and performance seen between the hottest and coldest years in a time series as indicative of the losses induced by heat (Lobell et al. 2013). On the other hand, correlating yields and temperatures of gridded datasets while accounting for variation in the availability of irrigation resulted in estimates that held weather in particular storm during harvest times to be responsible for maximum yield variations of irrigated wheat while heat stress explained yield variation in rain fed wheat (Zaver & Lobel 2019). The Indian agricultural community typically blames the yield and performance differences between timely sown wheat for cultivars sown at the same location at different times entirely on the difference in heat exposure. Only few studies have evaluated the impact of heat stress on yield and performance using controlled experiments in which heat exposure happens on specified days and a control is kept.

Table 4: Differences between late sown and timely sown PBW550 reported by different authors

Source	Sowing	Days to maturity	Effective tillers per plant	Grains per spike	1000 grain weight	Grain yield per plant
Kant et al. 2014	Average	123.4	15.54	43.06	38.6	36.72
Bala et al. 2010	Normal sown		22.7±1.5	56.0±2.3	43.7±2.4	
	Late sown		31.3±1.6	50.3±1.9	33.8±1.6	
Singh et al. 2018	Late sown	128.5		29.64	36.8	46.15
	Very late sown	111		30.60	34.1	39.33
Coventry et al. 2011	Early				40-42.8	
	Normal				39.2-39.9	
	Late				36.3-38.7	

High temperatures affect wheat crop growth through several mechanisms at different stages of production. Grain yields depends on two major factor- change in grain number and change in grain size. Grain number is determined at the time when reproductive growth starts, approximately 30 days before flowering (Lobell, Sibley, and Ivan Ortiz-Monasterio 2012), and during flowering due to heat induced flower sterility during flowering (Aiqing et al. 2018) , whereas grain size is determined during grain filling (States, Mauzerall, and Wang 2001). Heat stress during the onset of reproductive growth can be identified by short heads. Flower sterility can be identified by completely unfilled grains. In such cases, while the head is long the number of grains is not proportional to the head length. Wheat is sensitive to heat shock induced.

anthesis (within the first 10 days) can also lead to abortion of up to 20% of the grains. High temperature during grain filling can be identified by shrivelled grains with a low weight. In several countries, the end of the winter season results in warmer climate,

the most pronounced effect of heating is to shorten the length of grain filling.

Hot temperatures at high temperatures (above 300 C) slow grain filling speeds, in sections as the photosynthetic components of the leaf can be harmed at extreme high temperatures , resulting in an acceleration of senescence.(Zhao et al. 2007). In general, many studies have found a correlation of the length of the grain filling period with the average yield

(Ritchie and Nesmith 2015). Exposing wheat plant to extreme high temperature during grain filling results in reduction in grain size. Cellular damage in grains due to high temperature results in shrivelled grains. Cellular damage in grains is maximum when heat stress is maximum around booting stage to 8 days after flowering. Events of heat stress during early grain filling reduces duration of grain filling that result in smaller size grains. High temperature exponentially increases the rate of development that result in breakdown of asymptotic relationship of duration of grain filling with temperature. Therefore, we must find the maximum temperature not just the daily average temperature.

According to a report decrease in individual grain yield and individual grain weight are due to increased drought severity and high temperature. Drought conditions affect plant very badly as it affects relative humidity and canopy temperature in crops. Heat stress during grain filling will affect 1000 grain weight.

1.4 Impact of tropospheric ozone on wheat – a history of ozone yield loss relationship established

Surface ozone (O3) is the key smog product formed by the catalytic reactions of nitrogen oxides (NO_X), carbon monoxide (CO) and volatile organic compounds (VOCs) in the troposphere when sunlight is present.

CO and VOC are oxidized by OH (hydroxyl radical) forms RO_2 and (HO_2) Peroxyl Radical that further reacts with (NO) to produce NO_2 and then NO_2 is photolyzed by sunlight and forms NO and O Radical and this O radical reacts with oxygen to produce Ozone(O_3).

$$\text{VOCs} + \text{OH}\cdot \rightarrow RO_2 + HO_2$$

$$HO_2 + NO \rightarrow NO_2$$

$$NO_2 + hv \rightarrow NO + O\cdot$$

$$O\cdot + O_2 \rightarrow O_3$$

CO, VOC_S and NO_X are the major precursors of ozone. They are formed by various anthropogenic activity like industrial emissions, chemical solvent and vehicle exhaust.

Surface ozone exposure for long-time period lead to declines in crop yield, reducing growth, increased senescence and reduced reproductive capability in agricultural crops and plants and it has scandalous effect on human health. This has been proved by various field experiments in different part of the world(States, Mauzerall, and Wang 2001). O3 enters the leaf through the stomata and creates reactive oxygen stress inside the plant (Avnery et al. 2011). This stress can negatively affect yields.

Investigating how much yield is lost due to ozone exposure alone requires carefully set up experiments where one set of plants is exposed to elevated ozone levels, while other plants are exposed to clean filtered air. All plants need to be grown in the same soil in the same field and need to be watered and fertilized equally so that all differences in yield can only be explained by the differences in ozone exposure alone, while any confounding factors such as unfavourable weather in a particular growth stage (e.g. heat stress) can be ruled out. The earliest such experiments were conducted through the Open top chamber programmes (OTC) of America and Europe. These experiments typically involved clean air controls chambers and several chambers where plants were exposed to different ozone levels between average daytime concentrations of 30 ppb and 150 ppb. These experiments allow to construct a dose response function. With time it became clear that plants can handle the exposure to low ozone mixing ratio as they have a certain detoxifying capacity which varies from crop to crop. Only the exposure over a critical threshold appeared to impact the plant yield. With this discovery came two new metrics to measure the ozone exposure. A sigmoid function W90 which attaches weights to the hourly concentrations while summing them in the US and the AOT40 metric in Europe. These two metrics have the unit ppb h or ppm h as they are summed over the entire critical exposure period. However, when different regions within Europe started to work collaboratively on the effect of ozone on rain fed wheat yields they started to face the challenge that data from Southern Europe (Italy & Greece) and Northern Europe (Sweden, UK and Poland) was difficult to reconcile. Cultivars in Southern Europe appeared to be more resilient to elevated ozone mixing ratios. However, researchers realized that these cultivars had a tendency to close their stomata during midday when the air is dry. This happens to be the time when the ozone is highest. Hence it became clear that not the ozone in the air but the ozone that enters the plant by going into the stomata damages the plant. After this point efforts focussed on determining the phytotoxic flux dose with models that simulate the stomatal aperture and the ozone uptake of the plant.

In addition, it was also found that chambers can have an impact on the outcome of dose-response function studies. They become even more challenging to manage when researchers have to enter the chamber to measure the stomatal conductance of plants. As researchers had started to study the impact of elevated CO2 mixing ratios on plants using free air enrichment setups these setups were adapted to enable free-air Ozone elevation (O3-FACE) exposure studies. This approach makes it easier to eliminate confounding effects, but at the same time makes the experiment much more complex and expensive to conduct as the amount of ozone added has to be constantly adapted as a function of wind speed and wind direction. Face experiments generally lack clean air control but rather include a set of 3-4 plots where the ozone exposure is half between double of the ambient ozone. In O3-FACE the exposure to the ozone was higher in OTC experiments. It has been proposed that the difference could be due to factors that impact the stomatal conductance. For example, chambers have strong edge effects due to the nozzels that bring in the air. Often the air added is also dry. Chambers tend to heat up which depending on the ambient temperature can have a positive or negative impact on yield. In addition, it is harder to maintain equal input application in a set of different chambers than it is in an open field (Feng et al. 2018).

1.5 The Novel of the study:

All these studies have strong evidence of wheat being sensitive to yield loss caused by ozone. None of them had experimented any relay seeding experiment and looked at the yield and the phenology at different stages of wheat plant (as yield losses happens at different stages of wheat growing plant) and none of study shows any leaf porometer data which can explain wheat plant phenology and also none of them had studied cultivar PBW-550 which is most suited for cultivation in the plains of Punjab, Uttar Pradesh, Uttarakhand which yields 45-50 quintals per hectare, under optimal conditions. All these studies have such a uniform approach assumes all different wheat cultivars in the world are equally sensitive to e.g. high temperatures, low temperatures, lack of soil moisture and dry air, irrespective of whether the breeding happened in Rajasthan, India or in Sweden, one of the coldest countries where wheat is grown. Keeping all these things in mind I performed the experiment which shows delay sowing shifts the grain filling during high temperature. By virtue of different sowing times cultivars are also exposed to different ambient ozone levels during specific periods in their development.

Chapter 2

Materials and Methods

2.1 Site description

Figure 1 displays the site of Mohali in India and the wheat field site in the IISER Mohali campus.

Figure 1: Location of Mohali in the India, IISER campus and location of wheat field in the campus.

This wheat field experiment was performed in the south-eastern corner of residentiary campus of IISER Mohali which is in Mohali and Mohali is one of the districts of Punjab and Punjab is in the north-western part of India. Mohali lies in Indo-Gangetic alluvial plains. Its latitude and longitude are 30^0.40 N and 72^0.73E. Soil of this land contains loam to slit clay and is extremely rich in nutrients that results in fertile land that fit for wheat cultivation.

Wheat production in Punjab (2018) was around 178 metric tonnes and yield 50.09 quintals per hectare. Food and Supplies Department reveal that wheat procure is 9.22 lakh metric tonnes. Agriculture department is assuming 180 lakh tonnes of wheat production in 2019. (Department of Food, Civil Supplies and Consumer Affairs, Govt. of Punjab)

Mohali's climate is warm and temperate, and it lies 324m above sea level. There is more rainfall in winter compare to summer. The temperature in Mohali in winter is around 7^0 to 15^0 while in summer is 35^0 to 42^0C and the usual rainfall is 940 mm.

2.2 Agricultural methodologies

Sowing was done on 1^{st} of November(plot-1),15^{th} of November (plot-2) and 1^{st} of December (plot-3) in 2018.

Each plot includes Row-A, Row-B, Row-C (ensures that the amount of error is minimised).

In each Row 60 wheat seed were sown and the weaker sampling was removed when both were germinated.

They were harvested in the late spring (April-May).

Random sampling of plants was done and yield data (number of effective tillers, length of head, number of grains per head, average weight of grain per head, and 1000 grain weight) was analysed.

2.3 Statistical Methodologies

Statistical techniques are mathematical formulae that are used in statistical assessment of data from the analysis. The application of statistical methods derives data from examination data an provides various ways of assessing the quality of study outputs.

Arithmetic Mean

When there is a huge set of data, arithmetic mean calculates average of data. That average is called Arithmetic Mean. Mean is the measure of central tendency. In easier words, Mean is equivalent to addition of all the values in a data divided by number of values that had added. It can be denoted as follows,

$$\mu_x = \frac{x_1 + x_2 + x_3 \ldots . x_n}{n} = \frac{\sum_{i=1}^{n} x_i}{n}$$

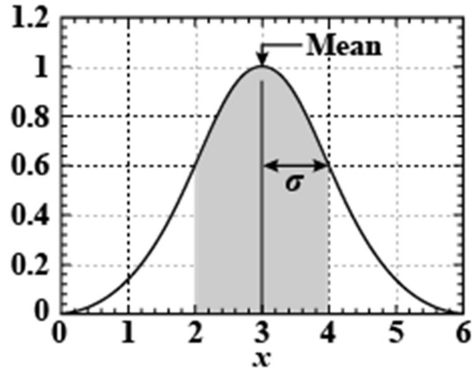

Figure 2: Mean and 1σ range

Standard deviation

Standard deviation is a brief measure of differences of mean from each observation. Sum of these differences is equal to zero. Therefore, Square of these differences are added up and divided by number of observations minus one to get mean and then square root is taken. (we use number of observations minus one instead of observations itself because " number degrees of freedom" must be used. In these situations, they are one less than the total.)

$$\sigma_x = \sqrt{\frac{\sum (X - \tilde{X})^2}{n - 1}}$$

Standard error of mean

When we sample a population to determine the mean the uncertainty of that mean depends on the number of samples drawn. When only one random sample is drawn we could be lucky and the value could be very close to the mean or we could be unlucky and the value could be very far from the mean. There is a 32% probability that a randomly drawn sample could be out of the 1 sigma range and a 5% chance that a single sample could be outside the 2 sigma range. In statistics a flawed conclusion arrived at based on a very small sample number is called a “small numbers fallacy”. However, when we draw a very large number of samples the uncertainty of the mean that we calculate reduces. The error of the mean expresses how likely it is that the calculated mean is close to the true mean as a function of the number of samples drawn. It derives from the central limit theorem.

$$\sigma_{\mu} = \frac{\sigma}{\sqrt{n}}$$

95% confidence interval:

A confidence interval is a range of numbers that we believe contains the estimated population parameter, in our case the mean. For any statistical parameter that folloes a normal distribution, 95% of randomly picked samples from the population will be within two standard deviation of the mean. However, as stated above, thanks to the central limit theorem our estimate of the mean itself increases with an increase in the number of samples drawn. Moreover, repeated random sampling makes the estimated parameter (the mean) follow a normal distribution even if the parameter in the original population is not normally distributed.

If the sample size is sufficiently large to avoid the small number fallacy, we can use the following formula to determine a 95% confidence interval:

$$\bar{x} = \mu \pm 1.96\frac{S_{\mu}}{\sqrt{n}}$$

For example: A sample is taken, and it is determined that the mean of the sample is 45 and the standard deviation is 6.5. Its 95% confidence interval will be if sample size is 40

95% CI = $45 \pm 1.96 \times \frac{6.5}{\sqrt{40}}$ = 45 ± 2.01 = (42.99, 47.01)

Let us take another example of a shopkeeper who says their latest LED television would last for 60000 hours. A research group on the market is choosing to test the argument. The party chooses 50 LEDs at random for research. The data from this study shows that a television has a mean lifetime of 57000 hours, with a standard deviation of 1200 hours.

95%CI = $57000 \pm 1.96 \times \frac{1200}{\sqrt{50}} = 57000 \pm 333 = (56667\,, 57333)$

95% does not support the shopkeeper as this range does not include 60000.

For example, there are students in a class, ,each student collects a sample of three measurement, the 95% confidence is calculated by each student, what we get on average 5 student out of 100 will calculate confidence interval that does not contain true population mean. As already explained, bigger the sample size is tighter the error bar will be but still on average 5% of those will miss true population mean. So, in general statisticians have said 5% of α = 0.05 is a nice happy median for representing error bars and this is how we report 95% confidence interval.
95% confidence interval is given by:

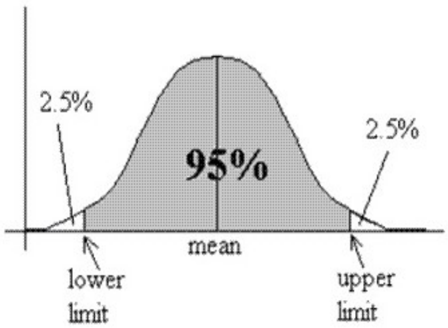

Figure 3: Mean and 2σ range. We used the 2σ range to calculate errors in this study

I had calculated standard error of mean; this will infer how well the mean estimated from a limited number of samples matches the true population mean. The error of the mean is impacted by the standard deviation and the sample size.

Weighted Average:

Weighted average is the mean of each value when it is multiplied by some weight.

$$\bar{x} = \frac{\sum w_i x_i}{\sum w_i}$$

Here is an example, let's say that we have a group of 5 kids each weigh 50 kg, and we have a man whose weight is 100 kg. Regular average weight of kids and man would be 75kg. So, the average weight is right in the middle of those two weights. It feels like we should able to consider the fact that there are many more kids who weight a lot less than the one man. This is where the idea of the weighted average comes into play. Weighted average considers how many things in each group we have. Thus, weighted average for this group of people would take the fact that there are five kids and they weigh 50 kg each and one man who weigh 100 kg. Therefore, weighted average would be:

$$\frac{50 + 50 + 50 + 50 + 50 + 100}{6} = 58.33$$

Now if we notice, this weighted average 58.33 is much less than 75(regular average) and 58.33 is a lot closer to the weight of the kids. That is because of the weighted average as it pulls down the average closest to the whatever we have most.

For weighted average we can have several different things more than two we can have a bunch of them. In this case, we are having three different kinds of cars in a parking lot-small sized which weigh 1200kg, mid-sized who weigh 1600kg and large sized cars whose weigh 2000kg. There are 32 small-sized cars, 5 mid-sized cars and 7 large sized cars. So, weighted average for this case will be:

$$\frac{32 \times 1200 + 5 \times 1600 + 7 \times 2000}{44} = 1372.72$$

This weighted average is closest to the small sized cars and that makes sense as small sized cars are much more than mid-sized or large sized cars. Regular average for these cars will be 1600 which lie exact at middle but once again weighted average gives us a number that takes into account how many of each type of these cars are there and gives a number that is much closer to the type of car which is the most.

In this wheat field experiment I had calculated weighted mean

$$\bar{x} = \frac{\sum w_i x_i}{\sum w_i}$$

where $w_i = \frac{1}{\sigma^2}$

$$x = \frac{\frac{1}{\sigma_1^2} x_1 + \frac{1}{\sigma_2^2} x_2 + \frac{1}{\sigma_3^2} x_3}{\frac{1}{\sigma_1^2} + \frac{1}{\sigma_2^2} + \frac{1}{\sigma_3^2}}$$

First the standard error of mean of row-A row-B and row-C for all the data was calculated in excel then the Weighted average of all data was calculated in excel for plot-1, plot-2 and plot-3.

2.4 Cultivar in this study

In this study I analyze the yield of two years of wheat sowing experiments for the cultivar PBW550 to estimate the impact of temperature and ozone exposure on the yield.

PWD-550 is considered most suitable for 85-95cm rainfall or irrigation areas in the plains of Punjab, Uttarakhand, Uttar Pradesh. It is considered to have a potential yield of 45-50 quintal per hectare under the optimum conditions.

Each ear heads are white and ideal period of sowing is from 2nd week of November to 2nd week of December. In our experiment we sowed on 1st and 15th November and on 1st of December 2018.

Chapter 3

Results and Discussion

3.1 Plant growth and development in the three treatments during 2018-19 growing season

Table 5 shows crop sown on 1st November (plot-1) took higher number of days from emergence to maturity than of plot-2 and plot-3. Reduction in number of days is due to delayed sowing. Thermal sum from emergence to maturity is higher in plot-3 as its grain filling period was shifted toward summer season. Number of days from emergence to flag leaf were higher in plot-3 as plant need optimum temperature for photosynthesis and the number of such days was low. Number of days from flag leaf to anthesis were also lower in plot 2 and plot-3, however, it does not appear that this is related to temperature stress as the time is shortest for plot 2. Instead it could be that the onset of reproductive growth is controlled by parameters (e.g. reaching a certain length of day for a certain number of hours after reaching a certain growth stage). When that occurs could fluctuate from cultivar to cultivar e.g. due to the cloudiness associated with the passing of a Western Disturbance. Number of Days from anthesis to maturity in plot 3 is decreased, possibly due heat stress triggering flag leaf death. However, when compared to the literature all three plots experienced the heat wave that affected the flag leaf and terminated grain filling relatively late in their grain filling period. Thermal sum during grain filling period was higher in plot 3 which results in plot 3 significantly lower the grain yield as compared to other two plots.

Table 5: Time in number of days and thermal sum taken by PBW550 to reach certain growth stages as a function of sowing date

	Plot 1 (1st Nov)	Plot 2 (15th Nov)	Plot 3 (1st Dec)	Kant et al. 2014	Singh et al 2018	
					late sown	very late sown
Number of days from emergence to maturity	160	149	139	123.41	128.5	111.0
Thermal sum from emergence to maturity	2334.16	2211.44	2441.19			
Number of days from emergence to flag leaf stage	68.0±0.2	81.7±0.3	80±2	89.26	97.08	83.5
Thermal sum from emergence to flag leaf stage	930.04	847.72	564.72			
Number of days from flag leaf stage to anthesis	24.5±0.8	16.6±0.1	19±3			
Thermal sum from flag leaf stage to anthesis	291.22	201.46	241.14			
Number of days from anthesis to maturity (grain filling period)	67.5±0.8	50.7±0.1	40±3	31.7		
Thermal sum from anthesis to maturity (grain filling period)	1112.88	1162.25	1635.32			

3.2 Meteorological parameters in relation to the growing season

Table 6: Different sowing dates of plot-1, plot-2 and plot-3 had significantly affected number of days to flag leaf, number of days to anthesis, number of days to maturity. Plot2 and plot 3 got higher number of days with optimum temperature for photosynthesis from emergence to flag leaf stage (12) and plot1 got higher number of days from flag leaf stage to anthesis (4). In plot-3 due to late sowing plant development period is reduced and crop matures early due to higher temperature. There was no heat stress during emergence to anthesis but during grain filling period there were number of days with heat stress plot-3 experienced 12 days of heat stress as it was late sown.

Table 6: Time in number of days PBW550 was exposed to particularly favourable and particular adverse temperature conditions during different growth stages

	Plot 1 (1st Nov)	Plot 2 (15th Nov)	Plot 3 (1st Dec)
Number of days with heat stress (T_{max}>34 °C) from emergence to flag leaf stage	0	0	0
Number of days with optimum temperatures for photosynthesis from emergence to flag leaf stage	8	12	12
Number of days with heat stress (T_{max}>34 °C) from flag leaf stage to anthesis	0	0	0
Number of days with optimum temperatures for photosynthesis flag leaf stage to anthesis	4	1	2
Number of days with heat stress (T_{max}>34 °C) from anthesis to maturity (grain filling period)	6	10	12
Number of days with optimum temperatures for photosynthesis anthesis to maturity (grain filling period)	14	14	13
First day of heat stress (T_{max}>34 °C) in days after sowing	156	141	126
First day of heat stress (T_{max}>34 °C) in days after anthesis	63	57	56

3.3 Air pollution exposure in relation to plant phenology during 2018-19 growing season:

Table 7 shows average 7 hour average ozone mixing ratio (M7) during different crop growth stages. Ozone mixing ratios is larger in plot-1 as plot-1 plants started emerging from 1st of November and experience more sunlight hours that results in crop loss in compare to plot-2. Plot-3 were late sown that is reason its plant development period is reduced and due to high ozone ratio during anthesis to maturity crop matures early with more no of shrivelled grains.

Table 7: Exposure of PBW550 was exposed to elevated ozone mixing ratios during different growth stages

	Plot 1 (1st Nov) (ppb)	Plot 2 (15thNov) (ppb)	Plot 3 (1st Dec) (ppb)
M7 from emergence to flag leaf stage	51.37	48.12	46.77
M7 from flag leaf stage to anthesis	40.42	33.81	34.09
M7 from anthesis to maturity (grain filling period)	47.31	50.45	52.50

3.4 Yield parameters during 2018-19 growing season:

First the standard error of mean of row-A row-B and row-C for all the data was calculated in excel then the Weighted average of all data was calculated in excel for plot-1, plot-2 and plot-3.

Table 8 shows plot-1 includes Row-A, Row-B and Row-C. Number of tillers per plant is largest in Row-A that means it had constant temperature and good light conditions while length of tillers is largest in Row-C that means it had good water source and nutrient management. Number of grains per tiller is comparatively same in all three Rows. Number of shrivelled is largest in Row-C this is because of prevalence of hot and dry winds during grain filling stage. Weight of the grains per plant is largest in Row-A is because of higher photosynthetic accumulation due to favourable temperature that results in high yield.

Table 8: Yield parameters of PBW550 in plot 1 sown on 1st of November

	ROW-A	**ROW-B**	**ROW-C**	**Weighted average**
No of tiller /plant	15±2	14±2	13±1	14±1
Length of tillers	10.8±0.4	11.0±0.2	12.5±0.4	11±1
No. of Grains/tiller	54±4	55±5	55±5	55±3
No. of shrivelled grains	5±1	5±1	7±2	5±1
Weight of grains/tiller	2.3±0.2	2.5±0.3	2.5±0.3	2±0
Weight of grains/plant	34±5	33±5	32±2	32±2

Table 9 shows Number of tillers per plant is largest in Row-C that means it had constant temperature and good light conditions while length of tillers is comparatively same in all three rows. Number of grains per tiller is largest in Row-B that means it had good water source and nutrient management. Number of shrivelled is largest in Row-A this is because of prevalence of hot and dry winds during grain filling stage. Weight of the grains per plant is largest in Row-B is because of higher photosynthetic accumulation due to favourable temperature that results in high yield.

Table 9: Yield parameters of PBW550 in plot 2 sown on 15th of November

	ROW-A	**ROW-B**	**ROW-C**	**Weighted average**
No of tiller /plant	13±4	13±3	14±2	13±2
Length of tillers	10.4±0.2	10.6±0.3	10.3±0.3	10±0
No. of Grains/tiller	58±6	60±7	53±6	57±4
Weight of grains/tiller	2.7±0.2	2.7±0.2	2.3±0.4	3±0
No. of shrivelled grains	12±4	11±6	7±6	11±3
Weight of grains/plant	35±11	36±7	32±5	34±4

Table 10 shows Number of tillers per plant is largest in Row-A that means it had constant temperature and good light conditions while length of tillers is largest in Row-B that means it had good water source and nutrient management. Number of grains per tiller is largest in Row-B that means it had good water source and nutrient management. Number of shrivelled is largest in Row-C this is because of prevalence of hot and dry winds during grain filling. Weight of the grains per plant is largest in Row-B is because of higher photosynthetic accumulation due to favourable temperature that results in high yield.

Table 10: Yield parameters of PBW550 in plot 3 sown on 1st of December

	ROW-A	**ROW-B**	**ROW-C**	**Weighted average**
No of tiller /plant	12±2	9±1	10±2	10±1
Length of tillers	8.9±0.3	9.5±0.3	9.3±0.5	9±0
No. of Grains/tiller	47±10	54±6	53±10	52±5
No. of shrivelled grains	12±6	13±7	15±6	13±4
Weight of grains/tiller	1.6±0.3	2.3±0.3	1.7±0.4	2±0
Weight of grains/plant	19 ± 4	20 ± 2	17 ± 4	19±2

3.5 Crop loss assessment of wheat:

Heat stress and ozone can cause shortening of the development phases of the wheat plants.

During the reproductive process heat stress and high ozone can cause pollen sterility during flowering. Heat stress can cause tissue dehydration during grain filling or grain abortion. Both heat stress and high ozone exposure during grain filling can also result in accelerated senescence of the flag leaf and reduced grain filling period. When comparing the three plots it is clear that the first two plots have comparable yields per plant, while the third plot shows a reduced grain yield that is almost half (Figure 4). Comparing the parameters that impacted this reduced yield allows to

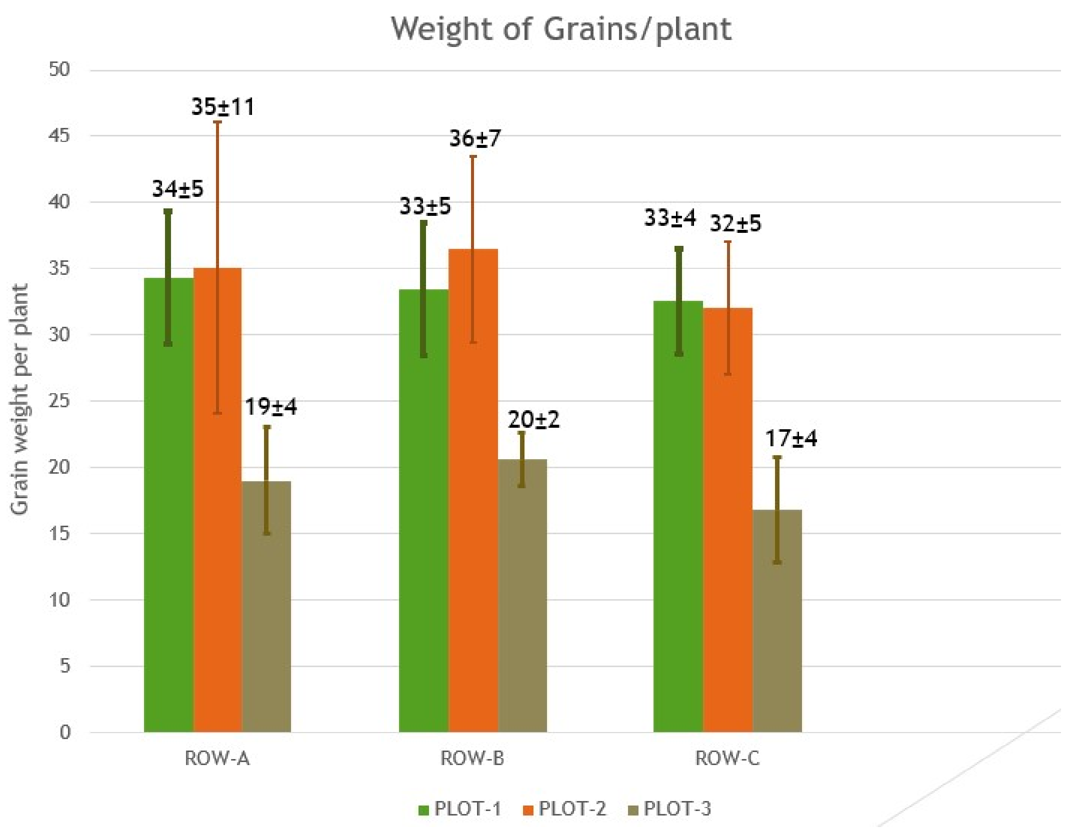

Figure 4: Grain yield per plant variations between the three plots

identify during which growth stage the wheat plant experienced stress either due to heat or due to elevated ozone.

By the above graph we can easily see the 41% loss of grain weight per plant of plot-3 in compare to plot-1 and plot-2. This is because of the more ozone concentration or the heat stress.

3.6 Estimating crop loss assessment of wheat:

Table 11: Comparison of yield parameters of PBW550 for the three plots

	Plot 1	Plot 2	Plot 3
Number of tillers/plant	14±1	13±2	10±1(-28%)
Length of head	11±1	10±0	9(-18%)
Number of grains/tiller	55±3	57±4	52±5 (-5%)
Number of grains/cm of tiller length	5±3	5.7±2	5.7±5(NA)
Grain weight per plant	32±2	34±4	19±2g/plant (-41%)
1000 grain weight	42.99	44.96	34.26 (-20%)

Loss can happen at three stages of plant development and table 11 helps to identify during which growth stages the plants in different plots experienced stress.

If environmental stress is there during the onset of reproductive growth that will reduce number of tillers and the tiller length. Plot 1 has the largest number of tillers and the longest tillers, indicating it experience the most favourable conditions during the onset of reproductive growth. Both plot 2 and plot 3 show reductions in comparison to plot 1 for these two parameters. Looking at the number of grains per cm of head length plot 2 and plot 3 show the largest number indicating that the crop in these plots suffered least stress during, immediately before and or after flowering, while plot 1shows a reduction in the number of grains per cm of head length indicating that the conditions around anthesis may have been less favourable in this plot. Plot 2 shows largest Grain weight per plant and 1000 grain weight indicating plot 2 was not affected by a significant amount of heat stress during grain filling, although plot 1 have been little stress during grain filling. Plot 3 experienced significant amount of stress during grain filling period.

As we already know that plot-3 was sown later that lead to crop loss. Crop loss happens at different stages and the above table shows that there was 28% loss due to the number of tiller per plant and the length of the tillers indicating the plant was stressed during the onset of reproductive growth. However, the reduced tiller length did not result in a significant reduction of the grain number in comparison to plot 1 as plot 1 seems to have been affected by some grain abortion or flower sterility which resulted in a lower number of grains despite the longer heads. Overall, the 41% loss in grain weight per plant in plot 3 is comprised half of the loss due to the lower number of tillers and half of the loss due to a 20% loss in 1000 grain weight and increase in the number of shrivelled grains.

3.7 Environmental parameters during the different growth stages

In this study I analyse the yield of two years of wheat sowing experiments for the cultivar PBW550 to estimate the impact of temperature and ozone exposure on the yield.

Tables summarizes the emergence data of plot -1, plot-2 and plot-3 of PWD-50 cultivar in terms of number of days from emergence to maturity, number of days T_{max} was more than 34^0C during grain filling, number of days with $T_{average}$ within optimum range for photosynthesis during grain filling , M7 during grain filling , number of shrivelled grains and 1000 grain weight. Number of days from emergence to maturity of wheat were least in Plot-3 as wheat seed were sown later in compare to plot-1 and plot-2. There were a greater number of days that plot-3 had gone through heat stress as its grain filling period was sifted toward summer season. M7 during grain filling of plot-3 is higher as there was more concentration of O_3. There were more shrivelled grains in Plot-3 also it has lesser 1000 grain weight because of the swift in grain filling period but Plot-2 has more 1000 grain weight than plot-1 this is probably because the soil was wet when plot-2 was sown as there had rained the night before.

Chapter 4

Conclusions

We observe that there is decrease in active number of tiller in plot 3 in compare to plot-1 and plot-2. The decrease in the number of active tillers correlates strongest (R=0.99) with the number of days with optimum temperatures for photosynthesis before the plant reaches the flag leaf stage. The total biomass generated which ends up getting translocated into grains during grain filling is hence a very important predictor of grain yield and explains half the observed yield reduction.

Plot 3 also shows shorter heads compared to plot-1 and plot-2. This head length correlates best with the first day of heat stress experience by the plant expressed in days after sowing (R=1) and is strongly anti-correlated with heat stress between anthesis and maturity (R=-0.98) and the ozone exposure (M7) from anthesis to maturity (R=-0.99). Anti-correlation means the stronger the stressor (heat wave and high ozone) the shorter head length. Overall the first heat wave in February or March appears to most strongly define the head length with head length explaining the other half of the yield reduction.

The number of grain normalized to the head length is perfectly anticorrelated with the ozone exposure from flag leaf stage to the anthesis stage (R=-1) indicating that ozone exposure just before the anthlers become visibly could most significantly reduce grain number.

The 1000 grain weight is weakly correlated with the number of days with optimum temperatures for photosynthesis before the plant reaches the flag leaf stage (R=0.67). This data seems to suggest that ozone exposure and heat stress after anthesis were not the only drivers of yield loss in late sown wheat and that the overall duration of optimum conditions for photosynthesis prior to the plant reaching flag leaf stage is the most important determinant of the final yield.

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Figure 5: M7 impacting no. of grain per cm of head length(flower sterility or grain absortion)

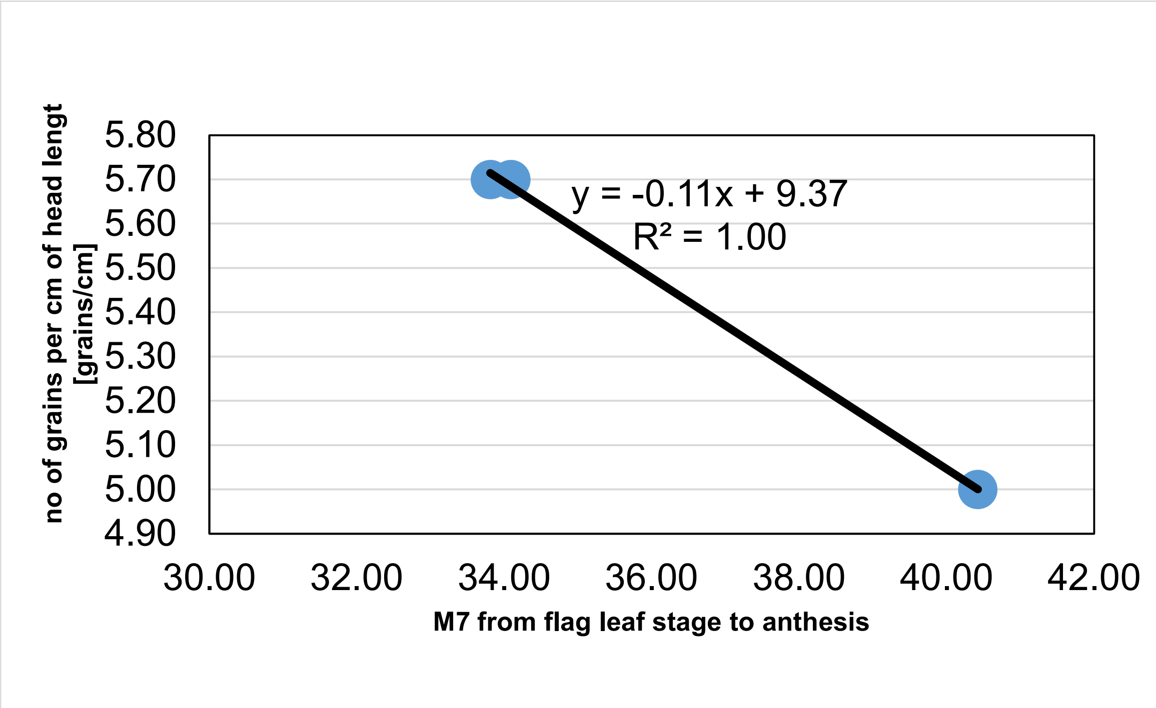


Figure 6: M7 impacting head length from anthesis to maturity(grain filling period)

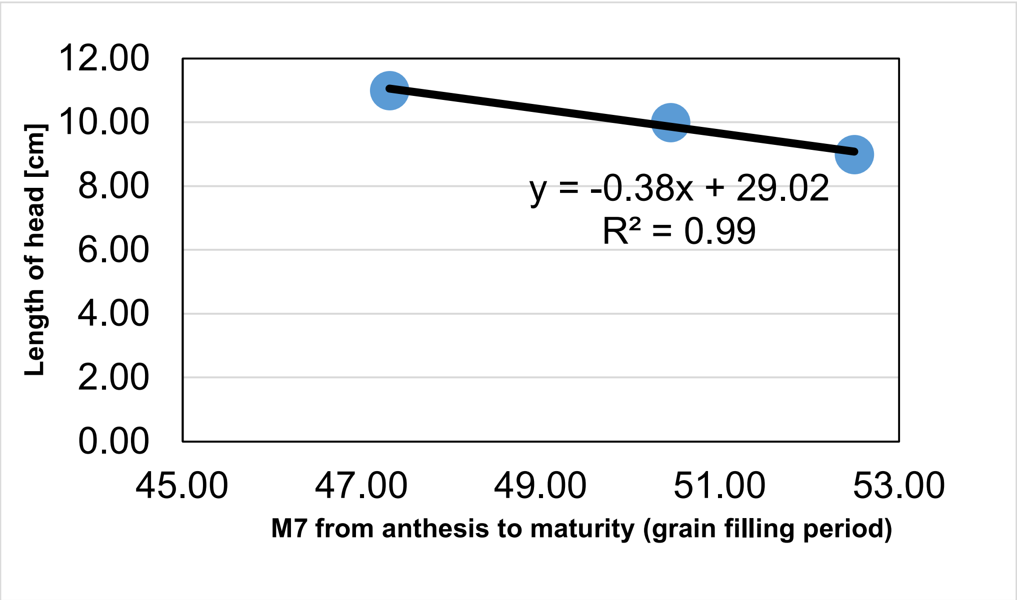


Figure 7: heat stress impacting head length from anthesis to maturity(grain filling period)

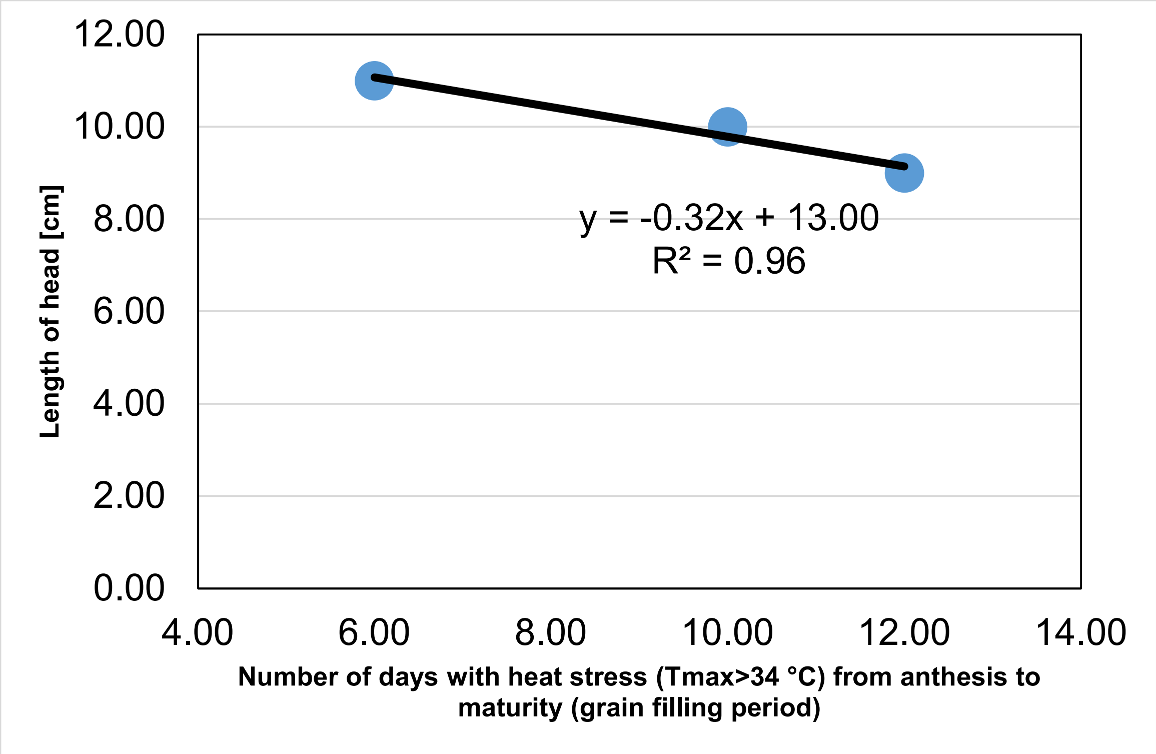


Figure 8: First day of heat stress impacting length of head(cm)

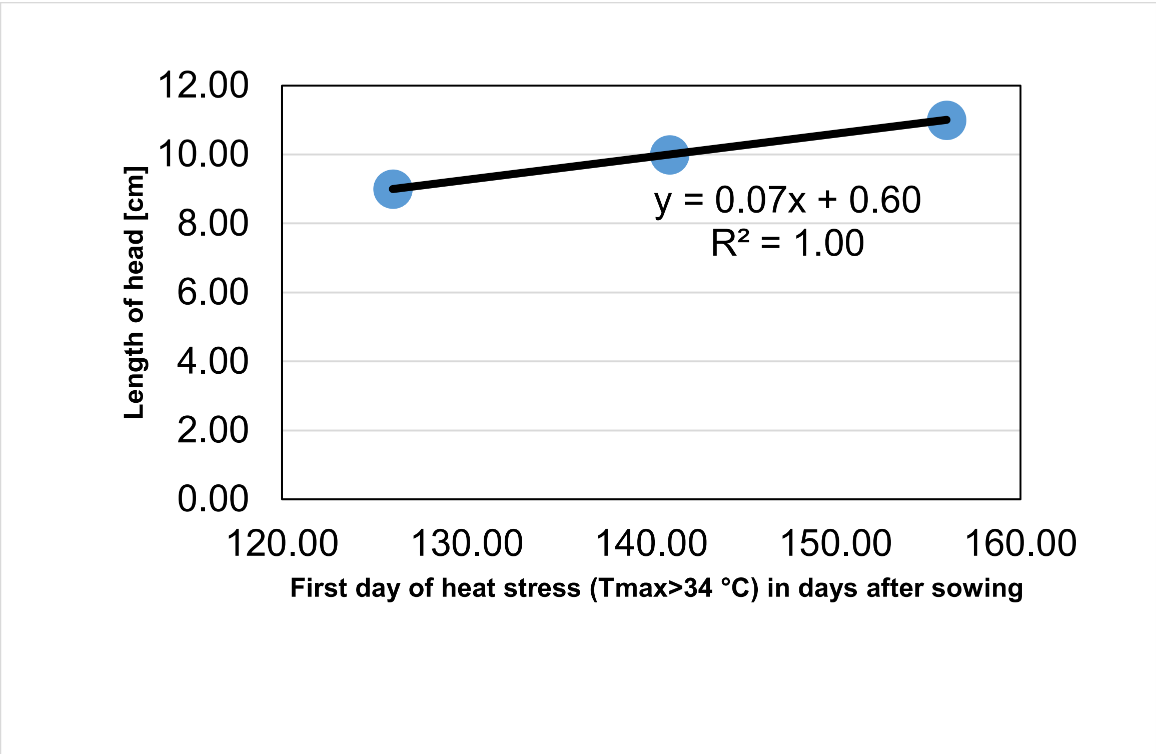
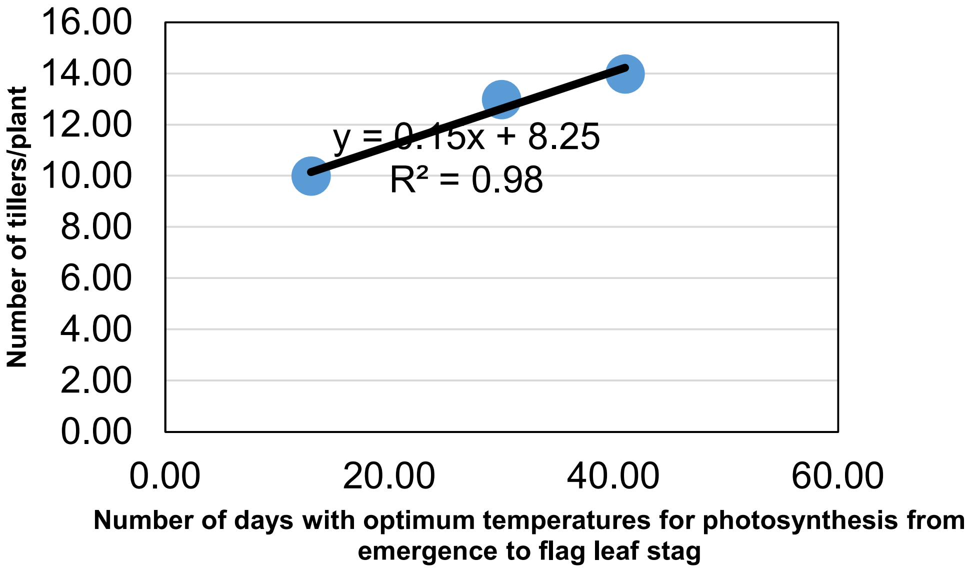


Figure 9: Environmental factors impacting number of active tillers



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