EFFECT OF HIGH TEMPERATURE STRESS ON SEED FILLING AND NUTRITIONAL QUALITY OF RICE (*Oryza sativa* L.)

by

KANDANULU PRAVALLIKA

(2018-11-162)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2020

EFFECT OF HIGH TEMPERATURE STRESS ON SEED FILLING AND NUTRITIONAL QUALITY OF RICE (*Oryza sativa* L.)

KANDANULU PRAVALLIKA

(2018-11-162)

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

K. Pravallika

Vellayani, **KANDANULU PRAVALLIKA.**

Date: (2018-11-162)

CERTIFICATE

Certified that this thesis entitled **"Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)"** is a record of research work done independently by Ms. Kandanulu Pravallika under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani,
Date:

Dr. Beena, R.
(Major advisor)
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani
Thiruvananthapuram

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Kandanulu Pravallika a candidate for the degree of **Master of Science in Agriculture** with major in Seed Science and Technology, agree that the thesis entitled **"Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)"** may be submitted by Ms. Kandanulu Pravallika, in partial fulfilment of the requirement for the degree.

Dr. Beena, R.
Assistant Professor
Department of Plant Physiology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Jayalekshmy V.G.
Professor and Head
Department of Seed Science & Technology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Roy Stephen
Professor
Department of Plant Physiology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

Dr. Geetha Lekshmi P.R
Assistant Professor
Department of Processing Technology
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522

External Examiner

ACKNOWLEDGEMENT

Firstly, I bow my head before the ***Creator*** *of the universe who has eased the difficulty and enlighten me at the times of despair.*

It is genuine pleasure to express my sense of thanks and deep sense of whole hearted gratitude to my major advisor ***Dr. Beena R.*** *Assistant Professor, Department of Plant Physiology, College of Agriculture, Vellayani, for her constant supervision as well as for providing necessary information regarding the research work and also for her major hand in helping to complete the thesis.*

I deem it to be my privilege to express my deep sense of reverence to ***Dr. Jayalekshmy V.G.,*** *Professor and Head, Department of Seed Science and Technology, College of Agriculture, Vellayani, for her continuous support and for her encouraging words that make me confident to further carry out the work.*

My heartfelt thanks to ***Dr. Roy Stephen,*** *Professor, Department of Plant Physiology, member of my advisory committee for his guidance, support and help in clarifying the doubts, for his valuable suggestions.*

I'm greatly indebted to ***Dr. Geetha Lekshmi*** *P.R., Department of Processing Technology, member of advisory committee for her kind heart and support through out the period of research work.*

I sincerely thank ***Dr. Umamaheswar****, Department of Plant Pathology, for his timely effort in observing the diseases in the plants and for suggesting control measures in maintaining the plant health.*

Wholeheartedly I thank ***Dr. Shahnas****, Department of Entomology, for his kind heart, support, suggestions and for taking effort in observing the plants and recommending some pest management practices, and for always giving a hand at the times of despair with some encouraging words.*

I express my special thanks to my friend ***Mr. Arunkumar C****. and to my senior* ***Mr. Vijay*** *without whose help my research work would be incomplete, none of the words are enough to express my thanks for their great contribution. I am greatly indebted to my bestie Arunkumar for his care, support, throughout my M.sc without whom I may feel that my days are boring and meaningless. Im greatly thankful to my friend* ***Akhilraj*** *for his support and care.*

Im thankful to my classmates ***Bhavana, Amrutha, Alekhya*** *my friend* ***Kusuma*** *my juniors* ***Diya, Keerthi, Jayashri, Sanith, Rajasekhar, Nikhil*** *and my seniors* ***Manikanta, Yogesh, Stephen, Arya, Afna, Shahiba, Gayathri, Manasa, Arvind, Anand, Arun, Vipin, Sayooj, Ragunath****, who made some contribution regarding doubts or some other help in carrying out the research work and in carrying out the thesis work without whom the work may not be successful.*

I acknowledge the boundless affection, unsolicited help, companionship and moral support rendered by my friends ***Himabindhu, Swathi, Alekhya, Bindhu, Mythri, Krishnan unni, Arun, Akhilraj Dikshitt, Sinchana, Remya, Veni, Nitasana, Anupama, Devapriya*** *whom I admire a lot. I warmly remember their role in making the period of my study here a memorable and cherished one.*

Im thankful to my friends ***Sailinga, Vikram, Arvind, Daniel, Arya, Nikitha, Priya, Mahathi, Laxmi, Kusuma, Medha, Vaitheeswari,*** *for their warth and timely support and for their kind heart towards me that always wish success.*

Words would fail to express the depth of my feelings for my family. My mom ***Kandanulu Maheswari****, my father* ***Kandanulu Maruthi Reddy****, who always care, love and wish for my good health and success. My thanks to my siblings* ***Aparna, Mounika, Hemanth****, and my brother in law* ***Ragunath*** *who always wish my happiness and guide me to the path of success with good, inspiring words. May god always shower his blessings on them.*

At last but not the least, I am greatly thankful to all those people who helped me either directly or indirectly during my degree programme.

THANK YOU *all very much.....*

K. Pravallika

Kandanulu Pravallika

CONTENTS

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

^{0}S	Degree south
^{0}N	Degree north
^{0}C	Degree Celsius
%	Percentage
IPCC	Intergovernmental Panel on Climate Change
ROS	Reactive Oxygen Species
Mg g^{-1}	Milligram/gram
PEG	Poly Ethylene Glycol
NaCl	Sodium chloride
WHO	World Health Organisation
AC	Amylose content
GBSS	Granule-Bound Starch Synthase
HWS	Hot water-soluble starch content
DAA	Days after anthesis
HSP	Heat stock proteins
HT	High temperature
TN	Taichung Native
TNG	Tainung
HS	Heat stress
NT	Normal temperature
µg kernel^{-1} weight	Microgram/kernel weight
BK	Black kernel
ml^{-1}	Per ml
n mole /kernel wt	Nano moles/kernel weight
SG	Suceptible Genotype
TG	Tolerant Genotype

CWIN	Cell wall invertase
VIN	Vacular invertase
DAF	Days after flowering
Mg	Milligram
Min^{-1} mg^{-1}	Per minute per milligram
Day/ngt	Day/Night
H	Hour
h^{-1} mg^{-1}protein	Per hour per milligram protein
g/plant	Gram/plant
DAS	Department of administrative services
FYM	Farm Yard Manure
DAT	Days after transplanting
CRD	Chronic respiratory disease
ML	Milli litre
µg/ml	Microgram/millilitre
Nm	Nanometer
Min	Minute
G	Gram
N	Normality
HSFs	Heat stress transcription factors
NaoH	Sodium hydroxide
HCL	Hydrogen chloride
PBS	Phosphate Buffer Saline
$Alcl_3$	Aluminium chloride
$NaNo_2$	Sodium nitrate
P^H	Potential of hydrogen
M	Molarity
Mg/OD	Milligram/Optical density
Mm	Millimetre
Min^{-1} g^{-1}	Per minute per gram
IKI	Iodine Potassium Iodide

KI	Potassium iodide
Mg g^{-1}	Milligram/gram
SPS	Sucrose Phosphate Synthase
NSC	Non-Structural Carbohydrate
UFGT	UDP glucose Flavonoid Glucosyl Transferase
mRNA	Micro Ribose Nucleic Acid
CWI	Cell Wall Invertase

INTRODUCTION

1. INTRODUCTION

Rice is the major cereal crop of the world and is the main source of food for greater than half of the world's population. Globally rice is cultivated over an area 158 million tons with total production of 700 million tons (Statista, 2018). In India rice is cultivated over an area of about 44.5 million hectares in the year 2018 with total production of 172.58 million tons and productivity of 3,878.2 kilogram per hectare (FAO STAT, 2018). In Kerala rice occupies an area of 0.20 million hectares in the year 2018-19 with total production of 0.57 million tons (ECOSTAT, 2018).

It belongs to the genus *Oryza* which constitute 25 species of which two species are cultivated and 23 are wild species. The cultivated species include *Oryza sativa* and *Oryza glaberrima*, among the two *Oryza sativa* is widely grown. Rice has wider adaptations with respect to altitude, geographical distribution, rainfall and atmospheric temperature hence it is grown in different types of ecosystems, such as tidal wetlands, irrigated areas, deep water, rainfed uplands and lowlands, around the world. Today rice is grown in more than 100 countries of the world across the north to south from 40^0S to 53^0 N latitude.

Rice occupies about 23.3% of gross cropped area of the country and plays a vital role in national food grain supply. It is the main source of energy and providing required amounts of zinc and niacin. Rice is mainly composed of oryzanol which act similar to vitamin E in accelerating growth, blood circulation and hormonal secretion. Rice bran is rich source of protein, fat, carbohydrates, antioxidants, micronutrients and vitamins (Devi and Arumughan, 2007).

Abiotic stress triggers a series of physiological, biochemical, morphological, and molecular fluctuations leading to yield loss in crops (Fahad *et al.,* 2015). Decrease in quality and productivity of food is mainly due to high temperature conditions, which poses a serious threat to agriculture (Zandalinas *et al.,* 2018). Occurrence of this stress, either individually or in combination, would severely reduce the crop productivity and food security, globally. There is considerable yield loss when the stress occurs during reproductive stage mainly at seed filling stage which is most critical stage.

Global food security is being distress by extreme changes in the climate. High temperature stress has become the most important limiting factors to crop productivity. Temperature stress affected rice yield by reducing the performance of different rice growth and yield traits and are most sensitive to high temperature at flowering stage. High temperature at this stage significantly increases the spikelet sterility in rice, or sometimes it can even lead to no harvest, which is due to inhibitions of anther dehiscence, pollen sterility and failed germination of pollen on the stigma (Snider *et al.,* 2011). In addition, cessation of pollen tube elongation in the pistil is another important factor causing pollination failure under heat stress (Sebastian *et al.,* 2017).

Seed filling is the crucial growth stage, which include transport processes that are required for importing various constituents for the synthesis of proteins, carbohydrates and lipids in the developing seeds. Heat stress affect the accumulation of various constituents, mainly starch and proteins through inhibiting the enzymatic processes of synthesis of starch and proteins. Seed filling stage is highly sensitive to environmental changes, which affect the qualitative and quantitative traits (Farooq *et al.,*2017). Temperature during grain filling period, decrease the grain filling duration, leading to the lower grain weight and yield in rice. Hence keeping all the facts in mind student has initiated the above research in rice.

REVIEW OF LITERATURE

2. Review of Literature

Rice (*Oryza sativa L.*) being the key source of food is a globally important cereal plant. For more than 3 billion humans, rice constitute for 35-75% of the calorie consumption. According to the population projections, due to increase in birth rate more proportional to the death rate, caused a rise in global inhabitants to grow 10 billion by 2050. Growth in population per hour is greatest in the rice-eating and rice cultivation zones of Asia, Africa, and the America that leads to faster growth in demand for rice compared to other crops (Battisti and Naylor, 2009). To meet the demand of this expeditious growing population, there will need to produce 70-100% more food worldwide, than the existing (Godfray *et al*., 2010). Hence to achieve sustainable food security it is imperative to expand the rice production at a scale of 1% annually (Ray *et al*., 2013). In irrigated rice in order to generate a kilogram of biomass, 500 L of water is essential. In terms of agricultural efficiency, irrigated rice is normally 100 times more productive than upland rice, 12 times more productive than deep-water rice, and five times more productive than rainfed-lowland rice. But this productivity is highly vulnerable, due to recurring episodes of high temperatures stress. A modern report projected that the matter of farthest temperature stress will increase in the upcoming days.

Global warming is one of the supreme factors in climatic variability. It increased the atmospheric concentration of greenhouse gases further increasing the earth's surface temperature. This high temperature is a threat to rice production. Heat stress is the temperature, over the ideal range for plant growth and development that can equally harm or permanently injure both vegetative and generative organs of rice. The best temperature for highest rice photosynthesis is 25-30 °C for daytime maxima and 20 °C for the night time maxima (Figueiredo *et al.,* 2015). Furthermore, increment in temperature above the optimum or incident of high temperatures during sensitive stages may lower the rice yields drastically. In tropical environments, high temperature is the prime environmental stress that restrict the rice productivity, causing reduction in grain mass and grade.

The temperatures above 33 ^{0}C cause yield deprivation in many fragments of the globe. In rice for every 1 ^{0}C hike in temperature the grain yield is reduced by 10% (Peng *et al.,* 2004). IPCC forecast a rise in mean annual temperature of 0.7-0.9 ^{0}C per decennium in Southeast Asia which relates to 4.8 ^{0}C by 2100 (Weiss, 2009). The predicted changes at the end of the twenty-first century, include the increment in surface air temperature around 1.4-5.8 ^{0}C, like the temperatures of 1980–1999 (IPCC, 2014).

Heat stress at grain-filling stage is source of detrimental effects on rice biomass and quality. Temperature is a crucial factor for photosynthesis. Inadequate temperature results in lowering of leaf photosynthesis in the plants and also cause reduction in the allocation of dry matter to shoots and roots. Temperature above optimum certainly affects the crop metabolism, and grain development. It also affects the yield and quality of the crop (Lyman *et al.,* 2013). Heat stress is injurious during reproductive and grain filling stages and the intensity, duration and timing of this stress determines the plant performance (Tenorio *et al.,* 2013).

2.1 IMPACT OF HIGH TEMPERATURE STRESS ON SEED QUALITY PARAMETERS

2.1.1 Reducing sugar

In response to various stresses different solutes are accumulated in plants among which sugars represent the major reserve in the seeds that helps in enhancing the response to environmental stresses. They take part in regulation of growth, photosynthesis, partitioning of carbon, metabolism of lipids and carbohydrate osmotic homeostasis, synthesis of protein and expression of genes during varied abiotic stresses, they also involve in stabilization of membranes (Rosa *et al.,* 2009).

Exogenous application of low levels of glucose, scavenge the surplus ROS which are produced under stress and protect from deadly activities, such as decay of chlorophyll, membrane damage and death of cell. Furthermore, sugars except glucose, such as sucrose, fructose and trehalose function as osmo protectants and regulate the

osmotic adjustment, protect the membrane protection and also scavenge the harmful ROS which are produced in response to varied kind of stresses (Singh *et al.,* 2015).

Sorghum bicolor CSH-6 is exposed to different treatments such as NaCl, PEG, heat and cold treatments and was noted that under heat stress (42°C) there is an increase in carbohydrate content compared to ambient conditions (37 °C). In the seed reducing sugar (185 mg g^{-1}) content was higher under heat treatments when compared to that of ambient condition (169 mg g^{-1}) (Gill *et al.,* 2001).

The wheat genotypes such as PBW 343, PBW 534 (heat susceptible), C 306 and C 273 (heat tolerant) were exposed to high temperature and thiourea with control maintained at 25 ^{0}C and high temperature maintained at 32 ^{0}C conditions. It was found that the reducing sugar content increased under high temperature in all the wheat cultivars with PBW 343 -60 mg g^{-1}; PBW 534-50 mg g^{-1}; C 306-43 mg g^{-1} and C 273-48 mg g^{-1} when compared with that of control with PBW 343 - 55 mg g^{-1}; PBW 534-48 mg g^{-1}; C 306-30 mg $g^{-1}$and C 273-33 mg g^{-1} (Asthir *et al.,* 2015).

2.1.2 Total carbohydrates

Carbohydrates are the building units and energy providers for the plants to produce biomass. Carbohydrates include hexoses (glucose and fructose), disaccharides (sucrose, trehalose), and oligosaccharides (raffinose, stachyose) which place a vital role as compatible osmolytes are soluble in water and are essentially involved in plant stress responses (Hussain *et al.,* 2011). Fructans which act as storage carbohydrates are highly effective than starch and were transported quickly under stress conditions (Keunen *et al.,* 2013).

Three rice varieties, N22 (HT-tolerant), Zhenshan 97B (Moderately heat tolerant), and Koshihikari (Heat sensitive), were grown in a pot trial under two N treatments and under ambient and high temperature condition. When pooled together, the data revealed that there is practically correlation of grain yield and percentage of seed set with the leaf photosynthetic rate and concentration of stem NSC under the heat stress treatment when compared with that of ambient treatment. At sufficient nitrogen condition that normally exist in soil and at high temperature, non-structural

carbohydrate content is increased (325 mg g^{-1}) in the panicle of N22 and Zhenshan compared to ambient temperature condition (305 mg g^{-1}) but the carbohydrate showed a decrease under high temperature in the panicle of Koshihikari (230 mg g^{-1}) when compared with ambient temperature condition, because it is highly heat sensitive cultivar (Xiong *et al.*, 2015).

Sorghum bicolor CSH-6 is exposed to different treatments such as NaCl, PEG, heat and cold treatments and was noted that under heat stress (42°C) there is an increase in carbohydrate content compared to ambient condition (37 °C). In the seed fructose (120 mg g^{-1}) amount was higher than that of glucose (31 mg g^{-1}) and sucrose (15 mg g^{-1}) under heat treatments when compared to ambient condition (fructose-100 mg g^{-1}; glucose-23 mg g^{-1}; sucrose-10 mg g^{-1}) (Gill *et al.*, 2001).

Source plants of two potato cultivars, Norchip and Up-to-Date, were exposed to high temperature of 29°C with control maintained at 17°C and found that temperature has little effect on carbohydrate. Plants from high temperature treatment showed increased glucose with 6.2 mg g^{-1} in norchip and up-to-date cultivars compared to ambient condition with 5.2 mg g^{-1} in norchip and 3.5 mg g^{-1} in up-to-date. In norchip sucrose showed 17.6 mg g^{-1} higher under high temperature treatment with 10 mg g^{-1} under control and up- to-date cultivar showed 17 mg g^{-1} under high temperature treatment with 12.5 mg g^{-1} under control (Basu and Minhas, 1991).

2.1.3 Starch

A principal part of dried rice grain was composed of starch (up to 90%) and hence it is, considered a prime benefactor of multiple grain quality characters. While the dietary patterns are continuously advancing, it is suggested that 50% of the calorie's intake of humans is procured from starch (WHO, 2003), and this rate may increase to 80% in developing nations (Burrell, 2003). Physiochemical properties and quality of starch influence different attributes of rice grain (Juliano, 2007).

In plants abiotic stresses cause a deleterious effect on starch by causing several modifications in its composition, cooking attributes and also affect the milling quality of the grains resulting in the proportion of chalkiness in the grains (Fabre *et al.*, 2005).

Satoh *et al.* (2003) reported that high temperature at the time of grain filling down regulate the genes related to starch synthesis in the rice grain thus resulting in adverse effect on starch quality.

Liu *et al.* (2007) reported that application of polyamines exogenously in rice plants improved the sucrose and starch contents by 28 and 13 % in grains that were unpolished and 36 and 22 % in polished grain at 105 days after sowing

Cheng *et al.* (2005) evaluated the performance of two early Indica rice varieties namely Jia 935 (low AC) and Jia 353 (high AC), which differ in amylose concentration under high temperature condition where the temperature maintained was 32 ^{0}C and with the control kept at a temperature of 32 ^{0}C. It was found that the starch content in the endosperm is reduced on exposure of crop to heat stress conditions which is due to reduced activity of granule bound starch synthase (GBSS) enzyme. The starch content in the endosperm of two rice varieties Jia 935 (13.41 mg endosperm^{-1}) and Jia 353 (13.36 mg endosperm^{-1}) was low under high temperature condition when compared with that of the ambient condition (13.67 mg endosperm^{-1}, 13.72 mg endosperm^{-1}).

Cao *et al.* (2015) proposed the effect of heat stress on grain starch properties of two non-waxy Indica rice cultivars 9311 and its mutant with greater high amylose content (9311 eha). The two rice genotypes were exposed to heat stress of 33 ^{0}C with the control kept at 23 ^{0}C and it was found that 9311 eha showed higher hot water-soluble starch content (HWS) than 9311 with rising extent of 73.54% (HWS) under optimum temperature. The hot water-soluble starch content of 9311 is reduced under heat stress (14.93%) when compared with that of the ambient condition (18.87%). The mutant 9311eha had high levels of actual amylose as reflected by HWS hence it is less sensitive to high temperature than those of 9311 so, it shows a slightest reduction in starch content under heat stress condition.

Kato *et al.* (2019) investigated the effect of high temperature stress on starch biosynthetic enzymes in japonica rice cultivar 'Akitakomachi' and reported that imposing heat stress of temperature 29^0C in artificial growth chamber and average daily temperature of 28^0C in high temperature greenhouse resulted in higher endosperm starch in both the conditions when compared to that of ambient condition (23^0C).

A well-known waxy maize variety 'Suyunuo 5' was analyzed in 2014 and 2015 by Yang *et al.* (2018). The results revealed that imposing high temperature of 35 ^{0}C at maturity showed a decrease in starch content by 3% and 3.3% in 2014 and 2015 and starch accumulation were reduced by 22.2% and 21.8% in 2014 and 2015 respectively when compared with their ambient condition (29 ^{0}C).

2.1.4 Amylose

Amylose controls the textural property of the rice grain (Singh *et al.*, 2005). The ratio of amylose–amylopectin is regulated by starch biosynthetic enzymes and it is a major factor that affects starch structure and properties in the amyloplasts of plant cells (Zeeman, Kossmann and Smith, 2010).

Amylose is the element of starch which affect the cooking and eating quality of rice (Futakuchi *et al.*, 2008). The quantity of amylose and amylopectin affects the rigidity of rice starch gel (Mariotti *et al.*, 2009).

Rice is grouped into four categories based on amylose content as glassy (0%-2%), very small (3%-9%), middle (20%-25%), and elevated (>25%) (Cruz and Khush, 2000).

Heat stress in rice result in chalky grain which is due to abnormal expression of the starch synthesizing enzymes, it also reduces the amylose content in the grain (Nishi etal, 2001; Tanaka *et al.*, 2004).

Chun *et al.* (2015) observed that imposing different temperature regimes (temperature of day/night of 22/14, 25/17, 28/20, and 31/23 °C) during grain filling in rice decreased the amylose content. Two rice varieties namely Ilpum and Chucheong

which are the japonica species were used. With the increase in temperature the amylose content is decreased. In both the varieties, Ilpum and Chucheong the amylose content is maximum at low temperatures (17.3%, 17.4% respectively) and minimum at high temperatures (14.8% and 14.7% respectively).

High temperature stress one day after heading in the commercial extra-long basmati rice variety (Basmati 2000) caused a reduction in amylose content by 22%. Optimum temperature of 22 ^{0}C for basmati was maintained at control with a temperature of 32 ^{0}C maintained in high temperature treatments. Amylose content of the hulled rice grain at maturity under high temperature with 3.2 mg grain^{-1} (21.7%) was lower than that of the ambient condition with 4.2 mg grain^{-1} (25.1%) (Ahmed *et al.,* 2015).

Wang *et al.* (2012) from his experiment wheat plants (*Triticum aestivum*, L. cv. Yang 9) reported that amylose content was lowered at maturity under high temperature condition. The study includes four treatments namely no heat-stress (CC) which is the control with 26/22 ^{0}C, and high temperature of 34/30 ^{0}C was imposed at pre-anthesis only (HC), heat at post-anthesis only (CH), and heat stress at both stages (HH). Amylose content influences the heat stress at post-anthesis which is crucially reduced from 15 to 25 DAA by heat stress at post anthesis only (14.43%) and at both pre and post anthesis stage (14.15%), whereas, amylose content was unchanged at 10 and 13 DAA by pre anthesis heat stress (17.46%) and ambient condition (16.70%).

Fang *et al.* (2019) performed a pot experiment using wheat cultivar ‘’Zhengmai 366” with two temperature regimes (Control-25^{0}C, High temperature-32^{0}C) during 2015-16 and 2016-17 and found that high temperature (14.2% and 14%) treatment decreased the amylose content when compared with their ambient condition (15.9% and 15.6%) during 2015-16 and 2016-17 respectively.

Two Japanese rice cultivars namely ‘Genkitskukushi’ (Heat tolerant) and ‘Tsukushiroman’ (Heat sensitive) were grown at a high temperature of 31^{0}C with the control maintained at 26^{0}C. The amylose content of cultivars Genkitskukushi and Tsukushiroman under high temperature (17.2% and 16.2%) condition was lower than that of ambient (18.8.% and 18.2%) condition (Ishimaru *et al.,* 2020).

2.1.5 Seed protein

Accumulation of proteins is a common phenomenon in many stressed plants, which help in protecting sub cellular structures by mediating osmotic adjustment, as protein are the major organic osmolytes (Ashraf and Foolad, 2007). Heat shock proteins (HSP) are the important classes of molecular chaperones, which act in response to various stresses (Swindell *et al.,* 2007; Al-Whaibi 2011).

The primary function of HSP is regulation of protein folding and unfolding, degradation of unfolded and denatured proteins (Singh *et al.,* 2016). HSP behave in connection with heat stress transcription factors (HSFs). In plant, HSFs are considered as important component of signal transduction cascade which moderate the appearance and adjustment of genes involves in several abiotic stress responses but some of the proteins are highly degraded and their content decreased under high temperature condition (Guo *et al.,* 2016).

Rice include storage proteins which are universally arranged into glutelins, prolamins, globulins, and albumins, constituting about 70, 3, 7, and 5% of rice grain nitrogen, respectively (Hamaker, 1993). They are the second huge number of macro molecules which are important for physicochemical properties and edible of the grains (Martin and Fitzgerald, 2002).

Two rice cultivars, one is an Indica type (Taichung Native 1/TN 1) and other is an japonica type (Tainung 67/TNG 67), with two treatments which include control at temperature 20^0C and HT imposed at 30^0C during grain filling stage and found that High temperature quickens the growth of prolamins and globulins during the advance filling stage but resulted in a decreased amount at the final mature stage. Prolamine content is higher under ambient condition with 7.3 mg g^{-1} in TN 1 and 7.6 mg g^{-1} in TNG 67 than that of high temperature with 4.8 mg g^{-1} in TN 1 and 5.5 mgg^{-1} in TNG 67. Globulin content under ambient condition is higher with 35.4 mg g^{-1}in TN 1 and 27.6 mg g^{-1} in TNG 67 when compared with high temperature where globulin content is lower with 16.7 mg g^{-1} in TN 1 and 18.7 mg g^{-1} in TNG 67(Lin *et al.,* 2010).

Lentil is exposed to heat stress by imposing a temperature of 28°C during seed filling in heat resistant genotype (FLIP2009) and thermo sensitive genotype (IG4242) with control at a temperature of 23°C and found that and protein contents declined by 26%, in the HT genotype, and by 42%, in the HS genotype. Different proteins fractions such as albumins, globulins, glutelins and prolamins were extremely affected by heat stress, with more depletion in HS genotypes, compared to HT genotype. Albumins appears in 21 % reduction in HT genotype, and 43% in HS genotype, globulins reduced by 14% in HT and 26% in HS genotypes, over their respective controls. Glutelins were found to reduce more in HS genotype (42%) than in HT genotype (22%), and likewise, prolamins reveals 40% decrease in HS genotype and 28.2% in HT genotype, above their respective controls (Sita *et al.*, 2018).

2.1.6 Anthocyanin content

Anthocyanins, are one of the most important classes of flavonoids together with pro anthocyanidins and flavonols, are important plant pigments responsible for different colours such as red, pink, purple, and blue colours in plants (Grotewold, 2006). In plants in response to stress anthocyanins are produced and they play a vital role as strong antioxidant molecules protecting plants from ROS damage and effective scavengers of ROS in vitro (Juadjur *et al.,* 2015)

Marko *et al.* (2004) proposed that part of anthocyanins during abiotic stresses include extinguish of ROS, photoprotection, stress signalling, and xenohormesis (i.e., the biological principle that relates bioactive compounds in environmentally stressed plants and the rise in stress resistance and extension in animals that feed from them). Anthocyanins are the antioxidants that are highly effective for quenching the excessively accumulated stress-induced ROS and the protection of osmotic balance (Wahid, 2007; Bita and Gerats, 2013).

Two Indica rice cultivars, 9311 and its corresponding mutant with the black kernel phenotype (9311bk), were used to contrast their variance in pigment content. The pots were deposit in a greenhouse under natural light cycles and mediate temperature environment (28 ^{0}C). At full heading stage, 30–40 panicles with uniform anthesis were

irregularly selected and identified. Afterward, the pots were advanced to two phytotrons to abuse the different temperature treatments till maturity. One phytotron was utilize for excessive temperature (High Temperature) and the other for ordinary temperature (Normal Temperature).The daily mean temperatures were set to 32 ^{0}C for HT and 22 ^{0}C for NT. Anthocyanins were analysed in seed and noted that 35 DAA the flavonoid content decreased in high temperature condition in both the mutant(10 µg kernel^{-1} weight in 9311 bk) and wild type (112 µg kernel^{-1} weight in 9311) when compared with that of their ambient condition (18 µg kernel^{-1} weight in 9311 bk and 128 µg kernel^{-1} weight in 9311) (Zaidi *et al.,* 2019)

Red sorghum is exposed to different temperature regimes and was found that with rise in temperature and there is decline in anthocyanin content. From 0^{0}C the temperature is raised every 10^{0}C until 70^{0}C and observed that half-life of anthocyanin content decreased at high temperature 70^{0}C with 0.1 h when compared to 0^{0}C with 3.7 h (Devi *et al.,* 2012).

According to Yuzbasioglu *et al.* (2017) gradually increasing temperature in maize resulted in dramatic increase in anthocyanin content. Temperature is imposed in 20 day old seedlings, every day temperature is raised by 5 ^{0}C until 45 ^{0}C with the control maintained at 25 ^{0}C and was found that minor increase occurred at 30 (1.2 µg ml^{-1}) and 35 ^{0}C (1 µg ml^{-1}), compared to the ambient condition (0.8 µg ml^{-1}), the increase at 40 (3.7 µg ml^{-1}) and 45^{0}C (2.3 µg ml^{-1}) was approximately 3-folds compared to the control.

Li *et al.* (2018) explored the effect of different abiotic stress on anthocyanin accumulation in the seeds of *Triticum aestivum* cv. Guizi and observed that the anthocyanin accumulation tends to decrease with maturity that is lowest value is recorded at 35 days of post anthesis (13.78%) when compared with that of 25 days of post anthesis (28.06%).

2.1.7 Flavonoids

Flavonoids are popular subordinate metabolites which harbour the classic soluble vacuolar compounds namely the anthocyanins (Neilson *et al.,* 2013). Lately

these compounds appear as crucial dictators of development and responses of plants to the stress conditions (Pourcel *et al.*, 2013).

Flavonoids has the ability to contribute to varied evolutionary pathways that are taken up by early land plants and help them to cope up with a range of stresses (Agati and Tattini, 2010). Fini *et al.* (2011) suggested that flavonoid synthesis is up-regulated upon exposure of crops to varied stresses.

Hernández *et al.* (2009) stated that in plants, at the time of stress, flavonoids played a vital role in accomplishing their function in restricting the production of ROS by chelating the metal ions, as they are the most effective antioxidants that can scavenge the ROS formed (Jaakola and Hohtola, 2010) .

Zaidi *et al.* (2019) studied the effect of heat stress in two Indica rice cultivars, 9311 and its corresponding mutant with the black kernel phenotype (9311bk). The pots were placed in a greenhouse under moderate temperature environment (28 ^{0}C). At full heading stage, 30–40 panicles with uniform anthesis were randomly selected and tagged. The plants were exposed to high temperature of 32 ^{0}C and the control was kept at 22 ^{0}C. Flavonoids were analysed in seed and noted that 35 DAA the flavonoid content increased in high temperature condition in both the mutant(0.0058 nmole/kernel wt in 9311 bk) and wild type (0.0048 nmole/kernel wt in 9311) when compared with that of their ambient conditions (0.0057 nmole/kernel wt in 9311 bk and 0.0046 nmole/kernel wt in 9311)

Two soybean genotypes: ‘‘Suceptible Genotype (SG)’’, a conventional, high yielding released line (DT97-429); and ‘‘Tolerant Genotype (TG)’’, an experimental line (04025-1-4-1-1) which has tolerance to heat induced seed degradation and was derived from plant introduction line PI 587982A (Smith *et al.*, 2008), were grown at ambient temperatures (28 ^{0}C). At the time of onset of flowering, a temperature of 36 ^{0}C was imposed. Seed metabolites were analysed by three metabolite profiling methods, and genotype-specific differences and temperature specific differences were identified and found that five out of thirteen flavonoids detected were higher in TG seed (For example, apigenin, glycitin, genistin, genistein and naringenin-7-Oglucoside were 4-

fold, 2.7-fold, 2.1-fold, 2.1-fold and 3-fold higher in abundance, respectively, in TG seed) but none were found to be higher in SG at 28 ^{0}C . At 36 ^{0}C, 10 out of the 13 analysed flavonoids were present at higher levels in TG as compared to SG seed (In addition to those flavonoids elevated in TG seed at 28 ^{0}C, daidzein, daidzin, glycitein, kaempferol 3-O-betaglucoside and naringenin-7-O-glucoside were also significantly higher with 2.9-fold, 2.9-fold, 2.3-fold, 2-fold and 2.9-fold respectively (Chebrolu *et al.*, 2016).

2.2 IMPACT OF HIGH TEMPERATURE STRESS ON ENZYME

2.2.1 Invertase

Invertase is the enzymes which cleave sucrose into hexoses and it play a diverse role in development and signalling (Koch, 2004; Weber *et al.*, 2005). It is the most important enzyme in sugar metabolism and is involved in the distribution of carbohydrates to sink organs, it regulates the source-sink transitions, amplifies the signals that control source-sink relationships, integrate the signals and defence responses, or responses to environmental changes (Roitsch and Gonzalez, 2004). Hence, invertase is considered as a central modulator of assimilation partitioning and plant responses to environments (Lou *et al.*, 2007).

CWIN (Cell Wall Invertase) and VIN (Vacuolar Invertase) activities are essential for seed and fruit set development (Zanor *et al.*, 2009).

Roitsch *et al.* (2003) reported that exposure of plants to different stress stimuli resulted in upregulation of cell wall-bound invertase (CWIN) activity.

Essmann *et al.* (2008) carried out a comparative analysis in tomato and revealed that activity of cell wall invertase activity was found to be maximum in heat tolerant varieties minimum in heat susceptible varieties under heat stress conditions.

Shi *et al.* (2017) observed the decrease in vacuolar invertase and cell wall invertase activity under high temperature condition across five rice genotypes namely N22, IR64, IR64 near – isogenic line and two hybrids, H2, H5. Under heat stress the

cell wall invertase activity was decreased in genotypes except for near isogenic line at 10 DAF and H2 at both 10 and 15 DAF and a significant increase in H5 at 10 DAF. Vacuolar invertase activity in the grain is also reduced with greater reduction recorded at 5 (61% to 91%) and 15 (68% to 92%) DAF, but there was less reduction at 10 DAF (5% to 47%) and an increase was recorded in N22 as it is tolerant to heat stress.

Sharma *et al.* (2018) based on his experimentation in two rice genotypes PAU-3699-13-2-1-1 and PR122 which were sown at two different dates (early and normal transplanting) are exposed to high temperature stress during grain filling stage and found that acid invertase activity was increased in both the genotypes that were transplanted early, while the alkaline invertase activity was decreased in both the genotypes that were transplanted early. Acid invertase activity in PAU-3699-13-2-1-1 and PR122 under normal transplanting condition was 1mg sucrose hydrolysed min^{-1} mg^{-1} protein and 0.50 mg sucrose hydrolysed min^{-1} mg^{-1} protein whereas under early transplanting condition at 15 DAA was increased showing 2mg sucrose hydrolysed min^{-1} mg^{-1} protein and 1mg sucrose hydrolysed min^{-1} mg^{-1} protein

Liu *et al.* (2016) examined whether increasing the native cell wall invertase activity genetically, would sustain fruit set in tomato under long term moderate heat stress condition for which he silenced the inhibitor gene of cell wall invertase activity and cultivated this transgenic tomato along with wild type under control (25/18°C, day/night, 14/10 h) and long term moderate heat stress conditions (25/18°C, day/night, 14/10 h). It was found that the cell wall invertase activity was increased under heat stress condition in transgenic tomato in both the ovaries (0.96 mg glucose h^{-1} mg^{-1} protein) and fruit (1.26 mg glucose h^{-1} mg^{-1} protein) when compared with that of the ovaries (0.68 mg glucose h^{-1} mg^{-1} protein) and fruit (0.95 mg glucose h^{-1} mg^{-1} protein) of the wild type tomato.

2.3 IMPACT OF HIGH TEMPERATURE STRESS ON YIELD PARAMETERS

2.3.1 Spikelet fertility percentage

Sterility of the spikelet's is mainly due to poor anther dehiscence and low pollen production, and hence low numbers of germinating pollen grains on the stigma (Weerakoon *et al.,* 2008)

Water deficit (Liu *et al.,* 2006) or heat stress (Jagadish *et al.,* 2007, 2008, 2010) at the time of flowering causes spikelet sterility in rice by affecting anther dehiscence, pollination, and pollen germination and reduced the spikelet fertility in all spikelet's by >55% in those outside the leaf sheath and by >90% in those trapped in the leaf sheath.

A key mechanism of high-temperature induced floret sterility in rice is the reduced ability of the pollen grains to swell thus resulting in poor thecae dehiscence (Matsui *et al.,* 2000).

In rice at heading stage a maximum temperature of 35.8 ^{0}C caused about 15% spikelet sterility (Hasegawa *et al.,* 2009), while a temperature of 38 ^{0}C induced 8–63% spikelet sterility across seven diverse rice genotypes (Jagadish *et al.,* 2014).

The effect of heat stress on spikelet fertility at the time of anthesis was studied in IR64 (lowland Indica) and Azucena (upland japonica) at 29.6 ^{0}C (ambient condition) and at 36 ^{0}C. It was found that in IR 64 the rate of spikelet's opening was increased by 20% but the spikelet fertility is reduced by 7% whereas in Azucena there is 36% reduction in number of spikelet's opening and 2.4% reduction in spikelet fertility under high temperature conditions (Jagadish *et al.,* 2007)

Matsui *et al.* (2001) conducted a study in rice tolerant and susceptible varieties and reported that heat stress at the time of reproductive stage decreased the spikelet number per unit dry weight, spikelet fertility, grain weight by accelerating senescence of panicle thus result in reduced grain yield. The difference of temperature between tolerant variety "Akitakomatch" (40 ^{0}C) and the susceptible variety "Hinohikari" (37 ^{0}C) that cause 50% spikelet fertility is 3 ^{0}C. Although heat tolerant varieties have been

found in both Indica and Japonica subspecies, it has been suggested that Indica types are more tolerant than Japonica ones to heat stress.

Jagdish *et al.* (2011) carried out an experiment in rice cultivar N22 by exposing it to heat stress alone and in combination with water stress and found that the spikelet fertility was lowest with heat stress. Under normal condition exerted spikelet showed higher spikelet fertility (>93%) than the trapped spikelet's (66%) but the fertility of exerted spikelet's was found to be lowest under heat stress (35%) than that of combined stress (44%). In the two experiments (1 and 2) followed the spikelet fertility of the exerted spikelet's under heat stress (28.7%, 43.5%) is lesser than that of the combined stress (65%, 73.8) from this it was concluded that heat stress had severe impact on spikelet fertility.

Krishnan *et al*. (2011) studied the effect of high temperature in two group of rice genotypes which include Group-I of Ranjit, Pooja, and Swarna that are suitable for lowland and Group-II of Annapurna, Khitish and Satabdi, are suitable for upland conditions and exposed these plants to six different temperature regimes such as 25/15,30/20, 35/25, 40/30, 45/35 and 50/40 ^{0}C with a constant diurnal temperature variation of 10 ^{0}C during panicle initiation and observed the decrease in number of spikelet's that flowered in a day with the increase in temperature, the decrease is more pronounced in low land genotypes compared to that of upland genotypes. At the temperature stress of above 40/30 ^{0}C the least number of spikelet flowering in a day was recorded by Swarna. The declines in spikelet fertility were more in Annapurna (5.03% per ^{0}C increase in temperature) and Khitish (5.13% per ^{0}C increase in temperature), with the extreme loss recorded in Swarna (6.26% per ^{0}C increase in temperature).

Two rice genotypes Nipponbare and its mutant High temperature susceptible (HTS) genotypes were subjected to high temperature stress of 40^{0}C at the time of anthesis and found that a sharp decrease in spikelet fertility was noted in Nipponbare (17.20%) and HTS (29.82%) when compared with their controls (90% and 80%) (Zhang *et al.,* 2018).

2.3.2 Yield per plant

Major part of the rice is presently cultivated in areas where the temperatures are found to be over the ideal for growth (28/ 22 ^{0}C). As far away rise in mean temperature during delicate stages cause drastic yield reduction in rice. In equatorial environments, elevated temperature is already one of the great environmental stresses restrict rice productivity, with relatively higher temperatures create depletion in grain weight and standard (Nagarajan *et al.,* 2010). Yields is estimated to reduce by 41% in rice under the exposure of high temperatures over short duration by the end of the 21st Century (Ceccarelli *et al.,* 2010). Nguyen 2014 reported that spikelet sterility under high temperatures during genital stage is one of the important factors to decrease rice yield under the projected global warming climate.

In rice yield is mostly depending on both vegetative (number of panicles per unit area) and reproductive (number of spikelets per panicle) phases. The real yield is to obtain at flowering and in the course of grain filling (filled spikelet percentage and weight per grain) phases (Abeysiriwardena *et al.,* 2002**)**.

Grain yield is a measurable quality that is considerably influenced by varied environmental factors. Rice yield is also set on by several agronomic characters which include days to heading, days to maturity, filled grain period, the number of reproductive tillers, number of grain filling per panicle, length of panicle, thousand grains pressure and grain force (Surek and Beser, 2005; Badshah *et al.,* 2014).

High temperature at the time of maturity in rice cause a reduction in tiller number when compared with that of ambient conditions (Oh-e *et al.,* 2007).

Highlevel temperatures during flowering and grain-filling phase declines the yield by giving rise to spikelet sterility and abridge the period of grain-filling phase (Xie *et al.,* 2009).

LU Guo-hua *et al.* (2013) imposed different temperature treatments such as 35 ^{0}C, 38 ^{0}C, 41 ^{0}C at flowering and grain filling stages of rice with the ambient condition maintained at 32 ^{0}C and found an average decrease in grain yield in all the treatments

from 15 to 73%. Lowest decrease in grain yield was recorded at flowering stage under 41 ^{0}C treatment with 0.62 g/plant when compared with that of grain filling stage under 41 ^{0}C temperature with 1.60 g/plant.

Lyman *et al.* (2013) proposed a study of cumulative exposure of rice crop to a day temperature of above 33 ^{0}C and to a night temperature of above 22 ^{0}C during the sensitive stages which include vegetative stage, early anthesis, early grain filling, and late grain filling and found that high temperature decreased the amount of paddy accessible for milling and also reduce the characteristic of outcome after milling. 1 ^{0}C rise in mean temperatures reduced the yield of paddy by 6.2%, head rice yield by 9.0% to 13.8%, total milled rice yield by 7.1% to 8.0%, and total milling revenue by 8.1% to 11.0%, across genotypes. Yield is divine to decrease by 13.5% and 30%, respectively with the rise in temperature by 2 ^{0}C and 3 ^{0}C.

Djanaguiraman *et al.* (2020) imposed short episodes of high temperature stress (32^{0}C) in spring wheat genotype Seri82 during anthesis and grain filling stage of development and found that the grain yield in the plants exposed to heat stress at grain filling stage is reduced by 44%.

Foliar application of polyamines remarkably increased the grain filling and yield, below conditions of high temperature pressure (increased by 51 % in field trials and 41 % in a greenhouse under heat strain at 10 days intervals) (Liu *et al.*, 2007).

2.3.3. Pollen viability

Pollen are the haploid gametophytes that are caused from diploid cells by meiosis in higher plants. In pollen development, meiosis is the stage that is really diplomatic to heat stress in plants (Oliver *et al.,* 2005). Heat tolerant genotypes translocate the assimilates from source to sink and unload those assimilates in the sink thus resulting in the production of fertile pollen and grain filling even under episodes of high temperature (Yang *et al.,* 2007).

Yield reduction in plants under heat stress is a result of spikelet degradation and sterility of pollen at the time of flowering (Yali and Zhiguo, 1997). Li *et al.* (2002)

appear that heat stress during early development stage resulted in sterile pollen production.

Huang *et al.,* (2004) stated that high temperature during rubric or flowering stage in rice had adverse result on pollen development but its effect on female gametophyte is little, hence pollen fertility acts as an index for heat-tolerance breeding and selecting in rice.

In rice high temperature of 33 ^{0}C disrupted the reproductive processes such as anther dehiscence, pollen shedding, pollen grains germination on stigma and expansion of pollen tubes as these processes are highly delicate. Similarly, high night temperature of 29 ^{0}C cause sterility in rice causing a subsequent decrease in seed set and grain yield (Ziska *et al.,* 1996).

Cao *et al.* (2008) treated two Indica rice varieties which differ in heat tolerance with high temperature and found that pollen viability was reduced significantly. Shuanggui 1 (heat-sensitive) and Huanghuazhan (heat-tolerant) were exposed to high temperature of above 35 ^{0}C with the control maintained at 33 ^{0}C resulted in reduced anther dehiscence in Shuanggui 1 whereas its outcome was little in Huanghuazhan as it is tolerant to heat it sustained under extreme temperature. The pollen viability in both Shuanggui 1 (63.7%) and Huanghuazhan (80.1%) was decreased under heat stress when compared with their ambient conditions (Shuanggui 1-77%; Huanghuazhan-82.9%) but the reduction was not significant in Huanghuazhan and it is minute.

Cao *et al.* (2009) in order to reveal the mechanism of heat tolerant cultivars in pollen development, conducted a study on two heat-delicate genotypes, Shuanggui 1 and T219, and two heat-forbearing genotypes, Huanghuazhan and T226, where he imposed heat stress of 33 ^{0}C temperature on the cultivars and found that there is significant reduction of the pollen viability in heat delicate genotypes. Under heat strain decrease in pollen viability is greater in heat delicate genotypes, Shuanggui 1 and T219 with 28.2% and 14.2%, respectively. There is not much influence of heat stress on heat tolerant genotypes hence the decrease in pollen viability is small in tolerant genotypes Huanghuazhan and T226 with 5.9% and 9.7% respectively.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled "Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)" was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani during the period from 2018-2020 with the objective to study the effect of high temperature stress on seed filling and nutritional quality of rice. The details of materials used and methods adopted for this experiment along with procedures carried out to do the laboratory analysis during course of experimentation are described in this chapter

3.1 TO STUDY THE EFFECT OF HIGH TEMPERATURE STRESS ON SEED FILLING AND NUTRITIONAL QUALITY OF RICE

3.1.1. Plant materials

Rice varieties used in the present study include Prathyasa, collected from Regional Agricultural Research Station, Moncombu, Hraswa and Manuratna collected from Agricultural Research Station, Mannuthy (Plate 1).

S.No	Variety	Salient features
1.	Hraswa	Obtained from IR-8 X T-140 Extra short duration: 75-80 days Bran and grain type: Red, Medium and bold Ideal as contingent variety for areas where there is crop loss, raised only as direct sown crop, susceptible to leaf folder
2.	Prathyasa (Mo 21)	Released from RRS, Moncombu Short duration: 100-110 days Bran and grain type: Red, Long bold

Plate 1. Germinated rice seeds of the rice varieties.

Hraswa

Prathyasa

Manuratna

		Non lodging, photo insensitive, semi tall variety, moderately tolerant to BPH, gall midge
3.	Manuratna	Released from ARS, Mannuthy Short duration: 100-105 days Meant for wet lands

3.1.2. Location

The study was conducted in the temperature controlled polyhouse maintained by Instructional Farm, Vellayani during 2018-2020.

3.1.3. Preparation of potting mixture and transplanting

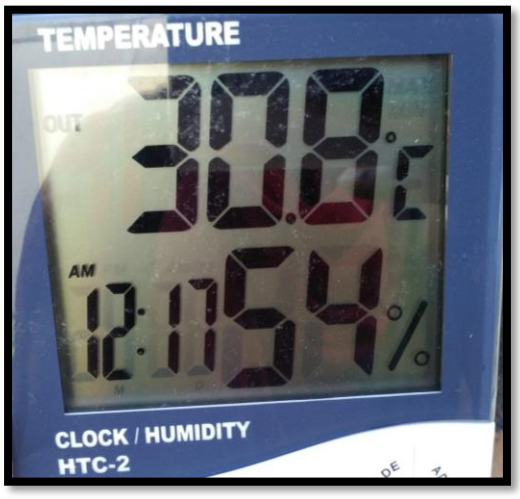
Earthen pots were filled with the potting mixture of soil, sand and FYM in the ratio of 1:1:1. Seeds were placed over the filter paper in glass bottles after attaining certain height the seedlings were raised in plastic pro-trays filled with soil and coir pith in the ratio of 2:1 (Plate 2). Foliar spray of 19:19:19 mixture was given on seedlings in pro-trays and also on 18DAS. Eighteen days old seedlings were transplanted to the pots at the rate of two seedlings per pot. Thinning and gap filling was done on 6^{th} DAT and one healthy seedling was maintained in each pot. Crop was applied with recommended dose of fertilizer as per package of practices of Kerala Agricultural University, Thrissur.

3.1.4. Methodology

The pots were kept under high temperature condition (5-6^0C more than ambient condition) in a temperature controlled polyhouse from seedling to maturity stage. Maximum and minimum temperature in control (31.61 ^{0}C and 23.97 ^{0}C respectively) and poly house (40.01 ^{0}C and 27.02 ^{0}C respectively) was measured daily using a thermo-hygrometer (Plate 3). Nutrient dosage was given as three splits and foliar spray of 19:19:19 mixture was given at tillering, panicle initiation, heading and flowering stage. Seed filling and enzyme parameters were taken at 30 and 50 days after 50% flowering and yield parameters were taken at harvest stage.

Plate 2. Seedlings of the rice varieties grown in bottles over the filter paper.

Plate 3. Thermo-hygrometer reading of maximum and minimum temperatures in control and polyhouse.



Outside polyhouse

Inside polyhouse

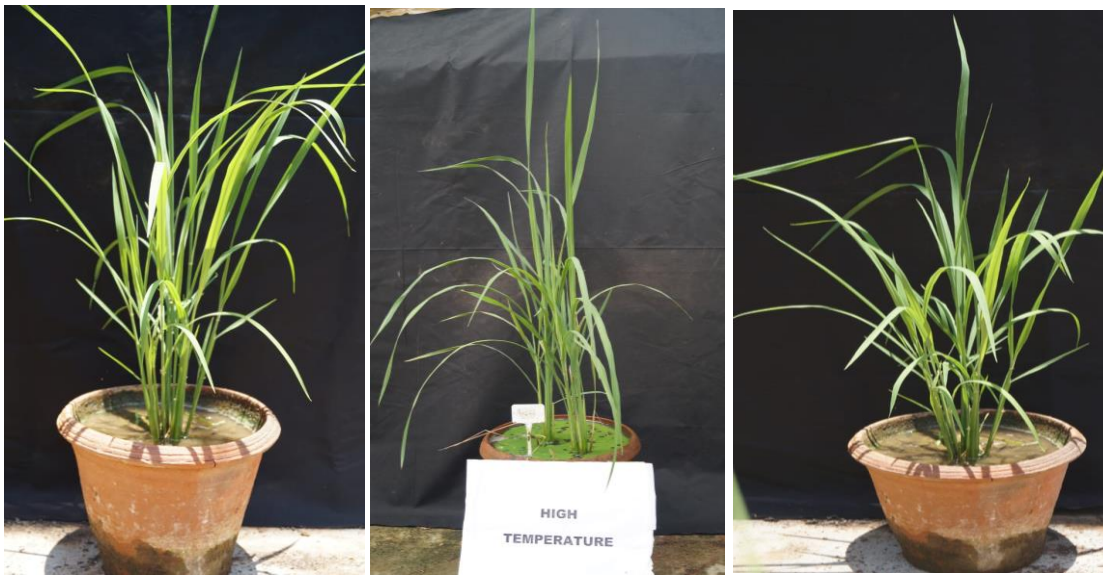
Plate 4. View of experimental unit of transplanted rice seedlings in control.

Plate 5. View of experimental unit of transplanted rice seedlings in polyhouse.

Plate 6. Rice varieties in control at tillering stage.

Hraswa **Prathyasa** **Manuratna**

Plate 7. Rice varieties in polyhouse at tillering stage.



Hraswa **Prathyasa** **Manuratna**

Particulars of pot culture experiment

1. Crop	Rice
2. Variety	1. Prathyasa 2. Hraswa 3. Manuratna
3. Design	CRD
4. Treatments	Two 1. Control 2. High temperature
5. Replications	Three

3.1.5. Observations

3.1.5.1. Seed quality parameters

3.1.5.1.1. Reducing sugars (Nelson and Somogyi method)

PRINCIPLE:

Reduced sugar when heated with alkaline copper tartrate lowers the copper from the cupric to the cuprous region and consequently the formation of cupide oxide. When cuprous oxide was treated with arsenomolybdic acid, a reduction of molybdic acid to molybdenum blue occurs. The enhanced blue colour was compared to the set of values in the colorimeter at 620 nm (Somogyi, 1952).

Materials

Alkaline Copper Tartrate

i) 2.5 g anhydrous sodium carbonate, 2 g sodium bicarbonate, 2.5 g potassium sodium tartrate and 20 g anhydrous sodium sulphate was dissolved in 80 mL water and the volume was made up to 100 mL.

ii) 15 g copper sulphate was dissolved in small volume of distilled water. One drop of sulphuric acid was added and made up to 100 mL.

4ML of B and 96 mL Solution A was mixed well before use.

Arsenomolybdate reagent: 2.5g ammonium molybdate was dissolved in 45 mL water. 2.5 mL sulphuric acid was added and mixed. Then 0.3 g di sodium hydrogen arsenate dissolved in 25 ml of water was mixed well and incubated at 37^0C for 24-48 hours.

Standard glucose solution: Stock: 100 mg in 100 mL distilled water.

Working standard: For working standard preparation 10 mL of stock was diluted to 100 ml with distilled water (100 µg / mL)

Procedure:

1.100 mg of sample was weighed and sugars with 80% hot ethanol extracted twice (5 ml, each time)

2 Supernatant was collected and kept on water bath at 80^0C for evaporation.

3. 10 mL of water was added to sugars to dissolve it.

4. Aliquots of 0.1 or 0.2 mL are pipetted to separate test tubes.

5. 0.2,0.4,0.6,0.8 and 1 mL of working standard solutions were pipetted into the series of test tube.

6. Sample and standard test tubes volumes were made upto 2 mL with distilled water.

7. 2 mL of distilled water was pipetted in a separate tube to set blank.

8. 1 mL alkaline copper tartrate reagent was added in each tube.

9. Tubes are placed in boiling water for 10 minutes.

10. Tubes were cooled and 1 mL of arsenomolybolic acid reagent was added to all the tubes.

11. Volume was made up to 10 ml in each tube with water.

12. Blue colour absorbance was read at 620 nm after 10 min.

13.A graph was drawn and amount of reducing sugars present in the sample were calculated.

Calculation

Absorbance was the same as 0.1 mL of test = x mg of glucose

$$10\text{mL contains} = \frac{X}{0.1} \times 10 \text{ mg of glucose} = \% \text{ of reducing sugars}$$

3.1.5.1.2. Carbohydrate (Anthrone method)

PRINCIPLE:

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. Glucose is dehydrated to hydroxymethyl furfural in hot acidic medium. Hydroxymethyl furfural forms a green coloured product with anthrone at an absorption maximum of 630 nm.

Materials:

- 2.5 N HCL
- Anthrone reagent: 200 mg of anthrone was dissolved in 100 mL of ice-cold 95% H_2SO_4.

  It was prepared fresh before the use.
- Standard glucose: Stock- 100 mg was dissolved in 100 mL water.

  Working standard- 10 mL of stock was diluted to 100 mL with distilled water. Few drops of toluene were added and the stored in refrigerator.

Procedure:

The total carbohydrates content was estimated by Anthrone method (Hodge and Hotreiter, 1962). 100 mg of sample was weighed and transferred into a boiling tube. It was hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5 N HCL and cooled to room temperature. The volume was made up to 100mL and centrifuged after neutralising it with solid sodium carbonate until the effervescence was stopped. 0.5 and 1mLof supernatant were used for the estimation of carbohydrates. The

volume was made up to 1 ml and 4 ml of anthrone reagent was added. After heating for 8 minutes, it was cooled rapidly and the optical density of the green to dark green colour was read in a spectrophotometer at 630 nm. The amount of carbohydrates present in the sample was estimated using the standard curve prepared from standard glucose and the amount of carbohydrate as mg per 100 mg of sample was computed as:

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

3.1.5.1.3 Starch

Starch accumulation in seed under control and stress condition was estimated using anthrone reagent as per the protocol given by Sadasivam and Manickam (1992).

PRINCIPLE:

The seed samples were treated with 80% hot alcohol to get rid of sugars and then extraction of starch was done using per chloric acid. In hot acidic medium starch gets hydrolysed to glucose and further dehydration leads to the formation of hydroxymethyl furfural. Hydroxymethyl furfural has the ability to form green coloured product with anthrone.

Material:

- 80% ethanol
- 52% per chloric acid
- Standard Glucose: Stock 100 mg in 100 mL water.
  Working standard – 10mL of stock diluted to 100mL with water.
- Anthrone: Dissolve 200mg anthrone in 100mL of ice-cold 95% sulphuric acid.

Methodology:

1. 0.5 g of sample was taken for extraction and homogenized in hot 80% ethanol to remove sugars. Residue after centrifuging was retained and the procedure was repeated

till the washings do not give colour with anthrone reagent. The residue obtained was dried well over a water bath.

2. To the residue obtained 5 mL of water and 6.5 mL of 52% per chloric acid was added.

3. Samples at 0°C for 20 min were extracted, centrifuged and supernatant was saved.

4. The same extraction was repeated using fresh per chloric acid. Centrifuged and supernatants were pooled and made up to 100 mL.

5. 0.1 mL of the supernatant was taken and made up the volume to 1mL with water.

6. Standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard and made up the volume to 1mL in each tube with water.

7. 4mL of anthrone reagent is added to series of tubes.

8. Heated for eight minutes in boiling hot water.

9. Cooled rapidly and read the intensity of green to dark green colour at 630nm.

3.1.5.1.4 Amylose

PRINCIPLE:

The iodine is adsorbed within the helical coils of amylose and produce a blue coloured complex which is measured colorimetrically (Mc Cready *et al.*, 1950)

Materials:

- 1 N NaOH
- 0.1% phenolphthalein
- Iodine reagent: 1 g iodine and 10 g KI was dissolved in water and made up to 500 mL.
- Standard: 100 mg amylose is dissolved in 10 mL 1N NaOH and made up to 100 mL with water.

Procedure:

100 mg of powdered sample was weighed and 1mL of distilled ethanol, 10 mL of 1N NaOH was added and left overnight. Volume was made up to 100 mL. 2.5 mL of the extract was taken and about 20 mL distilled water was added, in addition three drops of phenolphthalein was also added. 0.1 N HCL was added drop by drop to the above solution until the pink colour just disappeared. Then 1 mL of iodine reagent was added, and the volume was made up to 50 mL. Colour readings were taken at 590 nm. 1 mL of iodine reagent was diluted to 50 mL with distilled water forblank.0.2,0.4,0.6,0.8 and 1 ml of the standard amylose solution were taken for the colour development (like sample). Amylose content in the sample was calculated from the graph readings.

Calculation:

Absorbance corresponds to 2.5 mL of the test solution

= x mg amylose 100 mL contains

$$= \frac{X}{2.5} \times 100 \text{ mg amylose} = \% \text{ amylose}$$

3.1.5.1.5. Seed protein

Total soluble proteins were estimated using Bradford method (1976). The assay is based on the ability of proteins to bind Coomassie brilliant blue G 250 and a complex is formed whose extinction coefficient is much greater than that of free dye. Seed samples were collected at fifteen and thirty days after fifty percent flowering. The total soluble protein from 500 mg of seed samples were extracted using 10 mL of Phosphate buffer saline (PBS) solution. The extracts were collected after centrifugation and 0.1 mL of extract was taken and volume was made up to 3 mL by adding PBS. Dye (5 mL) solution was added to each sample. The solution was mixed well and allowed to develop blue colour. The red dye turned blue when it binds with protein and its absorbance was read at 595 nm. Bovine serum albumin was used as the protein standard and protein concentration was calculated and expressed as %.

3.1.5.1.6. Anthocyanin

Estimation of anthocyanin was done by as per the method described by Ranganna (1976). The initial step was alcoholic extraction of the seed material. One gram of the sample from each treatment was extracted with ethanolic HCL. The sample was later centrifuged and the supernatant was then diluted with ethanolic HCL to 50 mL to yield the optical density measurements within the optimum range of the spectrophotometer (535 nm). The anthocyanin content was then calculated using the following relationship and quantity was expressed as mg per 100 g of the sample.

$$\text{Total OD per 100g of sample(x)} = \frac{\text{Absorbance at 535 nm} \times \text{volume made up of the extract used for colour development} \times \text{Total Volume} \times 100}{\text{Volume (ml of the extract) used} \times \text{Weight of the sample taken}}$$

The absorbance of a solution containing 1 mg per mL is equal to 98.2 (constant)

Therefore,

$$\text{Total anthocyanin in mg per 100 g of the sample} = \frac{x}{98.2}$$

3.1.5.1.7. Total flavonoids

PRINCIPLE:

Flavonoids develop a brick red colour with $AlCl_3$ and $NaNO_2$ at alkaline pH. The absorbance of the complex is read at 510 nm.

Reagents

- 80% methanol
- 10% Aluminium chloride (AlCl3)

- 5% Sodium nitrite (NaNO2)
- 4N Sodium hydroxide (NaOH)
- Standard quercetin in water (20 - 100µg/mL)

Procedure:

Estimation of flavonoids was done as per the method described by Ordonez *et al.* (2006). Sample of 0.5g was homogenized with methanol (80%) at 40^0C and centrifuged at 4,500 rpm for 15 min after cooling it down to room temperature. 0.5 mL of the supernatant was taken and to it 0.5 mL of 80% methanol was added along with 4 mL distilled water. Then0.3 mL of 5% Sodium nitrite ($NaNO_2$) was added and incubated for 5 min later 0.3 mL of 10% Aluminium chloride ($AlCl_3$)was added and the solution was allowed to stand for 6 min. 2mL of 1 M Sodium hydroxide (NaOH) was added to stop the reaction and the volume was made up to 10 mL with distilled water. The sample was allowed to stand at room temperature for 15 minutes and the absorbance of pink colour at 510 nm was taken against blank. Catechin or quercetin was used as standard.

Calculation:

$$= \frac{\text{Total flavonoid content (mg Catechin equivalents/100g)} \times \text{Std. value (OD}_{510}\text{ (mg/OD))} \times \text{Total Volume of extract} \times 100}{\text{Assay volume} \times \text{Weight of the sample taken (g)}}$$

3.1.5.2. Enzymes

3.1.5.2.1. Invertase

Invertase activity was estimated as per the procedure given by Morris and Arthur (1984). 0.5 g of the sample was macerated with 2mL of ice cold 100 mM Acetate buffer (pH-5.0). The contents were centrifuged at 2500 g for 20 minutes at 4^0C. Take 0.2 mL of supernatant and to it add 0.8 mL of 0.1M sucrose and incubate for 30 minutes at 30^0C. With the addition of 1mL of Somogyi's copper reagent the reaction was stopped.

Later the contents were boiled for 10 minutes. Blank was run without sucrose. The reaction mixture was cooled and 1 mL of arsenomolybdate reagent was added. The absorbance was taken at 630nm. The enzyme activity was expressed as n moles min^{-1} g^{-1}.

3.1.5.3. Yield parameters

3.1.5.3.1. Spikelet fertility percentage (%)

The total numbers of filled and unfilled spikelets of three randomly selected primary tillers of the target plants in each treatment were counted. Then, Spikelet fertility (%) was calculated by using the formula

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of fertile spikelet's}}{\text{Total No. of spikelet's}} \times 100$$

3.1.5.3.2. Yield per plant

The grain yield obtained from each replication by total number of filled grains expressed in grams.

3.1.5.4. Pollen viability (%)

Pollen viability was measured by using 1% iodine- potassium iodide (IKI) solution which was prepared by dissolving 2.5 g of KI with 250 mg of iodine and made up to 125 mL. Just before anthesis, spikelets were collected from each treatment. Then the anthers were crushed and stained by using IKI solution in a glass slide. Fully stained pollen indicates the fertile pollen and unstained, shrivelled, empty grains indicate sterile pollen. Fertile pollen grains were visually counted under compound microscope, Leica. The pollen viability was calculated by using the formula given below and expressed as percentage.

$$\text{Pollen viability} = \frac{\text{Number of pollen grains stained}}{\text{Total number of pollen grains}} \times 100$$

RESULTS

4. RESULTS

This chapter describes the results obtained from the pot culture experiment conducted to study the effect of heat stress on seed filling and nutritional quality of rice (*Oryza sativa* L.). This experiment was carried out in the Department of Seed Science and Technology, College of Agriculture, Vellayani during 2018-20. The data obtained during the course of investigation were statistically analysed and the results are presented with suitable tables.

4.1 EFFECT OF HIGH TEMPERATURE ON SEED QUALITY PARAMETERS

4.1.1 Reducing sugars (mg g^{-1})

Significant varietal difference for reducing sugar content was observed in rice varieties under high temperature. There was significant variation for reducing sugars between the treatments. The overall increase in reducing sugar content for varieties under high temperature was 9.45% when compared to ambient condition. Under control and high temperature conditions of both stages, Hraswa recorded the highest and Prathyasa recorded the lowest reducing sugar content. Under milky stage of control condition, the reducing sugar content of Hraswa and Prathyasa were (12.79 mg g^{-1} fresh weight), (12.14 mg g^{-1} fresh weight) respectively. Under dough stage of control condition, the reducing sugar content of Hraswa and Prathyasa were (15.54 mg g^{-1} fresh weight) and (14.29 mg g^{-1} fresh weight) respectively. But under heat stress condition of milky stage, reducing sugar content of Hraswa and Prathyasa were (13.89 mg g^{-1} fresh weight), (13.36 mg g^{-1} fresh weight) respectively. Under heat stress condition of dough stage, reducing sugar content of Hraswa and Prathyasa were (16.71 mg g^{-1} fresh weight) and (15.61 mg g^{-1} fresh weight) respectively (Figure 1).

The percent increase in reducing sugar content was more in Manuratna and less in Hraswa at both milky and dough stages. During milky stage the percent increase in reducing sugar of Manuratna and Hraswa were (11.48 %), (8.55 %) respectively. During dough stage, percent increase in reducing sugar content of Manuratna and Hraswa were (9.79 %), (7.58%) respectively. The average reducing sugar content of the rice varieties were 13.54 mg g^{-1} fresh weight and 14.81 mg g^{-1} fresh weight under control and heat stress conditions respectively (Table 1).

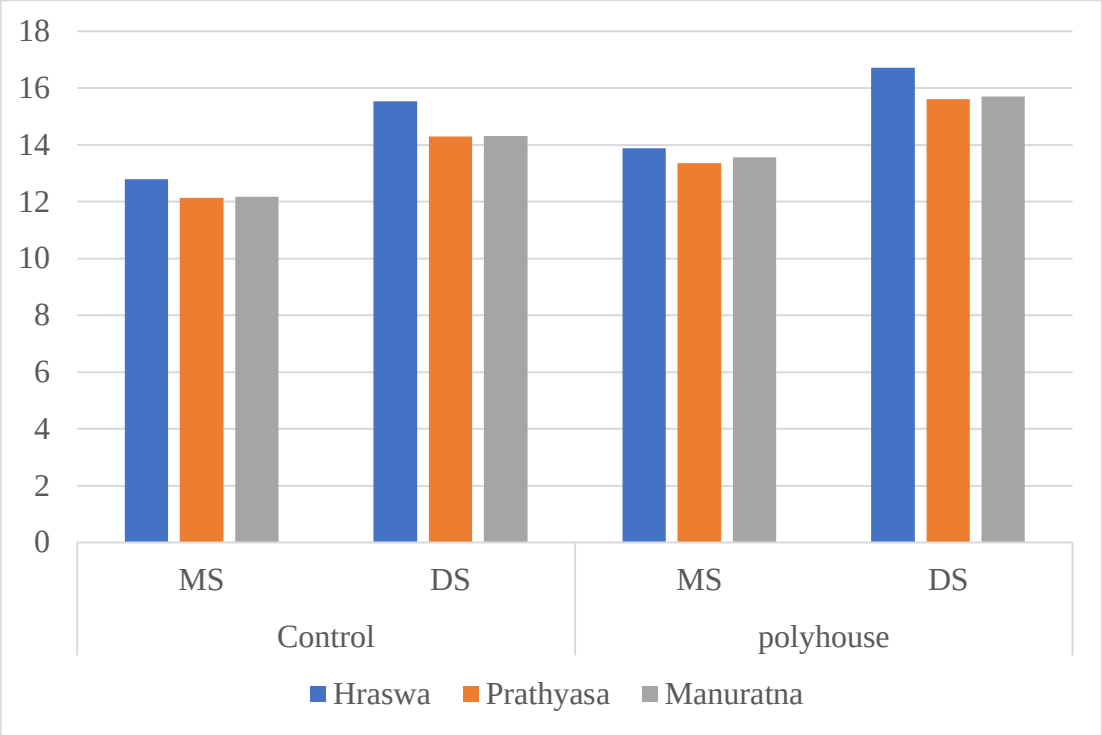


Figure 1: Variation in reducing sugar content (mg g^{-1}) of rice varieties at milky and dough stages under high temperature.

4.1.2 Carbohydrate (mg g^{-1})

The carbohydrate content showed an increment under high temperature in all the rice varieties as compared to ambient condition. Significant varietal difference for carbohydrates was observed among the rice varieties under high temperature. The overall increase in carbohydrate content under heat stress condition for varieties was 20.10% when compared with that of control. There was significant variation for carbohydrate content between the treatments. Under ambient condition and high temperature conditions of both stages, Prathyasa recorded the highest and Hraswa recorded the lowest carbohydrate content. Under milky stage of control condition, the carbohydrate content of Prathyasa and Hraswa were (751.40 mg g^{-1} fresh weight), (742.28 mg g^{-1} fresh weight) respectively. Under dough stage of ambient condition, the carbohydrate content of Prathyasa and Hraswa were (753.97 mg g^{-1} fresh weight) and (745.12 mg g^{-1} fresh weight) respectively. But under heat stress condition of milky stage, carbohydrate content of Prathyasa and Hraswa were (905.27 mg g^{-1} fresh weight), (891.78 mg g^{-1} fresh weight) respectively. Under heat stress condition of dough stage, carbohydrate content of Prathyasa and Hraswa were (903.38 mg g^{-1} fresh weight) and (889.89 mg g^{-1} fresh weight) respectively (Figure 2).

The percent increase in carbohydrate content was same for all varieties under milky and dough stages. The average carbohydrate content of the rice varieties were 747.93 mg g^{-1} fresh weight and 898.31 mg g^{-1} fresh weight under ambient condition and heat stress conditions respectively. (Table 2).

4.1.3 Starch (mg g^{-1})

The plants grown under heat stress conditions showed an increase in starch content when compared to control plants. Significant varietal differences for starch content was observed in rice varieties under high temperature. There was significant variation for starch content between the treatments. The overall increase in starch content for varieties under heat stress condition was 58.18 % when compared to ambient condition. Among the varieties under control conditions of both stages, Manuratna recorded the highest and Hraswa recorded the lowest starch content. Under milky stage of ambient condition, the starch content of Manuratna and Hraswa were (11.24 mg g^{-1}

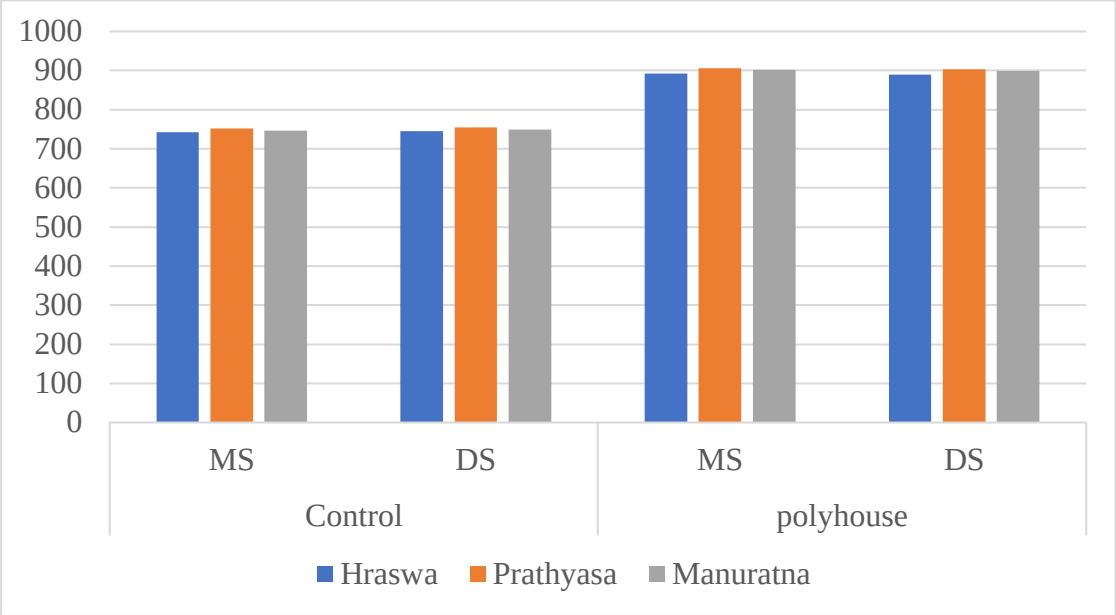


Figure 2: Variation in carbohydrate content (mg g^{-1}) of rice varieties at milky and dough stages under high temperature.

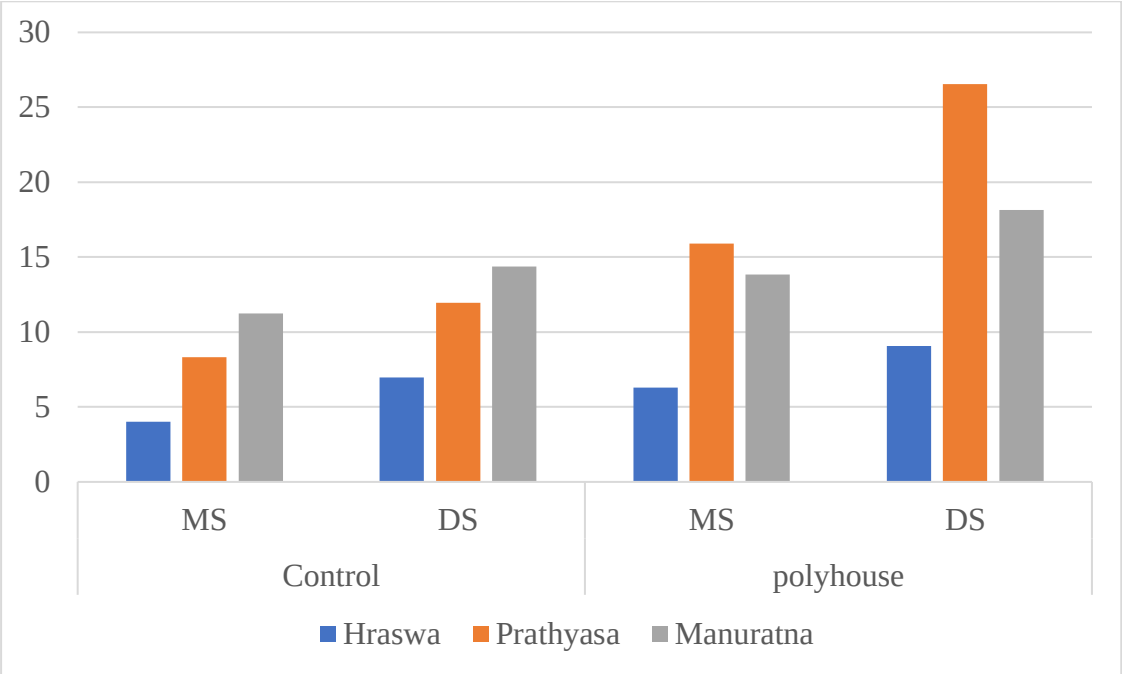


Figure 3: Variation in starch content (mg g^{-1}) of rice varieties at milky and dough stages under high temperature.

fresh weight), (4.01 mg g^{-1} fresh weight) respectively. Under dough stage of control condition, the starch content of Manuratna and Hraswa were (14.38 mg g^{-1} fresh weight) and (6.97 mg g^{-1} fresh weight) respectively. Under heat stress conditions of both stages, Prathyasa recorded the highest and Hraswa recorded the lowest starch content. Under milky stage of heat stress condition, the starch content of Prathyasa and Hraswa were (15.90 mg g^{-1} fresh weight), (6.30 mg g^{-1} fresh weight) respectively. Under heat stress condition of dough stage, starch content of Prathyasa and Hraswa were (26.53 mg g^{-1} fresh weight) and (9.06 mg g^{-1} fresh weight) respectively (Figure 3).

The percent increase in starch content was more in Prathyasa and less in Manuratna at both milky and dough stages. During milky stage the percent increase in starch of Prathyasa and Manuratna were (90.85 %), (23.01 %) respectively. During dough stage, percent increase in starch content of Prathyasa and Manuratna were (122.17 %), (26.01%) respectively. The average starch content of the rice varieties was 9.48 mg g^{-1} fresh weight and 14.96 mg g^{-1} fresh weight under ambient condition and heat stress conditions respectively (Table 3).

4.1.4 Amylose (%)

Starch include amylose and amylopectin of which amylose infers to the textural property of the grain. The amylose content significantly reduced at high temperature in all the rice varieties as compared to ambient condition. There was significant variation for amylose content between the treatments. The overall decrease in amylose content for varieties under high temperature conditions was 26.93% when compared to ambient temperatures. Significant varietal differences for amylose content was observed in rice varieties under high temperature. Among the varieties, Manuratna (22.53 % fresh weight) recorded the maximum amylose content and Hraswa (22.37 % fresh weight) recorded minimum amylose content under ambient condition at milky stage, while Hraswa (27.43 % fresh weight) and Prathyasa (25.35 % fresh weight) recorded maximum and minimum amylose content at dough stage for plants grown under control conditions. But under heat stress conditions, Hraswa (18.67 % fresh weight) recorded the maximum amylose content and Prathyasa (15.45 % fresh weight) recorded the minimum amylose content at milky stage while Hraswa (20.10 % fresh weight) and

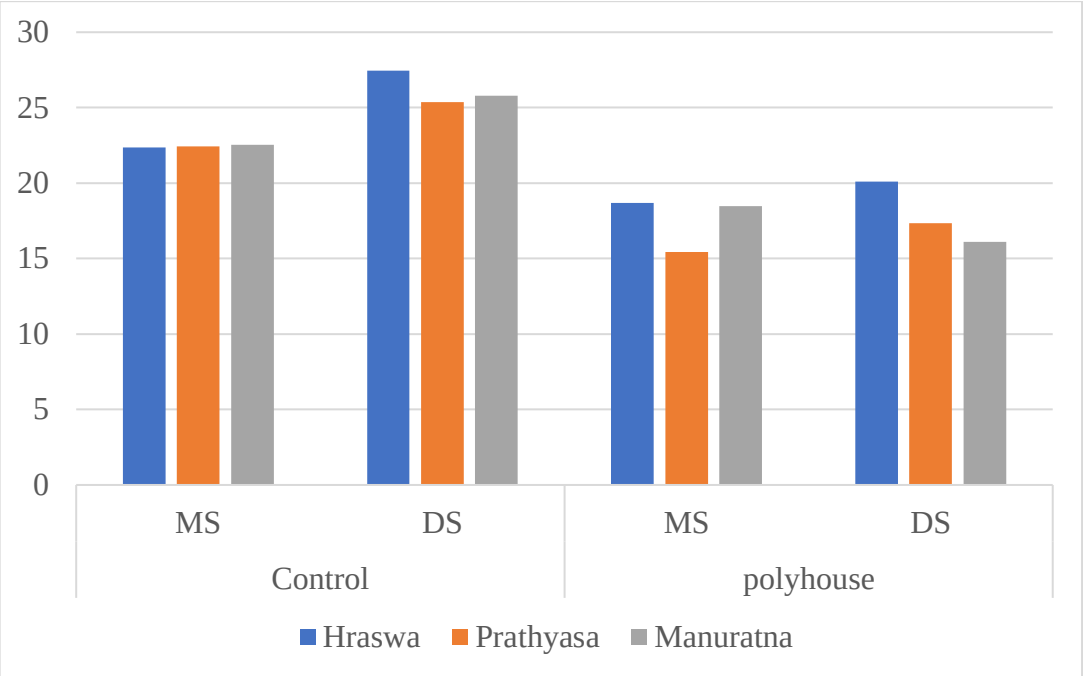


Figure 4: Variation in amylose content (%) of rice varieties at milky and dough stages under high temperature.

Manuratna (16.11 % fresh weight) recorded maximum and minimum amylose content for plants grown under high temperature conditions (Figure 4).

The percent decrease in amylose content was more in Prathyasa (31.15 %) and less in Hraswa (16.52 %) during milky stage and percent decrease in amylose content was more in Manuratna (37.54 %) and less in Hraswa (26.72 %) at dough stage. The average amylose content of the rice varieties was 24.32 % fresh weight under ambient condition and 17.69 % fresh weight under heat stress conditions respectively. (Table 4).

4.1.5 Seed Protein (%)

Seed protein content estimated in the grain decreased under heat stress condition. Significant varietal difference for seed protein content was observed in rice varieties under high temperature. There is significant variation for seed protein content between the treatments. The overall decrease in seed protein content for varieties under high temperature conditions was 41.45 % when compared with that of ambient condition. Among the varieties, Hraswa (7.21 % fresh weight) recorded the maximum seed protein content and Prathyasa (5.05 % fresh weight) recorded minimum seed protein content under control condition at milky stage, while Manuratna (9.04 % fresh weight) and Prathyasa (7.23 % fresh weight) recorded maximum and minimum seed protein content at dough stage for plants grown under ambient condition. But under heat stress conditions of both stages, Hraswa recorded the highest and Manuratna recorded the lowest seed protein content. Under milky stage of heat stress condition, the seed protein content of Hraswa and Manuratna were (6.59 % fresh weight), (1.93 % fresh weight) respectively. Under heat stress condition of dough stage, seed protein content of Hraswa and Manuratna were (6.08 % fresh weight) and (2.69 % fresh weight) respectively (Figure 5).

The percent decrease in seed protein content was more in Manuratna and less in Hraswa at both milky and dough stages. During milky stage the percent decrease in seed protein of Manuratna and Hraswa are (71.38 %), (8.65 %) respectively. During dough stage, percent decrease in seed protein content of Manuratna and Hraswa are (70.28 %), (23.50 %) respectively. The average seed protein content of the rice varieties were 7.20

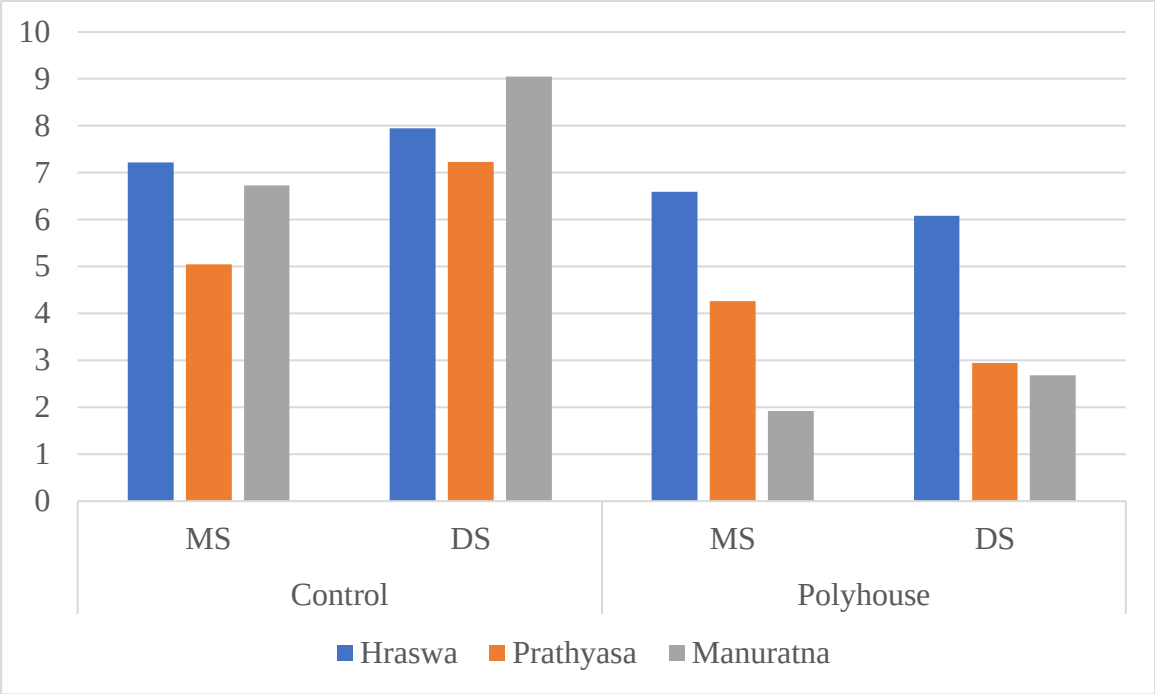


Figure 5: Variation in seed protein content (%) of rice varieties at milky and dough stages under high temperature.

% fresh weight and 4.08 % fresh weight under control and heat stress conditions respectively. (Table 5).

4.1.6 Anthocyanin (mg 100 g^{-1})

Anthocyanin is a pigment that provide colour to fruits and it is estimated in the grain and was found that it was decreased under high temperature condition. Significant varietal difference for anthocyanin content was observed in rice varieties under high temperature. There was significant variation for anthocyanin content between the treatments. The overall decrease in anthocyanin content for varieties under high temperature conditions was 29.09 % when compared with that of ambient condition. Among the varieties, Manuratna (30.80 mg 100 g^{-1} fresh weight) recorded the maximum anthocyanin content and Hraswa (24.86 mg 100 g^{-1} fresh weight) recorded minimum anthocyanin content under ambient condition at milky stage, while Prathyasa (16.94 mg 100 g^{-1} fresh weight) and Hraswa (14.73 mg 100 g^{-1} fresh weight) recorded maximum and minimum anthocyanin content at dough stage for plants grown under control conditions. But under heat stress conditions of both stages, Manuratna recorded the highest and Hraswa recorded the lowest anthocyanin content. Under milky stage of heat stress condition, the anthocyanin content of Manuratna and Hraswa are (23.55 mg 100 g^{-1} fresh weight), (17.89 mg 100 g^{-1} fresh weight) respectively. Under dough stage of heat stress, the anthocyanin content of Manuratna and Hraswa are (14.88 mg 100 g^{-1} fresh weight) and (8.21 mg 100 g^{-1} fresh weight) respectively (Figure 6).

The percent decrease in anthocyanin content was more in Prathyasa and less in Manuratna at both milky and dough stages. During milky stage the percent decrease in anthocyanin of Prathyasa and Manuratna are (33.07 %), (23.51%) respectively. During dough stage, percent decrease in anthocyanin content of Prathyasa was (45.80 %). The average anthocyanin content of the rice varieties was 21.82 mg 100 g^{-1} fresh weight and 15.49 mg 100 g^{-1} fresh weight under ambient condition and heat stress conditions respectively (Table 6).

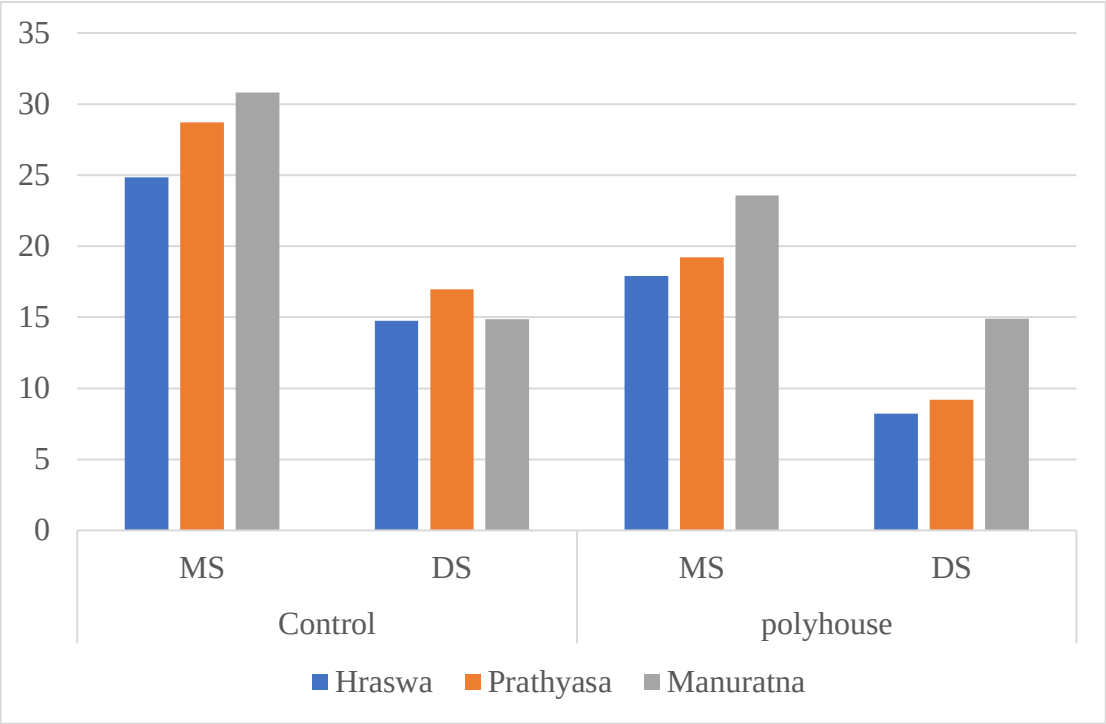


Figure 6: Variation in anthocyanin content (mg 100 g^{-1}) of rice varieties at milky and dough stages under high temperature.

4.1.7 Flavonoid (mg 100 g^{-1})

Significant varietal difference for flavonoid content was observed in rice varieties under high temperature. Flavonoids are the secondary metabolites that help in defence, were estimated in the grain. There was significant variation for flavonoid content between the treatments. The overall increase in flavonoid content for varieties under high temperature conditions was 35.61 % when compared with that of ambient condition. Among the varieties under control conditions of both stages, Hraswa recorded the highest and Manuratna recorded the lowest flavonoid content. Under milky stage of control condition, the flavonoid content of Hraswa and Manuratna were (17.40 mg 100 g^{-1} fresh weight), (12.48 mg 100 g^{-1} fresh weight) respectively. Under ambient condition of dough stage, flavonoid content of Hraswa and Manuratna were (7.93 mg 100 g^{-1} fresh weight) and (6.58 mg 100 g^{-1} fresh weight) respectively. But under heat stress conditions, Hraswa (20.08 mg 100 g^{-1} fresh weight) recorded maximum flavonoid content and Prathyasa (16.20 mg 100 g^{-1} fresh weight) recorded the minimum flavonoid content at milky stage while Manuratna (12.70 mg 100 g^{-1} fresh weight) and Prathyasa (9.03 mg 100 g^{-1} fresh weight) recorded maximum and minimum flavonoid content at dough stage for plants grown under high temperature conditions (Figure 7).

The percent increase in flavonoid content was more in Manuratna and less in Prathyasa at both milky and dough stages. During milky stage the percent increase in flavonoid of Manuratna and Prathyasa were (39.19 %), (5.24 %) respectively. During dough stage, percent increase in flavonoid content of Manuratna and Prathyasa are (92.85 %), (23.37 %) respectively. The average flavonoid content of the rice varieties was 11.18 mg 100 g^{-1} fresh weight and 14.38 mg 100 g^{-1} fresh weight under ambient condition and heat stress conditions respectively. (Table 7).

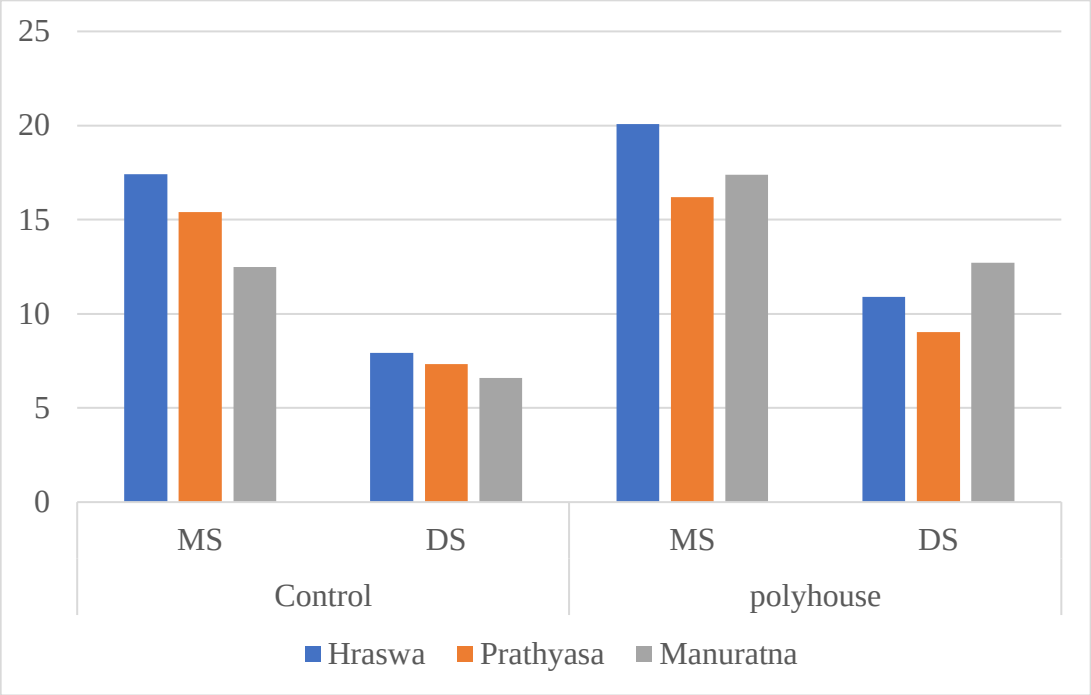


Figure 7: Variation in flavonoid content (mg 100 g^{-1}) of rice varieties at milky and dough stages under high temperature.

Table 1. Reducing sugar (mg g^{-1}) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	12.79	15.54	13.89	16.71	14.73
V_2	12.14	14.29	13.36	15.61	13.85
V_3	12.16	14.30	13.56	15.71	13.93
Mean	13.54		14.81		
	CD (0.05): C= 0.48, V= 0.58, CxB= N/A; CxV= N/A; BxV= N/A				
	SE±(m): B= 0.17, V= 0.20, CxB= 0.24; CxV= 0.29; BxV= 0.29				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

Table 2. Total carbohydrate (mg g^{-1}) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	742.28	745.12	891.78	889.89	817.27
V_2	751.40	753.97	905.27	903.38	828.50
V_3	746.11	748.72	901.13	898.40	823.59
Mean	747.93		898.31		
	CD (0.05): C= 0.70, V= 0.85, CxB= 0.99; CxV= 1.21; BxV= N/A				
	SE±(m): B= 0.24, V= 0.30, CxB= 0.35; CxV= 0.42; BxV= 0.42				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

Table 3. Starch content (mg g^{-1}) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	4.01	6.97	6.30	9.06	6.58
V_2	8.33	11.94	15.90	26.53	15.67
V_3	11.24	14.38	13.83	18.13	14.39
Mean	9.482		14.961		
	CD (0.05): C= 0.42, V= 0.51, CxB= 0.59; CxV=0.73; BxV= 0.73				
	SE±(m): B= 0.14, V=0.18, CxB= 0.21; CxV= 0.25; BxV= 0.25				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

Table 4. Amylose content (%) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	22.37	27.43	18.67	20.10	22.14
V_2	22.44	25.35	15.45	17.33	20.14
V_3	22.53	25.79	18.46	16.11	20.72
Mean	24.32		17.69		
	CD (0.05): C= 0.53, V= 0.65, CxB= 0.75; CxV=0.92; BxV= 0.92				
	SE±(m): B= 0.18, V= 0.23, CxB= 0.26; CxV= 0.32; BxV= 0.32				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

Table 5. Seed protein content (%) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	7.21	7.94	6.59	6.08	6.96
V_2	5.05	7.22	4.26	2.93	4.87
V_3	6.72	9.04	1.92	2.68	5.09
Mean	7.20		4.08		
	CD (0.05): C= 0.32, V= 0.39, CxB= 0.45; CxV=0.55; BxV= 0.55				
	SE±(m): B= 0.11, V= 0.13, CxB= 0.16; CxV= 0.19; BxV= 0.19				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

Table 6. Anthocyanin content (mg 100 g^{-1}) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	24.86	14.73	17.89	8.21	16.42
V_2	28.69	16.94	19.20	9.18	18.50
V_3	30.80	14.87	23.55	14.88	21.02
Mean	21.82		15.49		
	CD (0.05):C= 0.41, V= 0.50, CxB= 0.58; CxV= 0.71; BxV= 0.71				
	SE±(m): B= 0.14, V= 0.17, CxB= 0.20; CxV= 0.25; BxV= 0.25				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

Table 7. Flavonoid content (mg 100 g^{-1}) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	17.40	7.93	20.08	10.91	14.08
V_2	15.40	7.31	16.20	9.02	11.98
V_3	12.48	6.58	17.37	12.70	12.28
Mean	11.18		14.38		
	CD (0.05): C= 0.37, V= 0.45, CxB= 0.52; CxV= 0.64; BxV= 0.64				
	SE±(m): B= 0.13, V= 0.16, CxB= 0.18; CxV= 0.22; BxV= 0.22				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

4.2 EFFECT OF HIGH TEMPERATURE ON ENZYME

4.2.1 Invertase (nmoles min^{-1} g^{-1})

Invertase is an enzyme that was estimated in the leaf at 15 and 30 days after 50% flowering. Significant varietal difference for invertase activity was observed in rice varieties under high temperature. There was significant variation for invertase activity between the treatments. The overall decrease in invertase activity for varieties under high temperature conditions was 28.42 % when compared with that of ambient condition. Under control conditions of both stages, Hraswa recorded the highest and Prathyasa recorded the lowest invertase activity. Under milky stage of ambient condition, the invertase activity of Hraswa

and Prathyasa are (43.67 n moles min^{-1} g^{-1} fresh weight), (34.14 n moles min^{-1} g^{-1} fresh weight) respectively. Under dough stage of ambient condition, the invertase activity of Hraswa and Prathyasa are (38.54 n moles min^{-1} g^{-1} fresh weight) and (32.08 n moles min^{-1} g^{-1} fresh weight) respectively. But under heat stress conditions of both stages, Hraswa recorded the highest and Manuratna recorded the lowest invertase activity. Under milky stage of heat stress condition, the invertase activity of Hraswa and Manuratna were (31.05 n moles min^{-1} g^{-1} fresh weight), (24.96 n moles min^{-1} g^{-1}fresh weight) respectively. Under heat stress condition of dough stage, invertase activity of Hraswa and Manuratna were (33.71 n moles min^{-1} g^{-1} fresh weight) and (20.28 n moles min^{-1} g^{-1} fresh weight) respectively (Figure 8).

The percent decrease in invertase activity was more in Manuratna and less in Hraswa at both milky and dough stages. During milky stage the percent decrease in invertase activity of Manuratna and Hraswa are (33.12 %), (22.79 %) respectively. During dough stage, percent decrease in invertase activity of Manuratna and Hraswa were (40.20 %), (19.41 %) respectively. The average invertase activity of the rice varieties was 36.61 n moles min^{-1} g^{-1} fresh weight and 26.35 n moles min^{-1} g^{-1} fresh weight under control and heat stress conditions respectively. (Table 8).

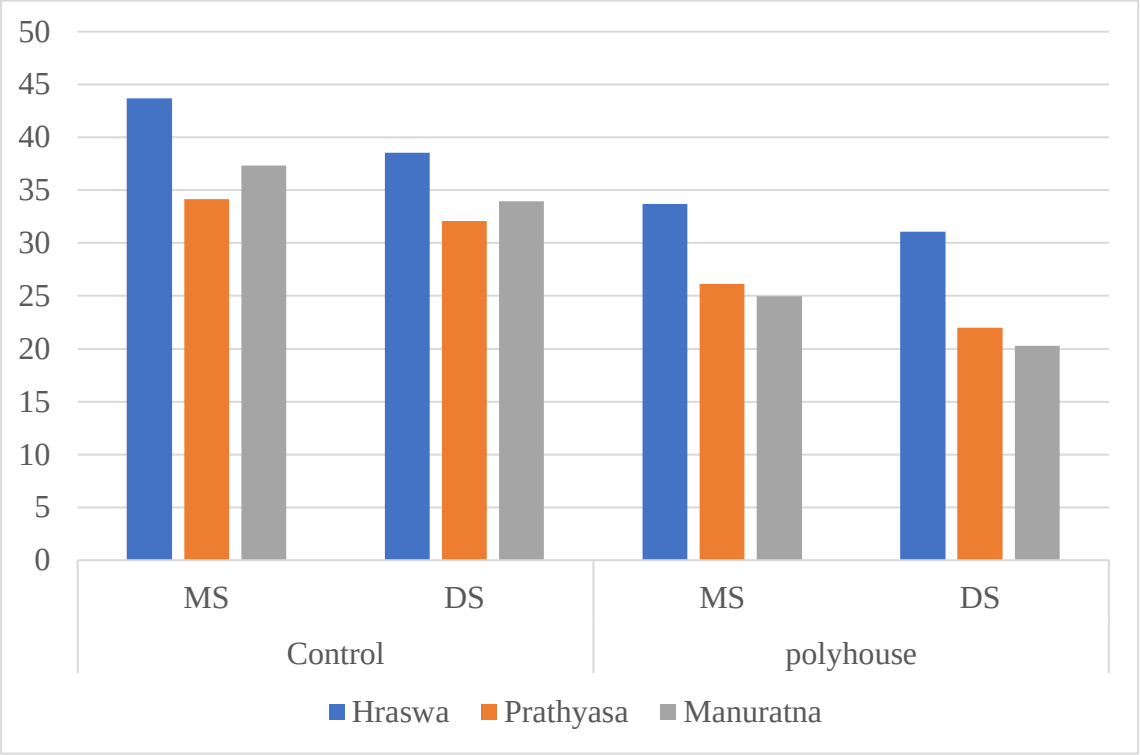


Figure 8: Variation in invertase activity (n moles min^{-1} g^{-1}) of rice varieties at milky and dough stages under high temperature.

Table 8. Invertase activity (n moles min^{-1} g^{-1}) at milky and dough stage in rice under high temperature:

	Control (C_1)		Polyhouse (C_2)		Mean (z)
VARIETIES	**Milky stage (B_1)**	**Dough stage (B_2)**	**Milky stage (B_1)**	**Dough stage (B_2)**	
V_1	43.67	38.54	31.05	33.71	36.74
V_2	34.14	32.08	26.11	21.97	28.58
V_3	37.33	33.93	24.96	20.28	29.12
Mean	36.61		26.35		
	CD (0.05): C= 0.61, V= 0.74, CxB= 0.86; CxV= 1.05; BxV= 1.05				
	SE±(m): B= 0.21, V= 0.26, CxB= 0.30; CxV= 0.37; BxV= 0.37				

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse; B_1- Milky stage, B_2- Dough stage.

4.3 EFFECT OF HIGH TEMPERATURE ON YIELD PARAMETERS

4.3.1 Spikelet fertility percentage (%)

Significant varietal difference for spikelet fertility percentage was observed in rice varieties under high temperature. These observations were taken at harvesting stage and found that spikelet fertility was decreased in rice plants under heat stress conditions. There was significant variation for spikelet fertility percentage between the treatments. The overall decrease in spikelet fertility percentage for varieties under high temperature conditions was 8.57 % when compared with that of ambient condition. Among the varieties both under control and heat stress conditions Prathyasa recorded highest and Hraswa recorded the lowest spikelet fertility. Under ambient condition the spikelet fertility of Prathyasa and Hraswa were (89.41 %), (74.86 %) respectively. But under high temperature condition the spikelet fertility of Prathyasa and Hraswa were

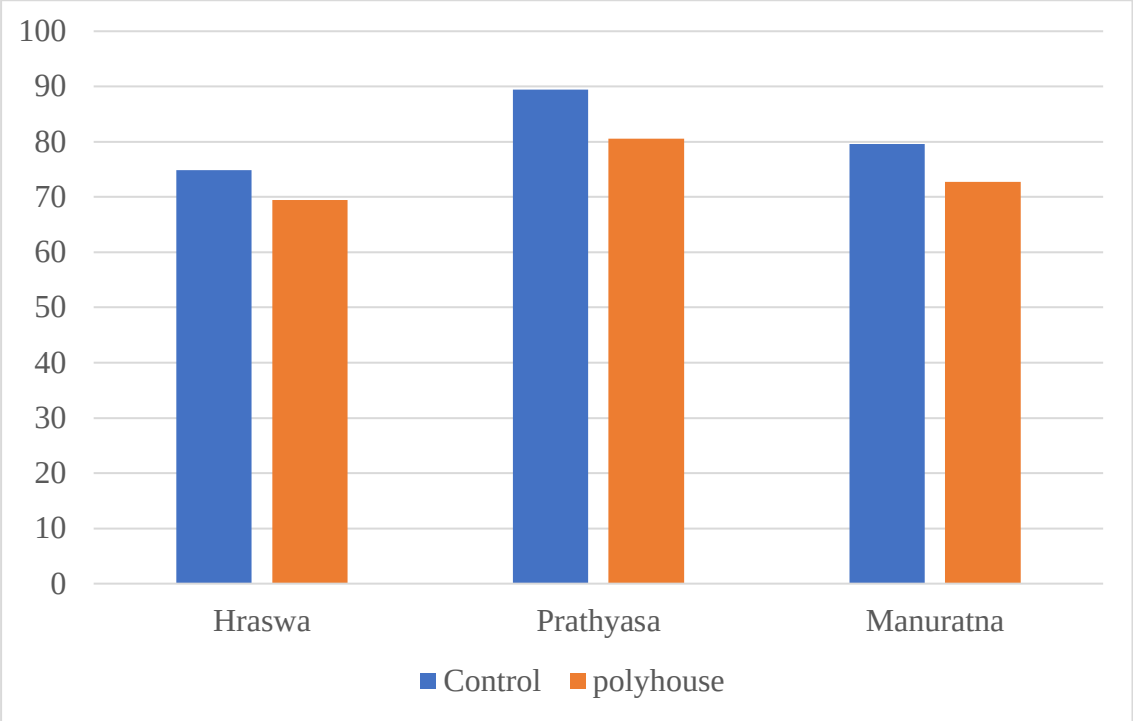


Figure 9: Variation in spikelet fertility percentage (%) of rice varieties under high temperature.

(80.59%), (69.44 %) respectively. Highest spikelet fertility percentage was recorded by Prathyasa variety (85 %) and the lowest spikelet fertility percentage was recorded by the variety Hraswa (72.15 %) under both ambient condition and high temperature conditions (Figure 9).

The percent decrease in spikelet fertility was more in Prathyasa (9.86 %) and less in Hraswa (7.23 %). The average spikelet fertility percentage of the rice varieties were 81.28 % and 74.24 % under control and heat stress conditions respectively. (Table 9).

4.3.2 Yield (g/ plant)

Significant varietal difference for yield content was observed in rice varieties under high temperature. There was significant variation for yield between the treatments and varieties. The overall decrease in yield for varieties under high temperature conditions was 23.29 % when compared with control. Among the varieties, Prathyasa (28.89 g/ plant) recorded the maximum yield content and Hraswa (20 g/ plant) recorded minimum yield content under ambient condition while under heat stress conditions, Prathyasa (22.79 (g/ plant) and Hraswa (14.48 (g/ plant) recorded the maximum and minimum yield content. Highest yield was recorded by Prathyasa variety (25.84 (g/ plant) and the lowest yield was recorded by the variety Hraswa (17.24 (g/ plant) under both control and high temperature conditions. Prathyasa recorded highest yield among all varieties as it is sustained under high temperature condition hence is noted as a tolerant variety (Figure 10).

The percent decrease in yield was more in Hraswa (27.57 %) and less in Prathyasa (21.12 %). The average yield content of the rice varieties was 24.30 g/ plant and 18.73 g/ plant under control and heat stress conditions respectively. (Table 10).

4.3.3 Pollen viability (%)

Significant varietal difference for pollen viability was not observed in rice varieties under high temperature. There was significant variation for pollen viability between the treatments (Plate 9). The overall decrease in pollen viability for varieties under high temperature conditions was 9.34 % when compared with that of ambient condition. Among the varieties, Hraswa (94.70 %) recorded the maximum pollen

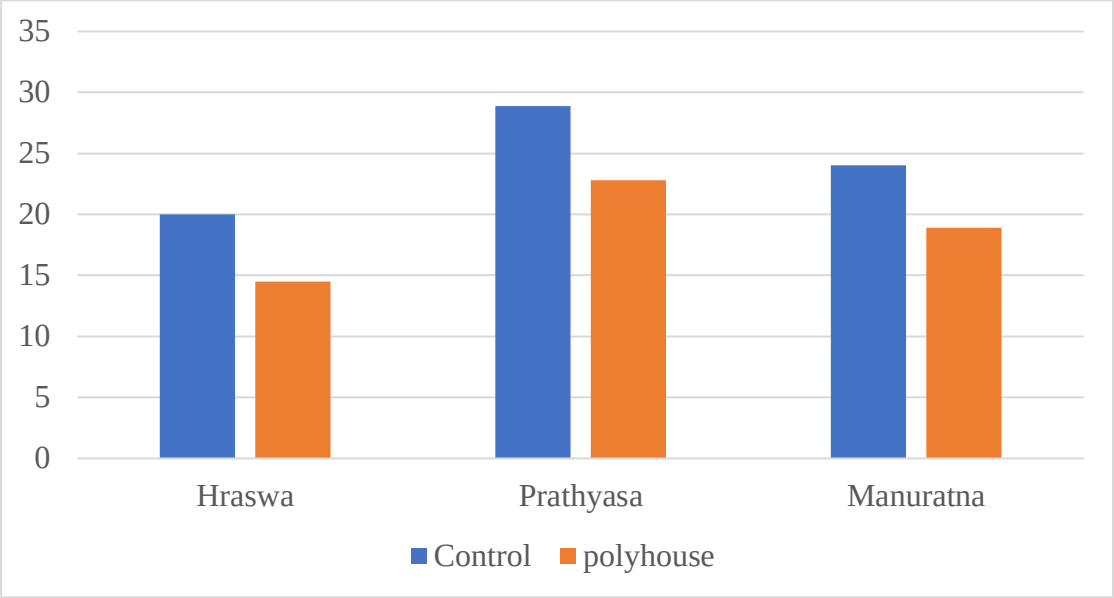


Figure 10: Variation in yield (g/plant) of rice varieties under high temperature.

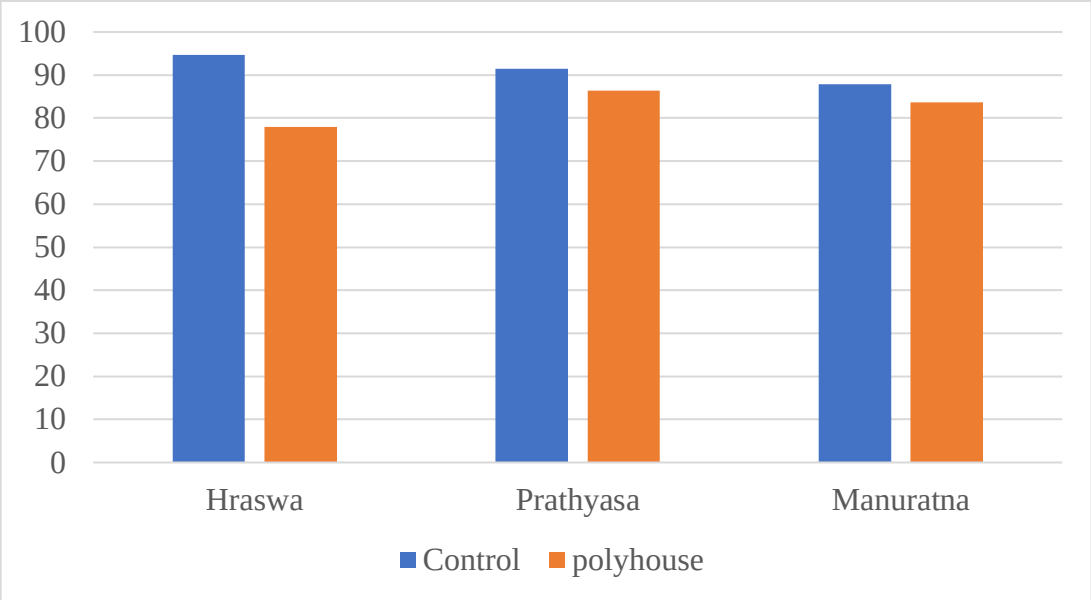


Figure 11: Variation in pollen viability (%) of rice varieties under high temperature.

Plate 8. View of experimental unit with rice plants at flowering stage inside polyhouse.

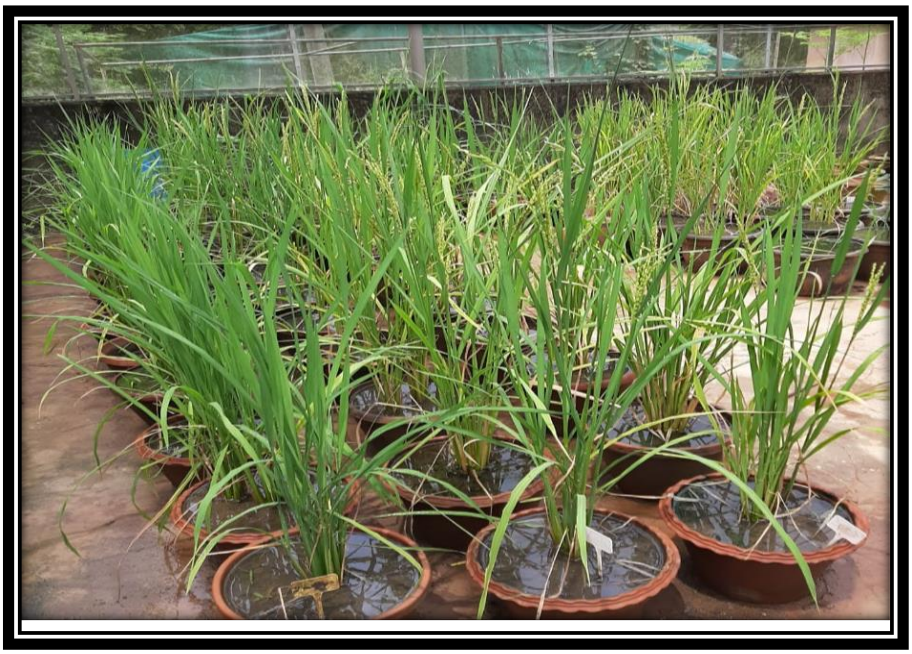
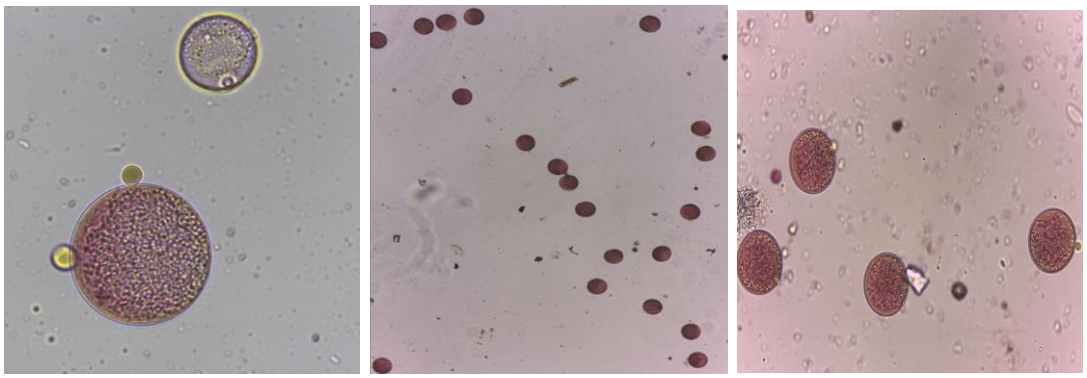


Plate 9. Microscopic view of viable pollen of the three rice varieties.



Hraswa **Prathyasa** **Manuratna**

viability and Manuratna (87.81 %) recorded minimum pollen viability under control condition while under heat stress conditions, Prathyasa (86.35 %) and Hraswa (77.94 %) recorded the maximum and minimum pollen viability respectively. Highest pollen viability was recorded by Prathyasa variety (88.92 %) and the lowest pollen viability was recorded by the variety Manuratna (85.73 %) under both control and high temperature conditions (Figure 11).

The percent decrease in pollen viability was more in Hraswa (17.69 %) and less in Manuratna (4.73 %). The average pollen viability of the rice varieties was 91.33 % and 82.65 % under control and heat stress conditions respectively (Table 11).

Table 9. Spikelet fertility (%) in rice under high temperature:

VARIETIES	**Control (C_1)**	**Polyhouse (C_2)**	**Mean (z)**
V_1	74.86	69.44	72.15
V_2	89.41	80.59	85.00
V_3	79.56	72.69	76.12
Mean	81.28	74.24	
	CD (0.05): C=0.85, V=1.05, CxV= 1.48		
	SE±(m):C=0.29, V=0.36, CxV=0.51		

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse;

Table 10. Yield (g/plant) in rice under high temperature:

VARIETIES	Control (C_1)	Polyhouse (C_2)	Mean (z)
V_1	20.00	14.48	17.24
V_2	28.89	22.79	25.84
V_3	24.00	18.92	21.46
Mean	24.30	18.73	
	CD (0.05): C=0.73, V=0.89, CxV= N/A		
	SE±(m):C=0.25, V=0.30, CxV=0.43		

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse;

Table11. Pollen viability (%) in rice under high temperature:

VARIETIES	Control (C_1)	Polyhouse (C_2)	Mean (z)
V_1	94.70	77.94	86.32
V_2	91.49	86.35	88.92
V_3	87.81	83.65	85.73
Mean	91.33	82.65	
	CD (0.05): C=2.90, V= N/A, CxV= 5.02		
	SE±(m):C=1.00, V=1.22, CxV=1.73		

V_1-Hraswa, V_2- Prathyasa, V_3- Manuratna; C_1- Control, C_2- Polyhouse;

4.4 Maximum and minimum temperature (^{0}C)

Maximum and minimum temperature inside polyhouse and in control were recorded using thermo-hygrometer from seedling to maturity stage. The average maximum and minimum temperature recorded in the control was 31.61 ^{0}C and 23.97 ^{0}C respectively. In polyhouse the mean maximum temperature recorded was 40.01 ^{0}C and the mean minimum temperature recorded was 27.02 ^{0}C. The difference of mean maximum and minimum temperatures was 8.4 ^{0}C and 3.05 ^{0}C.

Table12. Maximum and minimum temperature (^{0}C) recorded inside polyhouse and in ambient temperature condition:

Sl.No.	DAS	Minimum temperature (°C)		Maximum temperature (°C)	
		Inside the polyhouse condition	Ambient condition	Inside the polyhouse condition	Ambient condition
1	18	25.3	23.0	39.5	30.4
2	19	24.6	23.5	39.4	30.4
3	20	24.3	22.0	40.1	31.0
4	21	24.9	24.1	41.5	30.8
5	22	25.1	24.5	40.3	28.6
6	23	25.0	22.5	40.3	30.5
7	24	24.6	25.3	39.5	30.6
8	25	25.2	24.8	41.4	30.6
9	26	25.5	24.9	40.7	30.8
10	27	25.2	22.3	38.7	28.0
11	28	25.2	22.5	39.5	29.2
12	29	25.1	23.5	38.7	28.2
13	30	25	22.8	38.5	31.0
14	31	24.9	24.9	39.5	32.4
15	32	24.9	22.5	39.9	31.8
16	33	24.5	22.6	40.1	32.3

17	34	24.7	23.9	40.5	32.0
18	35	25.6	25.1	41.8	31.0
19	36	25.4	24.2	41.5	31.4
20	37	25.6	22.1	39.8	32.8
21	38	25.6	22.2	41.9	31.3
22	39	25.4	24.5	40.5	32.9
23	40	24.1	24.9	37.2	30.4
24	41	24.9	24.8	40.1	32.0
25	42	24.6	24.8	40.2	32.2
26	43	25.0	24.8	41.3	33.0
27	44	24.3	22.6	42.1	33.0
28	45	25.1	23.1	43.0	32.2
29	46	25.4	23.5	33.5	32.5
30	47	25.0	23.6	39.9	31.2
31	48	24.8	24.3	40.1	32.9
32	49	24.9	24.5	40.0	32.5
33	50	25.0	24.6	41.3	33.0
34	51	24.8	24.8	42.1	33.1
35	52	23.5	23.5	43.0	30.4
36	53	24.2	22.1	42.8	32.9
37	54	24.5	22.6	38.9	33.0
38	55	24.2	24.5	39.3	32.9
39	56	25.5	25.1	40.2	32.5
40	57	24.9	22.6	37.0	33.9
41	58	23.9	22.5	41.3	33.3
42	59	25.1	24.6	41.1	33.0
43	60	25.5	24.3	41.6	32.0
44	61	24.7	24.8	40.9	32.1

45	62	24.3	23.5	42.3	31.8
46	63	25.3	22.6	40.4	31.6
47	64	24.2	23.5	41.7	31.6
48	65	25.1	25.1	43.2	30.7
49	66	24.5	24.8	41.9	32.4
50	67	25.3	24.9	42.0	32.5
51	68	25.6	24.6	39.8	31.3
52	69	26.2	22.5	40.3	32.6
53	70	26.1	24.8	43.2	32.9
54	71	24.2	24.2	41.8	32.5
55	72	24.5	24.3	41.5	32.5
56	73	24.8	24.0	39.9	33.1
57	74	24.1	25.1	41.7	32.4
58	75	25.2	23.5	42.3	31.3
59	76	24.8	24.9	41.5	32.2
60	77	24.2	25.1	39.9	30.7
61	78	26.0	24.9	40.0	31.8
62	79	25.4	24.8	39.5	31.5
63	80	24.8	25.3	39.8	31.1
64	81	25.1	23.5	41.2	31.2
65	82	24.2	25.1	40.1	29.2
66	83	25.3	24.9	39.5	30.3
67	84	25.2	24.2	41.6	31.4
68	85	24.8	24.3	41.2	32.5
69	86	25	24.0	40.4	31.5
70	87	25.4	24.9	41.3	31.9
71	88	26.1	25.1	39.8	31.5
72	89	24.9	23.5	34.0	32.2

73	90	25	24.5	42.1	32.5
74	91	25.1	24.6	44.1	33.2
75	92	26.4	22.1	42.0	32.1
76	93	26.9	22.3	42.1	32.1
77	94	26.4	24.5	38.6	32.1
78	95	26.3	22.3	40.4	28.7
79	96	25.6	23.5	37.0	32.6
80	97	26	24.5	40.5	31.2
81	98	26.7	24.8	38.1	31.3
82	99	25.9	23.6	36.5	29.8
83	100	24.7	25.2	40.2	31.9
84	101	26.1	24.1	37.3	31.8
85	102	25.9	23.5	41.1	31.2
86	103	24.7	23.6	40.6	31.8
87	104	23.5	24.8	40.5	32.5
88	105	25.0	24.6	39.9	32.6
89	106	25.2	22.1	40.5	31.8
90	107	23.5	22.3	42.2	31.2
91	108	25.6	24.8	41.5	31.9
92	109	26.1	24.8	38.4	31.2
93	110	23.4	25.1	40.1	32.5
94	111	255	22.3	39.4	31.4
95	112	23.4	22.2	37.5	30.8
96	113	24.2	24.2	39.9	30.2
97	114	25.0	24.8	40.5	31.5
98	115	24.8	24.7	40.1	30.8
99	116	25.5	23.0	40.1	32.7
100	117	24.8	25.1	38.4	32.1

101	118	24.6	23.5	39.9	32.2
102	119	25.3	22.6	42.1	31.2
103	120	25.3	24.8	42.5	31.8
104	121	24.8	24.8	44.3	31.1
105	122	24.9	22.9	41.2	30.2
106	123	25.1	24.3	32.8	30.2
107	124	24.6	25.0	39.4	30.8
108	125	24.2	24.8	40.1	32.1
109	126	25.1	25.1	41.2	31.2
110	127	25.2	24.9	38.4	31.2
111	128	24.7	22.6	40.1	30.2
112	129	24.9	23.3	39.4	30.8
113	130	24.2	25.1	7.5	32.7
114	131	25.1	24.9	40.6	32.5
	Mean	27.02	23.97	40.01	31.61

4.5 Correlation analysis

Correlation analysis is a statistical method used to evaluate the strength of relationship between two quantitative variables. The data from varied parameters were recorded under ambient and high temperature conditions in rice varieties were subjected to correlation analysis. The results regarding the correlation between varied traits under both conditions were presented in the table 13 and 14.

Correlation between different traits and yield under control condition

The correlation between different traits and yield under control condition is presented in the table no. 13. Under control condition rice yield was found to be significant and positively correlated with seed quality parameters such as total carbohydrate (r = 0.922**), starch (r = 0.597**), anthocyanin (r = 0.839**). Similarly, negative correlation for yield was observed with seed quality parameters such as reducing sugar (r = -0.455), amylose (r = -0.596), seed protein (r= -0.365) and flavonoids (r = -0.271).

Yield was found to have significant negative correlation with enzyme such as invertase (r = -0.825**) under control condition. In case of yield parameters, significant positive correlation was observed for spikelet fertility (r = 0.954**) whereas negative correlation for yield was observed for pollen viability and it is nonsignificant (r = -0.370^{NS}).

Correlation between different traits and yield under high temperature condition

The correlation between different traits and yield under high temperature condition is presented in the table no. 14. Correlation study revealed that positive correlation for yield under high temperature condition was observed with seed quality parameters such as total carbohydrate (r = 0.888), starch (r = 0.935), anthocyanin (r = 0.180). Negative correlation for rice yield was recorded with seed quality parameters like reducing sugar (r = -0.354), amylose (r = -0.529), seed protein (r = -0.691), and flavonoids (r = 0.426). Significant correlation for yield was observed with total

carbohydrate (r = 0.888**), starch (r = 0.935**), amylose (r = -0.529*), seed protein (r = -0.691**).

Negative correlation for yield was observed with invertase (r = -0.783**) enzyme and the correlation recorded was significant. In case of yield parameters, significant positive correlation was observed with spikelet fertility (r = 0.894**) and pollen viability (r = 0.471*).

Table 13. Pearson Correlation Matrix (Control):

	Yield	Reducing sugar	Total carbohydrate	Starch	Amylose	Seed protein	Anthocyanin	Flavonoids	Invertase	Spikelet fertility	Pollen viability
Yield	1.000	-0.455^{NS}	0.922**	0.597**	-0.596**	-0.365^{NS}	0.839**	-0.271^{NS}	-0.825**	0.954**	-0.370^{NS}
Reducing sugar	-0.455^{NS}	1.000	-0.493*	-0.463^{NS}	0.608**	-0.186^{NS}	-0.384^{NS}	0.014^{NS}	0.669**	-0.396^{NS}	0.319^{NS}
Total carbohydrate	0.922**	-0.493*	1.000	0.489*	-0.697**	-0.375^{NS}	0.818**	-0.306^{NS}	-0.827**	0.912**	-0.364^{NS}
Starch	0.597**	-0.463^{NS}	0.489*	1.000	-0.567*	0.329^{NS}	0.223^{NS}	-0.668**	-0.687**	0.477*	-0.868**
Amylose	-0.596**	0.608**	-0.697**	-0.567*	1.000	-0.124^{NS}	-0.452^{NS}	0.372^{NS}	0.779**	-0.626**	0.440^{NS}
Seed protein	-0.365^{NS}	-0.186^{NS}	-0.375^{NS}	0.329^{NS}	-0.124^{NS}	1.000	-0.582*	-0.284^{NS}	0.058^{NS}	-0.434^{NS}	-0.384^{NS}
Anthocyanin	0.839**	-0.384^{NS}	0.818**	0.223^{NS}	-0.452^{NS}	-0.582*	1.000	0.097^{NS}	-0.577*	0.860**	0.015^{NS}
Flavonoids	-0.271^{NS}	0.014^{NS}	-0.306^{NS}	-0.668**	0.372^{NS}	-0.284^{NS}	0.097^{NS}	1.000	0.353^{NS}	-0.154^{NS}	0.760**
Invertase	-0.825**	0.669**	-0.827**	-0.687**	0.779**	0.058^{NS}	-0.577*	0.353^{NS}	1.000	-0.773**	0.581*
Spikelet fertility	0.954**	-0.396^{NS}	0.912**	0.477*	-0.626**	-0.434^{NS}	0.860**	-0.154^{NS}	-0.773**	1.000	-0.241^{NS}
Pollen viability	-0.370^{NS}	0.319^{NS}	-0.364^{NS}	-0.868**	0.440^{NS}	-0.384^{NS}	0.015^{NS}	0.760**	0.581*	-0.241^{NS}	1.000

Correlations @ * P <- 0.05, ** P <- 0.01, *** P <- 0.001; NS- Non significant

Table 14. Pearson Correlation Matrix (Polyhouse):

	Yield	Reducing sugar	Total carbohydrate	Starch	Amylose	Seed protein	Anthocyanin	Flavonoids	Invertase	Spikelet fertility	Pollen viability
Yield	1.000	-0.354^{NS}	0.888**	0.935**	-0.529*	-0.691**	0.180^{NS}	-0.426^{NS}	-0.783**	0.894**	0.471*
Reducing sugar	-0.354^{NS}	1.000	-0.223^{NS}	-0.309^{NS}	0.282^{NS}	0.365^{NS}	-0.125^{NS}	-0.025^{NS}	0.424^{NS}	-0.235^{NS}	-0.540*
Total carbohydrate	0.888**	-0.223^{NS}	1.000	0.953**	-0.596**	-0.784**	0.290^{NS}	-0.386^{NS}	-0.810**	0.869**	0.526*
Starch	0.935**	-0.309^{NS}	0.953**	1.000	-0.570*	-0.757**	0.156^{NS}	-0.475*	-0.786**	0.932**	0.541*
Amylose	-0.529*	0.282^{NS}	-0.596**	-0.570*	1.000	0.754**	-0.653**	-0.205^{NS}	0.818**	-0.370^{NS}	-0.369^{NS}
Seed protein	-0.691**	0.365^{NS}	-0.784**	-0.757**	0.754**	1.000	-0.610**	-0.087^{NS}	0.879**	-0.598**	-0.416^{NS}
Anthocyanin	0.180^{NS}	-0.125^{NS}	0.290^{NS}	0.156^{NS}	-0.653**	-0.610**	1.000	0.746**	-0.666**	-0.092^{NS}	0.157^{NS}
Flavonoids	-0.426^{NS}	-0.025^{NS}	-0.386^{NS}	-0.475*	-0.205^{NS}	-0.087^{NS}	0.746**	1.000	-0.097^{NS}	-0.666**	-0.173^{NS}
Invertase	-0.783**	0.424^{NS}	-0.810**	-0.786**	0.818**	0.879**	-0.666**	-0.097^{NS}	1.000	-0.628**	-0.552*
Spikelet fertility	0.894**	-0.235^{NS}	0.869**	0.932**	-0.370^{NS}	-0.598**	-0.092^{NS}	-0.666**	-0.628**	1.000	0.518*
Pollen viability	0.471*	-0.540*	0.526*	0.541*	-0.369^{NS}	-0.416^{NS}	0.157^{NS}	-0.173^{NS}	-0.552*	0.518*	1.000

Correlations @ * P <- 0.05, ** P <- 0.01, *** P <- 0.001; NS- Non significant

DISCUSSION

5. DISCUSSION

The world's population is growing at faster rate, but the cultivation of crops decreased due to the adverse changes in climate. This throws us in a critical condition of the lack of food for humans. Various random and unheralded disturbances in the environment adversely affect plant growth and limit the plant performance by compromising on productivity (Alcazar *et al.,* 2010). Global warming is one of the most important factors in climate change. The global average temperature has risen by 0.13^0C per decade since 1950 and an even more rapid pace of 0.2^0C per decade is expected in the next two or three decades (Lobell *et al*., 2011). Most of Earth's plants being sessile organisms are suffering from extreme temperature conditions (Duque *et al.,* 2013).

Climate change is the serious hazard to rice crop as it is the staple crop that helps in nutrition, mitigation of poverty and food protection globally (Allen *et al.,* 2010). Rice growth and development are severely affected below 15-20^0C and above 30^0C (Dufresne *et al.,* 2013).

Exposure of the crop to a temperature above the threshold level at least by 5^0C affect the metabolic responses that are required by the crop for survival under high temperature conditions (Wahid *et al.,* 2007). Rice is highly sensitive to rise in temperatures and its grain yield was reduced by 10% for every 1^0C increase in temperature (Zinn *et al.,* 2010). Impact of high temperature was greater at reproductive stage than that of vegetative stage (Li *et al.,* 2011). For rice previous study revealed that heat stress is a complex phenomenon affecting the morphological, physiological and biochemical changes in plants that finally result in reduced growth and yield of the crop. Thus, in the present experiment high temperature stress was imposed on rice plants, and the temperature in the control (mean minimum and maximum temperature-23.97^0C and 31.61^0C respectively) and polyhouse (mean minimum and maximum temperature-27.02^0C and 40.01^0C respectively) conditions were recorded from seedling to maturity stage using thermo-hygrometer. This study revealed the effect of high temperature stress in rice on seed quality parameters,

enzymes at milky and dough stages, and also on yield parameters at harvest stage. The basic results related to these observations are presented in this chapter.

5.1. EFFECT OF HIGH TEMPERATURE ON SEED QUALITY PARAMETERS

The days to first flowering for Hraswa variety was 45 days and days to 50 % flowering was 57 days. Manuratna, took 74 days to initiate the first flower and it took 83 days to 50% flowering. For Prathyasa days to first flowering were 80 days and it took 93 days to 50 % flowering.

Gill *et al.* (2001) investigated the effect of various abiotic stresses on reducing sugars in sorghum CSH-6 cultivar in different organs such as root, shoot, endosperm and reported that the reducing sugar content increased in the endosperm, on exposure to heat stress in light grown seedlings. These findings coincided with our present study where the reducing sugar content increased in rice plants under high temperature condition than that of ambient condition. Asthir *et al.* (2015) carried out an experiment in wheat cultivars PBW343, PBW 534 (heat susceptible), C306 and C273 (heat tolerant) and found an increased reducing sugars under high temperature condition when compared with that of the ambient condition and the increase was more profound in tolerant variety showing 45% increase in C273 cultivar with the least increase in reducing sugar recorded in susceptible variety showing 4% increase in PBW 534 cultivar under high temperature conditions. Our observations in the present study showed greater increase in reducing sugar in Prathyasa which showed tolerance towards high temperature and the least increase in reducing sugar was observed in susceptible variety thus this study support the earlier findings.

Studies revealed that exposure of rice crop to a temperature of 34^0C caused accumulation of carbohydrate in the rice grain (Rowland *et al.*, 1966). Our study is in line with this previous inference showing an increase in carbohydrate content under high temperature condition (5-6^0C above the ambient temperature) in all the rice varieties, which was related to increase in SPS (Suc-6-P synthase) activity that played a significant role in stimulating the pathway of sucrose synthesis at high temperatures causing the accumulation

of carbohydrates (Lafta and Lorenzen, 1995). Stem NSC (non-structural carbohydrate) play a vital role in grain filling and nearly 25% of carbohydrates supplied to rice kernels under heat stress is from the reserve stem NSC before heading as reported by Pan *et al*. (2011). In other findings in rice under extreme temperature conditions through enhancement of the fructan catalysing enzyme activities remobilization of carbohydrate reserve from stem to grain occurs under extreme temperatures resulting in accumulation of carbohydrates in grains (Wang *et al.,* 2012). Cao *et al*. (2000) reported that during the grain filling period, rice grains are strong carbohydrate sinks.

There was significant increase in starch content among the rice varieties under high temperature conditions. The variety with highest increase in starch content was a tolerant variety and with less starch content was a susceptible variety. Similar results were observed from our study too. Gunaratne *et al.* (2011) carried out an experiment in rice which showed an increment in starch content under high temperature when the crop was exposed to heat stress during grain filling. Several studies also reported an increase in starch accumulation under stress which include the halophyte *Thellungiella halophila* (Wang *et al.,* 2013), the green alga *Chlamydomonas reinhardtii* (Siaut *et al.,* 2011), and in some cases Arabidopsis also (Kaplan and Guy, 2004; Skirycz *et al.,* 2010). Wang *et al.* (2012) from his experiments in wheat cultivar Yang 9 reported that, plants which are exposed to heat stress at pre and post anthesis stage showed higher starch content which was a result of increased grain percentage of B type granule compared to ambient condition. Cao *et al.* (2015) showed that high temperature enhanced the proportion of large size starch granules in rice grain. Baun *et al.* (1970) reported that synthesizing starch from sucrose was thermodynamically favoured by starch synthase that result in accumulation of starch under high temperatures. Kaneko *et al.* (2014) reported that nucleotide pyrophosphatase were up regulated and had a great influence on starch accumulation under high temperature conditions mainly in the heat sensitive genotypes. Kato *et al.* (2019) reported an increase in endosperm starch in japonica rice variety 'Akitakamochi' under a heat stress of 29 ^{0}C.

In the present study amylose that infers to the textural property of the grain, was decreased under high temperature condition in rice plants which is supported by the previous study carried out by Jiang *et al.* (2003) where exposure of non-waxy Indica rice to high temperature and found a decrease in amylose content in the rice endosperm by 25%. In other study using two rice varieties namely Taichung Native 1 and Tainung 67, when exposed to high temperature stress of 30^0C showed a reduction in amylose content in the rice caryopsis which was due to decrease in the expression of waxy protein that result in lower expression of GBSS (Granule-Bound Starch Synthase I), which is necessary to generate amylose thus resulting in reduced amylose content in rice grain (Lin *et al.*, 2005). Asaoka *et al.* (1985) from his findings reported that when the rice crop is exposed to heat stress, amylose content was affected by high temperature from 5-15 days after pollination that is the early stage of grain filling period resulting in decreased amylose and this study supported out present experiment where the rice crop exposed to high temperature showed an decrease in amylose content and the decrease was more profound at 15 days after 50 % flowering. Fang *et al.* (2019) reported the decrease in amylose content under high temperature condition in wheat.

Reduction in protein fraction and composition under heat stress was associated with altered quantity of total nitrogen accumulated during grain filling (Triboi *et al.*, 2002). Sita *et al.* (2018) reported a decrease in the accumulation of protein in lentil by 26% in heat tolerant genotypes and 42% in heat sensitive genotypes. Huang and Lur (2000) carried out an experiment in Indica and Japonica rice varieties by exposing them to high temperatures, accelerated the accumulation of protein in early filling stages, but resulted in decreased protein at the maturity stage, it was also found that the protein content is higher in ambient condition than in treatment which is in line with our present study, where the rice crop exposed to high temperature recorded higher protein content in control and under high temperature the protein content was reduced, protein content was highest at milky stage and with maturity the protein content was found to decrease. Hasanuzzaman *et al.* (2013) reported that the reduction in storage proteins under high temperature in plants, was indicative of impaired protein synthesis, especially of albumins and globulins, probably

due to the lack of sufficient precursors and inactivity of biosynthetic enzymes. Similarly, in pea, high temperature denatured and aggregated the seed storage proteins such as globulins, legumin, and vicilin (Mession *et al*., 2013) which might be due to loss of covalent and non-covalent interactions (Sun and Arntfield, 2012) thus resulting in decreased seed storage protein.

In the present study anthocyanin content in rice decreased in the grains on exposure of the crop to heat stress. Our present result is in consistent with the previous study where Zaidi *et al.* (2019) imposed heat stress in two Indica rice at full heading stage and observed the decrease in anthocyanin accumulation in the kernel, which was a result of inhibition of transcriptional expression of different genes that were involved in anthocyanin biosynthetic pathway. Exposure of the grapes to higher temperature of 36^0C results in reduced anthocyanin content in berries, this reflects the combined impact of reduced biosynthesis and increased degradation, particularly the direct role of peroxidases in anthocyanin catabolism (Mohaved *et al.*, 2016). Cutanda-Perez *et al.* (2009) reported that lower activity of UFGT (which encodes the final enzyme in the anthocyanin biosynthesis pathway) in the high temperature berries all along ripening was recorded and *UFGT* and *MYBA1* (transcriptional regulator of UFGT) gene were downregulated in high temperature exposed grapes. Mori *et al.* (2007) carried out other experiment in grapes by exposing the berries to high temperature and found that the anthocyanin content in high temperature exposed berries is low which results from factors such as anthocyanin degradation as well as the inhibition of mRNA transcription of the anthocyanin biosynthetic genes. Li *et al.* (2019) studied the effect of high temperature on anthocyanin accumulation in wheat and observed that anthocyanin was found to be lower in treatment which is due to large consumption of anthocyanin as small molecule compound against stress.

Chebrolu *et al.* (2016) reported that in soybean imposing heat stress in susceptible and tolerant genotypes, resulted in increased flavonoids in treated plants compared to control and this increase was at higher levels in tolerant genotypes as compared to susceptible genotype seed. Shamloo *et al.* (2017) reported that elevation of the growing

temperature in six wheat genotypes yielded an increase in the flavonoid content through the activation of their catalytic enzymes. A study on rice revealed that the flavonoid content increased in the seed of rice plants on exposure of the crop to high temperature (Zaidi *et al.*, 2019). In our present investigation in rice revealed that exposure of this crop to high temperatures resulted in elevated levels of flavonoids in the seeds. It is noteworthy that these results are in concordance with the earlier studies.

5.2. EFFECT OF HIGH TEMPERATURE ON ENZYME

Effect of high temperature tolerance in relation to carbon partitioning and grain sink activity was studied in ten genotypes of wheat by Bavita *et al.* (2012) where, the activity of acid invertase was increased till 14 days after anthesis, then decreased till maturity and also noted that the activity of acid invertase in susceptible genotypes was more at initial stages. In other findings Yaliang *et al.* (2020) reported that imposing high temperature of 40^0C in rice genotypes resulted in lower activity levels of soluble acid invertase which was due to downregulation in the expression of related genes. Bahugana *et al*. (2017) conducted an experiment in five rice genotypes and noted the invertase activity at three stages on 5, 10 and 15 days after flowering and found that the grains had lower CWI (cell wall invertase) activity at 5 DAF, which was further reduced at 10 DAF when plants were exposed to high day temperatures, furthermore reduction in cell wall invertase activity under high day temperature at 15 DAF was highly significant and consistent across all five genotypes. These above findings support our study which state the invertase activity was decreased under high temperature conditions and its activity was higher at milky stage and decreased at dough stage under polyhouse and it was found to be higher in susceptible genotypes in the initial stages.

5.3. EFFECT OF HIGH TEMPERATURE ON YIELD PARAMETERS

Das *et al.* (2014) conducted an experiment in rice by exposing the crop to high temperature of above 35^0C before flowering and reported that this stress showed an adverse effect on the number of anthesing (opened) spikelet's and the pollen water content was

found to decrease causing poor fertilization and high spikelet sterility. This could be attributed to the asynchrony between pollination and stigma receptivity (Jagadish *et al.*, 2010; Rang *et al.*, 2011). Lee (2011) reported that the physiological conditions at flowering and before flowering after the temperature stress determines the subsequent spikelet fertility to a certain extent. Six japonica and two Indica cultivars were exposed to five different temperature levels during flowering season and found that spikelet sterility increased with temperature (Maruyama *et al.,* 2015). Similar results of rapid decline in fertility at higher temperatures were obtained by Matsui and Omasa (2002), Prasad *et al.* (2006) and Tian *et al.* (2009). Zhang *et al.* (2018) stated the decrease in spikelet fertility in rice genotypes under high temperature which is a result of cessation of pollen tube growth caused due to heat stress. Jagdish *et al.* (2010) studied the response of three rice varieties namely Moroberekan (heat sensitive), IR64 (moderately tolerant), N22 (highly heat tolerant) to high temperature at anthesis and found that Moroberekan the most susceptible genotype, had very poor pollen germination at 38^0C recording 18% spikelet fertility while N22, the most tolerant genotype, had higher levels of pollen germination close to those at control recording highest spikelet fertility of 71%. In our findings we observed that there is decrease in spikelet fertility in rice plants under high temperature conditions we also noted that the tolerant variety Prathyasa recorded highest spikelet fertility whereas Hraswa being susceptible to heat stress recorded lowest spikelet fertility percentage. This result is in line with the previous studies.

Matsui *et al.* (2000) reported that heat stress in rice caused significant reduction in yield which resulted poor seed setting percentage. Peng *et al.* (2004) suggested that yield declined in rice with minimum temperature by 10% per 1^0C and yield declined with average temperature by 15% per 1^0C and under long term exposure of crop to elevated temperatures yield reduction is due to decline in number of grains per panicle which decreases the biomass and affect the spikelet formation. Extreme temperatures during flowering and grain-filling phase caused reduction in yield by shortening the duration of grain-filling phase and resulting in spikelet sterility (Tian *et al.,* 2007). Cao *et al.* (2008) reported that in rice crop, heat stress during meiosis stage of pollen had a great impact on

development of anther and pollen grains, significantly reducing the anther dehiscence, pollen fertility, number of spikelet per panicle, seed setting rate, 1000 grain weight finally resulting in the reduced grain yield. Djanaguiraman *et al.* (2020) proposed the decrease in rice yield under high temperature condition which is due to decrease in yield associated traits such as seed set percentage and individual grain weight. All the above-mentioned literatures supported out present results of reduced yield under heat stress conditions and the variety which sustained this extreme temperature was the tolerant variety and it recorded highest yield.

From the study reduced pollen viability was recorded by the rice varieties under heat stress conditions and the variety Prathyasa recorded high pollen viability as it was tolerant to high temperature. This result is in line with the findings of Bahuguna *et al.* (2015) which stated that in rice, heat tolerant genotypes such as Nagina22 and Nerica-L44 showed less reduction in pollen viability as compared with highly sensitive cultivars such as Swarna and Pusa Sugandh 5. Porter (2005) reported that imposing high temperature stress before anthesis in rice had a severe impact on dehiscence of anther and release of pollen thus resulted in reduced pollen viability. The degeneration of the tapetum and decreased carbohydrate metabolism can influence the nourishment of pollen mother cells, leading to infertile pollen and decreased pollen viability under heat stress (Hedhly, 2011). Buitink and Leprince (2004) reported that in rice under high temperature conditions poor anther dehiscence occur due to tight closure of locules and pollen water content which play a significant role in pollen dispersal was reduced by 30-50% affecting cell differentiation and pollen viability resulting in reduced spikelet formation.

Our present study revealed that under high temperature stress some of the seed filling parameters were adversely affected, that caused a reduction of these parameters, and they include amylose, seed protein and anthocyanin. Some of the constituents which are involved in filling of the seed were increased under heat stress condition and they include reducing sugars, carbohydrates, starch, flavonoids. These constituents help the plants to tolerate the temperature stress and make them able to survive in such unfavourable

conditions. The activity of the invertase enzyme was inhibited or reduced under high temperature treatment. Pollen that are highly sensitive to heat stress, loss their viability, on exposure to high temperature conditions. The yield and fertility of spikelet is reduced among all varieties under heat stress condition when compared with their respective controls.

SUMMARY

6. SUMMARY

The thesis programme "Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)" was conducted with the objective to study the effect of high temperature stress on seed filling and nutritional quality in rice. The daily maximum and minimum temperatures in control and polyhouse from seedling to maturity was calculated using thermo-hygrometer and was found that the mean minimum and maximum temperatures in the control were 23.97 ^{0}C and 31.61 ^{0}C respectively. Under polyhouse the mean minimum and maximum temperatures recorded were 27.02 ^{0}C and 40.01 ^{0}C respectively. A set of three varieties namely Hraswa, Prathyasa and Manuratna were evaluated in this experiment. The plants were grown in pots under polyhouse where the high temperature was imposed. Later the samples from both control and polyhouse were taken and the seed filling and enzyme parameters were analysed at milky and dough stages and the yield observations were taken at the time of harvest.

Different methods were used to analyse different parameters. Reducing sugars were analysed using Nelson-Somogyi method. Starch and carbohydrates were estimated using anthrone method. Amylose was estimated using the iodine procedure. Seed protein was analysed as per the Bradford method. Anthocyanin content was estimated as per the protocol of Ranganna. Flavonoids were analysed as per procedure laid out by Ordonez and co-workers. Invertase activity in the leaf was estimated as per the procedure given by Morris and Arthur. The viability of the pollen collected from the spikelet's, before anthesis was observed under microscope using potassium iodide. The number of filled and unfilled spikelets from the primary tiller were noted and the percentage of spikelet fertility was calculated. The yield observation was taken at harvest from the dried panicles. Statistical analysis was carried out using Opstat analysis. The salient findings of the study are given below.

The constituents that fill the seed were increased under heat stress conditions and they provide the ability to tolerate the high temperatures in plants. Some of these

parameters include reducing sugars, carbohydrates, starch, and flavonoids. The rice varieties Prathyasa, Hraswa and Manuratna maintained higher reducing sugar content under high temperature stress both at the milky stage and the dough stage when compared to ambient conditions. They showed an increase with maturity under ambient temperatures and recorded highest amount at the dough stage under high temperatures. The carbohydrate content significantly increased at high temperature for all the rice varieties as compared to ambient condition. At high temperature conditions carbohydrate was maximum at the milky stage and less at the dough stage. Under heat stress condition, an increment in starch content was observed for all the three rice varieties. The starch content was higher for plants at the dough stage under polyhouse and the least starch content at milky stage. In the case of flavonoid content of rice varieties, when grown under heat stress conditions they exhibited significant increase in its content. Under heat stress flavonoid content was highest at the milky stage and less at the dough stage.

Some the seed filling parameters were adversely affected by high temperatures and hence they were reduced under heat stress conditions, they include amylose, seed protein, and anthocyanin. The amylose content significantly reduced at high temperature in all the rice varieties as compared to ambient condition. Highest amylose percentage was recorded at dough stage under heat stress. All the three varieties under high temperature showed a decrease in seed protein content from that of control. Maximum seed protein content was recorded at milky stage and the minimum was recorded at dough stage under high temperature conditions. The anthocyanin content of seeds was reduced significantly for rice varieties when grown under heat stress conditions. Anthocyanin pigment decreased with maturity under ambient temperatures and it decreased under high temperature with maturity and thus at dough stage it recorded the lowest anthocyanin content

The activity of invertase enzyme gets stopped or gets partially reduced under temperature stress condition. Invertase activity was highest at the early seed filling stage and decreased at the maturity stage in ambient condition but under polyhouse its activity was higher in milky stage and less activity of invertase was recorded at the dough stage.

The yield parameters like spikelet fertility percentage and yield per plant showed significant decrease in its rate when grown under high temperature stress conditions when compared to ambient temperatures. Pollen viability showed a reduction under high temperature conditions. Among the rice varieties Manuratna recorded the lowest pollen viability. Yield, spikelet fertility and pollen viability were recorded highest in Prathyasa and Hraswa recorded the lowest yield and spikelet fertility.

The correlation analysis revealed that under high temperature condition yield showed positive and significant correlation with total carbohydrate, starch, anthocyanin, spikelet fertility and pollen viability. Hence it is important to identify the rice varieties which possesses the major seed filling parameters which play a vital role in increasing thermotolerance that result in giving a satisfactory grain yield even under conditions of heat stress.

It is therefore concluded that high temperature stress result in accumulation of some of the constituents which are involved in filling of the seed and they also make the plant able to survive under the unfavourable condition. Some of such constituents that are found to accumulate in the seed under high temperature conditions in this study include carbohydrates, reducing sugars, starch, and flavonoids. While the remaining seed filling parameters such as amylose, seed protein and anthocyanin were adversely affected in the seed on exposure of the plants to high temperature conditions. The activity of invertase was reduced in the heat stress condition. Under high temperature maximum amounts of reducing sugars, carbohydrates, starch, and amylose were recorded at dough stage while the maximum amount of seed protein, anthocyanin, flavonoids and invertase were recorded at milky stage. Pollen that is highly heat sensitive showed a reduction in its viability under heat stress condition. Yield and fertility of the spikelet were adversely affected by high temperature, hence recorded lower levels under heat stress condition. But the variety Prathyasa recorded highest yield and spikelet fertility from which we can conclude that this variety was able sustain under high temperature condition, hence it is a tolerant variety

while the Hraswa with the lowest yield and spikelet fertility was considered as heat susceptible variety.

REFERENCES

Reference

Abeysiriwardena, D.D.Z., Ohba, K., and Maruyama, A. 2002. Influence of temperature and relative humidity on grain sterility in rice. *J. Natl. Sci. Found. Sri Lanka*, 30(1-2): 33-41.

Agati, G. and Tattini, M. 2010. Multiple functional roles of flavonoids in photoprotection. *New Phytologist*, 186(4), pp.786-793.

Ahmed, N., Tetlow, I.J., Nawaz, S., Iqbal, A., Mubin, M., Nawaz ul Rehman, M.S., Butt, A., Lightfoot, D.A., and Maekawa, M. 2015. Effect of high temperature on grain filling period, yield, amylose content and activity of starch biosynthesis enzymes in endosperm of basmati rice. *J. Sci. Food Agric.* 95(11): 2237-2243.

Alcázar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P., and Tiburcio, A.F. 2010. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta*. 231(6): 1237-1249.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.T., and Gonzalez, P. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manag*. 259(4): 660-684.

Al-Whaibi, M.H. 2011. Plant heat-shock proteins: a mini review. *J. King Saud Univ. Sci.* 23(2): 139-150.

Asaoka, M., Okuno, K., and Fuwa, H. 1985. Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). *Agric. Biol. Chem.* 49(2): 373-379.

Ashraf, M.F.M.R. and Foolad, M.R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 59(2): 206-216.

Asthir, B., Kaur, R., and Bains, N.S. 2015. Variation of invertase activities in four wheat cultivars as influenced by thiourea and high temperature. *Acta Physiol. Plant.* 37(1): 1712.

Badshah, M.A., Naimei, T., Zou, Y., Ibrahim, M., and Wang, K. 2014. Yield and tillering response of super hybrid rice Liangyoupeijiu to tillage and establishment methods. *Crop J.* 2(1): 79-86.

Bahuguna, R.N., Jha, J., Pal, M., Shah, D., Lawas, L.M., Khetarpal, S., and Jagadish, K.S. 2015. Physiological and biochemical characterization of NERICA-L-44: a novel source of heat tolerance at the vegetative and reproductive stages in rice. *Physiol. Plant.* 154(4): 543-559.

Bahuguna, R.N., Solis, C.A., Shi, W., and Jagadish, K.S. 2017. Post-flowering night respiration and altered sink activity account for high night temperature-induced grain yield and quality loss in rice (*Oryza sativa* L.). *Physiol. Plant.* 159(1): 59-73.

Basu, P.S. and Minhas, J.S. 1991. Heat tolerance and assimilate transport in different potato genotypes. *J. Exp. Bot.* 42(7): 861-866.

Battisti, D.S. and Naylor, R.L. 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. *Sci.* 323(5911): 240-244.

Baun, L.C., Palmiano, E.P., Perez, C.M., and Juliano, B.O. 1970. Enzymes of starch metabolism in the developing rice grain. *Plant Physiol.* 46(3): 429-434.

Bavita, A., Puneet, K.R., Navtej, S.B., and Virinder, S.S. 2012. Genotypic variation for high temperature tolerance in relation to carbon partitioning and grain sink activity in wheat. *Am. J. Plant Sci.* 3: 381-390.

Bita, C. and Gerats, T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* 4: 273.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72(1-2): 248-254.

Buitink, J. and Leprince, O. 2004. Glass formation in plant anhydrobiotes: survival in the dry state. *Cryobiology* 48(3): 215-228.

Burrell, M.M. 2003. Starch: the need for improved quality or quantity-an overview. *J. Exp. Bot.* 54(382): 451-456.

Cao, J., Henry, P.R., Guo, R., Holwerda, R.A., Toth, J.P., Littell, R.C., Miles, R.D., and Ammerman, C.B. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.* 78(8): 2039-2054.

Cao, Y.Y., Duan, H., Yang, L.N., Wang, Z.Q., Liu, L.J., and Yang, J.C. 2009. Effect of high temperature during heading and early filling on grain yield and physiological characteristics in Indica rice. *Acta Agron. Sin. 35*(3): 512-521.

Cao, Y.Y., Duan, H., Yang, L.N., Wang, Z.Q., Zhou, S.C., and Yang, J.C. 2008. Effect of heat stress during meiosis on grain yield of rice cultivars differing in heat tolerance and its physiological mechanism. *Acta Agron. Sin.* 34(12): 2134–2142.

Cao, Z.Z., Pan, G., Wang, F.B., Wei, K.S., Li, Z.W., Shi, C.H., Geng, W., and Cheng, F.M. 2015. Effect of high temperature on the expressions of genes encoding starch synthesis enzymes in developing rice endosperms. *J. Integr. Agric.* 14(4): 642-659.

Ceccarelli, S., Grando, S., Maatougui, M., Michael, M., Slash, M., Haghparast, R., Rahmanian, M., Taheri, A., Al-Yassin, A., Benbelkacem, A., and Labdi, M. 2010. Plant breeding and climate changes. *J. Agric. Sci.* 148(6): 627-637.

Chebrolu, K.K., Fritschi, F.B., Ye, S., Krishnan, H.B., Smith, J.R., and Gillman, J.D. 2016. Impact of heat stress during seed development on soybean seed metabolome. *Metabolomics* 12(2): 28.

Cheng, F., Zhong, L., Zhao, N., Liu, Y., and Zhang, G. 2005. Temperature induced changes in the starch components and biosynthetic enzymes of two rice varieties. *Plant Growth Reg.* 46(1): 87-95.

Chun, A., Lee, H.J., Hamaker, B.R., and Janaswamy, S. 2015. Effects of ripening temperature on starch structure and gelatinization, pasting, and cooking properties in rice (*Oryza sativa*). *J. Agric. Food Chem.* 63(12): 3085-3093.

Cruz, N.D. and Khush, G.S. 2000. Rice grain quality evaluation procedures. In: Singh, R.K., Singh, U.S. and Khush, G.S. (eds), *Aromatic rice's*, Oxford and IBH Publishing Co Pvt. Ltd, New Delhi pp.15-28.

Cutanda-Perez, M.C., Ageorges, A., Gomez, C., Vialet, S., Terrier, N., Romieu, C., and Torregrosa, L. 2009. Ectopic expression of VlmybA1 in grapevine activates a narrow set of genes involved in anthocyanin synthesis and transport. Plant. Mol. Biol. 69(6): 633-648.

Das, S., Krishnan, P., Nayak, M., and Ramakrishnan, B. 2014. High temperature stress effects on pollens of rice (*Oryza sativa* L.) genotypes. *Environ. Exp. Bot.* 101: 36-46.

Djanaguiraman, M., Narayanan, S., Erdayani, E., and Prasad, P.V. 2020. Effects of high temperature stress during anthesis and grain filling periods on photosynthesis, lipids and grain yield in wheat. *BMC Plant Biol.* 20(1): 1-12.

Devi, P.S., Saravanakumar, M., and Moh, S. 2012. The effects of temperature and pH on stability of anthocyanins from red sorghum (*Sorghum bicolor*) bran. *Afr. J. Food Sci.* 6(24): 567-573.

Devi, R.R. and Arumughan, C. 2007. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. *Bioresource Technol.* 98(16): 3037-3043.

Dufresne, J.L., Foujols, M.A., Denvil, S., Caubel, A., Marti, O., Aumont, O., Balkanski, Y., Bekki, S., Bellenger, H., Benshila, R., and Bony, S. 2013. Climate change projections using the IPSL-CM5 Earth System Model: from CMIP3 to CMIP5. *Clim. Dyn.* 40(9-10): 2123-2165.

Duque, A.S., de Almeida, A.M., da Silva, A.B., da Silva, J.M., Farinha, A.P., Santos, D., Fevereiro, P., and de Sousa Arau´ jo, S. 2013. Abiotic stress responses in plants: unraveling the complexity of genes and networks to survive. In: Vahdati, K., Leslie, C. (eds), *Abiotic Stress-Plant Responses and Applications in Agriculture*. InTech, London, UK, pp. 49–101.

ECOSTAT [Economics and Statistics]. 2018. *Kerala Aging Survey* [Internet]. Kerala Government. Available from: http://www.ecostat. kerala.gov.in/index.php/reports/75.html [cited 2017 Oct 5].

Essmann, J., Schmitz-Thom, I., Schön, H., Sonnewald, S., Weis, E., and Scharte, J. 2008. RNA interference-mediated repression of cell wall invertase impairs defense in source leaves of tobacco. *Plant Physiol.* 147(3): 1288-1299.

Fabre, D., Siband, P. and Dingkuhn, M. 2005. Characterizing stress effects on rice grain development and filling using grain weight and size distribution. *Field Crops Res.* 92(1): 11-16.

Fahad, S., Hussain, S., Saud, S., Tanveer, M., Bajwa, A.A., Hassan, S., Shah, A.N., Ullah, A., Wu, C., Khan, F.A., and Shah, F. 2015. A biochar application protects rice pollen from high-temperature stress. *Plant Physio. Biochem*. 96: 281-287.

Fang, L.H., Hu, Y., Wang, C., Liu, W., Ma, G., Han, Q., and Ma, D. 2019. Effects of high temperature and drought stress on the expression of genes encoding enzymes and

the activity of key enzymes involved in starch biosynthesis in wheat grains. *Front. Plant Sci.* 10: 1414.

FAOSTAT. 2018. FAOSTAT database collections. Rome: Food and Agriculture Organization of the United Nations. Available: http://www.fao.org/faostat/ home/E [01 Aug.2018].

Farooq, M., Nadeem, F., Gogoi, N., Ullah, A., Alghamdi, S.S., Nayyar, H., and Siddique, K.H. 2017. Heat stress in grain legumes during reproductive and grain-filling phases. *Crop Pasture Sci.* 68(11): 985-1005.

Figueiredo, N., Carranca, C., Trindade, H., Pereira, J., Goufo, P., Coutinho, J., Marques, P., Maricato, R., and de Varennes, A. 2015. Elevated carbon dioxide and temperature effects on rice yield, leaf greenness, and phenological stages duration. *Paddy Water Environ.* 13(4): 313-324.

Fini, A., Brunetti, C., Di Ferdinando, M., Ferrini, F., and Tattini, M. 2011. Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. *Plant Signalling Behav.* 6(5): 709-711.

Futakuchi, K., Watanabe, H., and Jones, M.P. 2008. Relationship of grain protein content to other grain quality traits in interspecific *Oryza sativa* L. x *Oryza glaberrima Steud.* progenies. *Agric. J.* 3(1): 50-57.

Gill, P.K., Sharma, A.D., Singh, P., and Bhullar, S.S. 2001. Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulg. J. Plant Physiol*. 27(1-2): 72-84.

Godfrey, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., and Toulmin, C. 2010. Food Security: The challenge of feeding 9 billion people. *Sci.* 327: 812-818.

Grotewold, E. 2006. The genetics and biochemistry of floral pigments. *Annu. Rev. Plant Biol.* 57: 761-780.

Gunaratne, A., Sirisena, N., Ratnayaka, U.K., Ratnayaka, J., Kong, X., Vidhana Arachchi, L.P., and Corke, H. 2011. Erratum: Effect of fertilizer on functional properties of flour from four rice varieties grown in Sri Lanka. *J. Sci. Food Agric.* 91(9): 1728-1728.

Guo, M., Liu, J.H., Ma, X., Luo, D.X., Gong, Z.H., and Lu, M.H. 2016. The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. *Front. Plant Sci.* 7: 114.

Hamaker, B.R. 1993. The influence of rice protein on rice quality. In: Marshall, W.E. and Wadsworth, J. I. (eds), *Food Science and Technology*. Dekker, New York, pp. 177-177.

Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., and Fujita, M., 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*. 14(5): 9643-9684.

Hasegawa, T., Yoshimoto, M., Kuwagata, T., Ishigooka, Y., Kondo, M., and Ishimaru, T. 2009. The impact of global warming on rice production: lessons from spikelet sterility observed under the record hot summer of 2007. *NIAES Annual Report 2008*. pp. 23–25.

Hedhly, A. 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environ. Exp. Bot.* 74: 9–16.

Hernández, I., Alegre, L., Van Breusegem, F., and Munné-Bosch, S. 2009. How relevant are flavonoids as antioxidants in plants? *Trends Plant Sci.* 14(3): 125-132.

Hodge, J.E. and Hofreiter, B.T. 1962. *Methods in Carbohydrate Chemistry*. In: Whistler, R.L. and BeMiller, J.N. (eds) Academic Press, New York.

Huang, J.J. and Lur, H.S. 2000. Influences of temperature during grain filling stages on grain quality in rice (*Oryza sativa* L.) 1. Effects of temperature on yield components, milling quality, and grain physicochemical properties. *J. Agric. Assoc.* 1: 370-389.

Huang, Y.D., Cao, L.J., Wu, L.Q., Yan, P., Chen, D.P., and Yang, A.Z. 2004. Investigation and analysis of heat damage on rice at blossoming stage in Anhui Province in 2003. *J. Anhui Agric. Univ*. 31: 385–388.

Hussain, S.S., Ali, M., Ahmad, M., and Siddique, K.H. 2011. Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnol. Adv*. 29(3): 300-311.

IPCC. 2014. Intergovernmental Panel on Climate Change. Fifth Assessment Report of the Intergovernmental Panel on Climate Change: The Impacts, Adaptation and Vulnerability. Cambridge University Press, United Kingdom and New York, NY, USA.

Ishimaru, T., Miyazaki, M., Shigemitsu, T., Nakata, M., Kuroda, M., Kondo, M., and Masumura, T. 2020. Effect of high temperature stress during ripening on the accumulation of key storage compounds among Japanese highly palatable rice cultivars. *J. Cereal Sci.* p.103018.

Jaakola, L. and Hohtola, A. 2010. Effect of latitude on flavonoid biosynthesis in plants. *Plant Cell Environ.* 33(8): 1239-1247.

Jagadish, K.S., Craufurd, P., Shi, W., and Oane, R. 2014. A phenotypic marker for quantifying heat stress impact during microsporogenesis in rice (*Oryza sativa* L.). *Funct. Plant Biol.* 41(1): 48-55.

Jagadish, S.K., Muthurajan, R., Rang, Z.W., Malo, R., Heuer, S., Bennett, J., and Craufurd, P.Q. 2011. Spikelet proteomic response to combined water deficit and heat stress in rice (*Oryza sativa* cv. N22). *Rice* 4(1): 1-11.

Jagadish, S.V.K., Craufurd, P.Q., and Wheeler, T.R. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *J. Exp. Bot.* 58(7): 1627-1635.

Jagadish, S.V.K., Craufurd, P.Q., and Wheeler, T.R., 2008. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Sci.* 48(3): 1140-1146.

Jagadish, S.V.K., Muthurajan, R., Oane, R., Wheeler, T.R., Heuer, S., Bennett, J., and Craufurd, P.Q. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *J. Exp. Bot.* 61(1): 143-156.

Jiang, H., Dian, W., and Wu, P. 2003. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. *Phytochem.* 63(1): 53-59.

Juadjur, A., Mohn, C., Schantz, M., Baum, M., Winterhalter, P., and Richling, E. 2015. Fractionation of an anthocyanin-rich bilberry extract and in vitro antioxidative activity testing. *Food Chem.* 167: 418-424.

Juliano, B.O. 2007. Structure and gross composition of the rice grain. *Rice Chemistry and Quality.* Philippine Rice Research Institute, Munoz, Nueva Ecija, Philippines, pp. 21-45.

Kaneko, K., Inomata, T., Masui, T., Koshu, T., Umezawa, Y., Itoh, K., Pozueta-Romero, J., and Mitsui, T. 2014. Nucleotide pyrophosphatase/ phosphodiesterase 1 exerts a negative effect on starch accumulation and growth in rice seedlings under high temperature and CO_2 concentration conditions. *Plant Cell Physiol.* 55(2): 320-332.

Kaplan, F. and Guy, C.L. 2004. β-Amylase induction and the protective role of maltose during temperature shock. *Plant Physiol.* 135(3): 1674-1684.

Kato, K., Suzuki, Y., Hosaka, Y., Takahashi, R., Kodama, I., Sato, K., Kawamoto, T., Kumamaru, T., and Fujita, N. 2019. Effect of high temperature on starch biosynthetic enzymes and starch structure in japonica rice cultivar 'Akitakomachi' (*Oryza sativa* L.) endosperm and palatability of cooked rice. *J. Cereal Sci.* 87: 209-214.

Keunen, E.L.S., Peshev, D., Vangronsveld, J., Van Den Ende, W.I.M., and Cuypers, A.N.N. 2013. Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. *Plant Cell Environ.* 36(7): 1242-1255.

Koch, K. 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr. Opin Plant Biol.* 7(3): 235-246.

Krishnan, P., Ramakrishnan, B., Raja Reddy, K., and Reddy, V.R. 2011. High temperature stress effects on rice plant growth and yield. *Adv. Agron.* 111: 87-206.

Lafta, A.M. and Lorenzen, J.H. 1995. Effect of high temperature on plant growth and carbohydrate metabolism in potato. *Plant Physiol.* 109(2): 637-643.

Lee, J.S. 2011. Combined effect of elevated CO_2 and temperature on the growth and phenology of two annual C_3 and C_4 weedy species. *Agric. Ecosyst. Environ.* 140(3-4): 484–491.

Li, H., Chen, Z., Hu, M., Wang, Z., Hua, H., Yin, C., and Zeng, H. 2011. Different effects of night versus day high temperature on rice quality and accumulation profiling of rice grain proteins during grain filling. *Plant Cell Rep.* 30(9): 1641-1659.

Li, X., Lv, X., Wang, X., Wang, L., Zhang, M., and Ren, M. 2019. Effects of abiotic stress on anthocyanin accumulation and grain weight in purple wheat. *Crop Pasture Sci*. 69(12): 1208-1214.

Li, X.Z., Liang, M.Z., Zhou, G.Q., and Chen, L.B. 2002. Effect of environment condition on pollen vigor and seed set during flowing time of rice. *Acta Agron. Sin*. 28(3): 417–420.

Lin, C.J., Li, C.Y., Lin, S.K., Yang, F.H., Huang, J.J., Liu, Y.H., and Lur, H.S. 2010. Influence of high temperature during grain filling on the accumulation of storage proteins and grain quality in rice (*Oryza sativa* L.). *J. Agric. Food Chemi.* 58(19): 10545-10552.

Lin, S.K., Chang, M.C., Tsai, Y.G., and Lur, H.S. 2005. Proteomic analysis of the expression of proteins related to rice quality during caryopsis development and the effect of high temperature on expression. *Proteomics* 5(8): 2140-2156.

Liu, J.H., Kitashiba, H., Wang, J., Ban, Y., and Moriguchi, T. 2007. Polyamines and their ability to provide environmental stress tolerance to plants. *Plant Biotechnol.* 24(1): 117-126.

Liu, J.H., Wang, W., Wu, H., Gong, X., and Moriguchi, T. 2015. Polyamines function in stress tolerance: from synthesis to regulation. *Front. Plant Sci*.6: 827.

Liu, J.X., Liao, D.Q., Oane, R., Estenor, L., Yang, X.E., Li, Z.C., and Bennett, J. 2006. Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. *Field Crops Res.* 97(1): 87-100.

Liu, Y.H., Offler, C.E., and Ruan, Y.L. 2016. Cell wall invertase promotes fruit set under heat stress by suppressing ROS-independent cell death. *Plant Physiol.* 172(1): 163-180.

Lobell, D.B., Schlenker, W., and Costa-Roberts, J. 2011. Climate trends and global crop production since *1980. Sci.* 333(6042): 616–620.

Lou, Y., Gou, J.Y., and Xue, H.W. 2007. PIP5K9, an Arabidopsis phosphatidylinositol monophosphate kinase, interacts with a cytosolic invertase to negatively regulate sugar-mediated root growth. *Plant Cell* 19(1): 163-181.

Lu, G.H., Wu, Y.F., Bai, W.B., Bao, M.A., Wang, C.Y., and Song, J.Q. 2013. Influence of high temperature stress on net photosynthesis, dry matter partitioning and rice grain yield at flowering and grain filling stages. *J. Integr Agric.* 12(4): 603-609.

Lyman, N.B., Jagadish, K.S., Nalley, L.L., Dixon, B.L., and Siebenmorgen, T. 2013. Neglecting rice milling yield and quality underestimates economic losses from high-temperature stress. *PloS one*. 8(8): e72157.

Mariotti, M., Sinelli, N., Catenacci, F., Pagani, M.A., and Lucisano, M. 2009. Retrogradation behaviour of milled and brown rice pastes during ageing. *J. Cereal Sci.* 49(2): 171-177.

Marko, D., Puppel, N., Tjaden, Z., Jakobs, S., and Pahlke, G. 2004. The substitution pattern of anthocyanidins affects different cellular signalling cascades regulating cell proliferation. *Mol. Nutr. Food Res.* 48(4): 318-325.

Martin, M. and Fitzgerald, M.A. 2002. Proteins in rice grains influence cooking properties!. *J. Cereal Sci.* 36(3): 285-294.

Maruyama, A., Hamasaki, T., Sameshima, R., Nemoto, M., Ohno, H., Ozawa, K., and Wakiyama, Y. 2015. Panicle emergence pattern and grain yield of rice plants in response to high temperature stress. *J. Agric. Meteorol.* 71(4): 282-291.

Matsui, T. and Omasa, K. 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: anther characteristics. *Ann. Bot.* 89(6): 683-687.

Matsui, T., Omasa, K., and Horie, T. 2000. High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Prod. Sci.* 3(4): 430-434.

Matsui, T., Omasa, K., and Horie, T. 2001. The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Prod. Sci.* 4(2): 90-93.

McCready, R.M., Guggolz, J., Silviera. V., and Owens, H.S. 1950. Determination of starch and amylose in vegetables. Anal. Chem. 22(9): 1156-1158.

Mession, J.L., Sok, N., Assifaoui, A., and Saurel, R. 2013. Thermal denaturation of pea globulins (*Pisum sativum* L.) molecular interactions leading to heat-induced protein aggregation. *J. Agric. Food Chem*. 61(6): 1196-1204.

Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. 2007. Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58(8): 1935-1945.

Morris, D.A. and Arthur, E.D. 1984. Invertase activity in sinks undergoing cell expansion. *Plant Growth Regul*. 2: 327-37.

Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., Cavallini, E., D'Incà, E., Tornielli, G.B., and Filippetti, I. 2016. The grapevine VviPrx31 peroxidase as a candidate gene involved in anthocyanin degradation in ripening berries under high temperature. *J. Plant Res.* 129(3): 513-526.

Nagarajan, S., Jagadish, S. V. K., Hari Prasad, A. S., Thomar, A. K., Anand, A., Pal, M., and Agarwal, P. K. 2010. Local climate affects growth, yield and grain quality of aromatic and non-aromatic rice in north western India. *Agric. Ecosyst. Environ*. 138(3-4): 274–281.

Neilson, E.H., Goodger, J.Q., Woodrow, I.E., and Møller, B.L. 2013. Plant chemical defense: at what cost? *Trends Plant Sci.* 18(5): 250-258.

Nishi, A., Nakamura, Y., Tanaka, N., and Satoh, H. 2001. Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. *Plant Physiol.* 127(2): 459-472.

Oh-e, I., Saitoh, K., and Kuroda, T. 2007. Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. *Plant Prod. Sci.* 10(4): 412-422.

Oliver, S.N., Van Dongen, J.T., Alfred, S.C., Mamun, E.A., Zhao, X., Saini, H.S., Fernandes, S.F., Blanchard, C.L., Sutton, B.G., Geigenberger, P., and Dennis, E.S. 2005. Cold-induced repression of the rice anther-specific cell wall invertase gene OSINV4 is correlated with sucrose accumulation and pollen sterility. *Plant Cell Environ.* 28(12): 1534-1551.

Ordonez, A.A.L., Gomez, J.D., Vattuone, M.A., and Isla, M.I. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chem.* 97: 452-458.

Pan, J., Cui, K., Wei, D., Huang, J., Xiang, J., and Nie, L. 2011. Relationships of non-structural carbohydrates accumulation and translocation with yield formation in rice recombinant inbred lines under two nitrogen levels. *Physiologia Plant.* 141(4): 321-331.

Peng, S., Huang, J., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X., Centeno, G. S., Khush, G. S., and Cassman, K. G. 2004. Rice yields decline with higher night temperature from global warming. *Proc. Natl. Acad. Sci.* 101(27): 9971–9975.

Porter, J.R. 2005. Rising temperatures are likely to reduce crop yields. *Nature* 436(7048): 174.

Pourcel, L., Irani, N.G., Koo, A.J., Bohorquez-Restrepo, A., Howe, G.A., and Grotewold, E. 2013. A chemical complementation approach reveals genes and interactions of flavonoids with other pathways. *Plant J.* 74(3): 383-397.

Rang, Z.W., Jagadish, S.V.K., Zhou, Q.M., Craufurd, P.Q., and Heuer, S. 2011. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. *Environ. Exp. Bot.* 70(1): 58-65.

Ranganna, S. 1976. *Manual of Analysis of Fruits and Vegetable Products*. McGraw Hill, New Delhi, 77p.

Ray, D.K., Mueller, N.D. Paul, C., and Foley, J.A. 2013. Yield trends are insufficient to double global crop production by, 2050. *PloS one*. 8: e66428.

Roitsch, T. and Gonzalez, M.C. 2004. Function and regulation of plant invertases: sweet sensations. Trends Plant Sci. 9(12): 606–613.

Roitsch, T., Balibrea, H.E., Hofmann, M., Proels, R., and Sinha, A.K. 2003. The upregulation of extracellular invertase was suggested to be a common response to various biotic and abiotic stress related stimuli like pathogen infection and salt stress. *J. Exp. Bot*. 54: 513-524.

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J.A., Hilal, M., and Prado, F.E. 2009. Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signalling Behav.* 4(5): 388-393.

Rowland-Bamford, A.J., Baker, J.T., Allen Jr, L.H., and Bowes, G. 1996. Interactions of CO_2 enrichment and temperature on carbohydrate accumulation and partitioning in rice. *Environ. Exp. Bot.* 36(1): 111-124.

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods for Agricultural Sciences*. Wiley eastern limited, New Delhi, pp.5-6.

Satoh, H., Nishi, A., Yamashita, K., Takemoto, Y., Tanaka, Y., Hosaka, Y., Sakurai, A., Fujita, N., and Nakamura, Y. 2003. Starch-branching enzyme I-deficient mutation specifically affects the structure and properties of starch in rice endosperm. *Plant Physiol.* 133(3): 1111-1121.

Sebastian, J.S.V., Somayanda, I.M., Chiluwal, A., Perumal, R., Prasad, P.V., and Jagadish, S.K. 2017. Resilience of pollen and post-flowering response in diverse sorghum genotypes exposed to heat stress under field conditions. *Crop Sci.* 57(3): 1658-1669.

Shamloo, M., Babawale, E.A., Furtado, A., Henry, R.J., Eck, P.K., and Jones, P.J. 2017. Effects of genotype and temperature on accumulation of plant secondary metabolites in Canadian and Australian wheat grown under controlled environments. *Sci. Rep.* 7(1): 1-13.

Sharma, K.P. and Sharma, N. 2018. Influence of high temperature on sucrose metabolism in chalky and translucent rice genotypes. *Proc. Natl. Acad. Sci.* 88(3): 1275-1284.

Shi, W., Yin, X., Struik, P.C., Solis, C., Xie, F., Schmidt, R.C., Huang, M., Zou, Y., Ye, C., and Jagadish, S.K. 2017. High day-and night-time temperatures affect grain growth dynamics in contrasting rice genotypes. *J. Exp. Bot.* 68(18): 5233-5245.

Siaut, M., Cuine, S., Cagnon, C., Fessler, B., Nguyen, M., Carrier, P., Beyly, A., Beisson, F., Triantaphylides, C., Li-Beisson, Y. and Peltier, G. 2011. Oil accumulation in the model green alga *Chlamydomonas reinhardtii:* characterization, variability between common laboratory strains and relationship with starch reserves. *BMC Biotechnol.* 11(1): 1-15.

Singh, M., Kumar, J., Singh, S., Singh, V.P., and Prasad, S.M. 2015. Roles of osmo protectants in improving salinity and drought tolerance in plants: a review. *Rev. Environ. Sci. Bio/Technol.* 14(3): 407-426.

Singh, N., Kaur, L., Sodhi, N.S., and Sekhon, K.S. 2005. Physicochemical, cooking and textural properties of milled rice from different Indian rice cultivars. *Food Chem.* 89(2): 253-259.

Singh, R.K., Jaishankar, J., Muthamilarasan, M., Shweta, S., Dangi, A., and Prasad, M. 2016. Genome-wide analysis of heat shock proteins in C $_4$ model, foxtail millet identifies potential candidates for crop improvement under abiotic stress. *Sci. Rep.* 6(1): 1-14.

Sita, K., Sehgal, A., Bhandari, K., Kumar, J., Kumar, S., Singh, S., Siddique, K.H., and Nayyar, H. 2018. Impact of heat stress during seed filling on seed quality and seed yield in lentil (*Lens culinaris Medikus*) genotypes. *J. Sci. Food Agric*. 98(13): 5134-5141.

Skirycz, A., De Bodt, S., Obata, T., De Clercq, I., Claeys, H., De Rycke, R., Andriankaja, M., Van Aken, O., Van Breusegem, F., Fernie, A.R., and Inzé, D. 2010. Developmental stage specificity and the role of mitochondrial metabolism in the response of Arabidopsis leaves to prolonged mild osmotic stress. *Plant Physiol.* 152(1): 226-244.

Smith, J.R., Mengistu, A., Nelson, R.L., and Paris, R.L. 2008. Identification of soybean accessions with high germinability in high-temperature environments. *Crop Sci.* 48: 2279–2288.

Snider, J.L. and Oosterhuis, D.M. 2011. How does timing, duration, and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant Signal Behav.* 6(7): 930-933.

Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem. 195*: 19-23.

STATISTA. 2018. The Portal for statistics 2017-18. Available: https://www.statista.com/statistics/263977/world-grain-production-by-type [25 April 2018].

Sun, X.D. and Arntfield, S.D. 2012. Molecular forces involved in heat-induced pea protein gelation: effects of various reagents on the rheological properties of salt-extracted pea protein gels. *Food Hydrocoll.* 28(2): 325-332.

Sürek, H. and Beşer, N. 2005. Selection for grain yield and its components in early generations in rice (*Oryza sativa* L.). *Trakya Univ. J. Sci*. 6(1): 51-58.

Swindell, W.R., Huebner, M., and Weber, A.P. 2007. Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. *BMC Genomics* 8(1): 125.

Tanaka, N., Fujita, N., Nishi, A., Satoh, H., Hosaka, Y., Ugaki, M., Kawasaki, S., and Nakamura, Y. 2004. The structure of starch can be manipulated by changing the expression levels of starch branching enzyme IIb in rice endosperm. *Plant Biotechnol. J.* 2(6): 507-516.

Tenorio, F.A., Ye, C., Redoña, E., Sierra, S., Laza, M., and Argayoso, M.A. 2013. Screening rice genetic resources for heat tolerance. *Sabrao J. Breed. Genet*. 45(3): 371-381.

Tian, X., Luo, H., Zhou, H., and Wu, C. 2009. Research on heat stress of rice in China: progress and prospect. *Chin. Agric. Sci.* 25(22): 166-168.

Tian, X.H., Matsui, T., Li, S.H., and Lin, J.C. 2007. High temperature stress on rice anthesis: Research progress and prospects. *Chin. J. Appl. Ecol*. 18(11): 2632–2636.

Triboi, E. and Triboi-Blondel, A.M. 2002. Productivity and grain or seed composition: a new approach to an old problem. *Eur. J. Agron.* 16(3): 163-186.

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M.R. 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.* 61(3): 199-223.

Wang, X., Cai, J., Liu, F., Jin, M., Yu, H., Jiang, D., Wollenweber, B., Dai, T., and Cao, W. 2012. Pre-anthesis high temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. *J. Cereal Sci.* 55(3): 331-336.

Wang, X., Chang, L., Wang, B., Wang, D., Li, P., Wang, L., Yi, X., Huang, Q., Peng, M., and Guo, A. 2013. Comparative proteomics of *Thellungiella halophila* leaves from plants subjected to salinity reveals the importance of chloroplastic starch and soluble sugars in halophyte salt tolerance. *Mol. Cell. Proteomics* 12(8): 2174-2195.

Weber, H., Borisjuk, L., and Wobus, U. 2005. Molecular physiology of legume seed development. *Annu. Rev. Plant Biol. 56*: 253-279.

Weerakoon, W.M.W., Maruyama, A., and Ohba, K. 2008. Impact of humidity on temperature-induced grain sterility in rice (*Oryza sativa* L). *J. Agron. Crop Sci.* 194(2): 135-140.

Weiss, J. 2009. The economics of climate change in southeast asia: a regional review. Asian Development Bank, Manila, Philippines.

WHO, 2003. Diet, Nutrition and the Prevention of Chronic Diseases. In: *WHO Technical Report Series.* World Health Organisation of the Food and Agriculture Organization of the United Nations, Geneva.

Xie, X.J., Li, B.B., Li, Y.X., and Shen, S. H. 2009. High temperature harm at flowering in Yangtze River basin in recent 55 years. *Jiangsu J. Agric. Sci.* 25(1): 28–32.

Xiong, D., Yu, T., Ling, X., Fahad, S., Peng, S., Li, Y., and Huang, J. 2015. Sufficient leaf transpiration and non-structural carbohydrates are beneficial for high-temperature tolerance in three rice (*Oryza sativa*) cultivars and two nitrogen treatments. *Funct. Plant Biol.* 42(4): 347-356.

Yali, M. and Zhiguo, Z. 1997. Relationship between rice grain quality and temperature during seed setting period. *Chin. J. Rice Sci.* 11: 51–54.

Yaliang, W.A., Yikai, Z.H., Qinghua, S.H., Huizhe, C.H., Jing, X.I., Guohui, H.U., Yanhua, C.H., Xiaodan, W.A., Junke, W.A., Zihao, Y.I., and Defeng, Z. 2020. Decrement of sugar consumption in rice young panicle under high temperature aggravates spikelet number reduction. *Rice Sci*. 27(1): 44-55.

Yang, T.M. and Chen, J.H. 2007. Effects of hot disaster of high temperature in summer on rice growth in Central Anhui. *J. Anhui Agric. Sci*. 35(27): 8530–8531.

Yang, H., Gu, X., Ding, M., Lu, W., and Lu, D. 2018. Heat stress during grain filling affects activities of enzymes involved in grain protein and starch synthesis in waxy maize. *Sci. Rep.* 8(1): 1-9.

Yüzbaşıoğlu, E., Dalyan, E., and Akpınar, I. 2017. Changes in photosynthetic pigments, anthocyanin content and antioxidant enzyme activities of maize (*Zea mays* L.) seedlings under high temperature stress conditions. *Trakya Univ. J. Nat. Sci.* 18(2): 97-104.

Zaidi, S.H.R., Zakari, S.A., Zhao, Q., Khan, A.R., Shah, J.M., and Cheng, F. 2019. Anthocyanin accumulation in black kernel mutant rice and its contribution to ROS detoxification in response to high temperature at the filling stage. *Antioxidants* 8(11): 510.

Zandalinas, S.I., Mittler, R., Balfagon, D., Arbona, V., and Gomez, C.A. 2018. Plant adaptations to the combination of drought and high temperatures. *Physiol. Pl.* 162(1): 2–12.

Zanora, M.I., Osorio, S., Nunes-Nesi, A., Carrarib, F., Lohse, M., Usadel, B., Kühn, C., Bleiss, W., Giavalisco, P., Willmitzer, L., and Sulpice, R. 2009. RNA interference of LIN5 in *Solanum lycopersicum* confirms its role in controlling Brix content,

uncovers the influence of sugars on the levels of fruit hormones and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. *Plant Physiol*. 150: 1204–1218.

Zeeman, S.C., Kossmann, J., and Smith, A.M. 2010. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu Rev. Plant Biol.* 61: 209-234.

Zhang, C., Li, G., Chen, T., Feng, B., Fu, W., Yan, J., Islam, M.R., Jin, Q., Tao, L., and Fu, G. 2018. Heat stress induces spikelet sterility in rice at anthesis through inhibition of pollen tube elongation interfering with auxin homeostasis in pollinated pistils. *Rice,* 11(1): 1-12.

Zinn, K.E., Tunc-Ozdemir, M., and Harper, J.F. 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. *J. Exp. Bot.* 61(7): 1959-1968.

Ziska, L.H., Manalo, P.A., and Ordonez, R.A. 1996. Intraspecific variation in the response of rice (*Oryza sativa* L.) to increased CO_2 and temperature: growth and yield response of 17 cultivars. *J.* Exp. Bot. 47(9): 1353-1359.

APPENDICES

APPENDICES

Acetate Buffer (pH - 5):

A: 0.2 M solution of acetic acid (11.55 mL in 1000 mL)

B: 0.2 M solution of sodium acetate (16.4 g of C_2H_2Na or 27.2 g of $C_2H_3Ona.3H_2O$ in 1000 mL).

14.8 mL of A, 35.2 of B, diluted to a total of 100 mL

Phosphate Buffer Saline:

A: 0.2 M solution of monobasic sodium phosphate (27.8 g in 1000 mL)

B: 0.2 M solution of dibasic sodium phosphate (53.65 g of $Na_2HPO_4.7\ H_2O$ or 71.7 g of $Na_2HPO_4.12\ H_2O$ in 1000 mL).

51 mL of A, 49 of B, diluted to a total of 200 mL

ABSTRACT

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE, VELLAYANI
DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY
MASTER'S DEFENSE SEMINAR

ABSTRACT

Kandanulu Pravallika **Date: 26-06-2020**
2018-11-162 **Time: 2.00- 2.30 pm**

EFFECT OF HIGH TEMPERATURE STRESS ON SEED FILLING AND NUTRITIONAL QUALITY OF RICE (*Oryza sativa* L.)

The present study entitled "Effect of high temperature stress on seed filling and nutritional quality of rice (*Oryza sativa* L.)" was conducted in the Department of Seed Science and Technology, College of Agriculture, Vellayani during the period from 2018-2020 with the objective to study the effect of high temperature stress on seed filling and nutritional quality of rice.

The extent of variation for various seed quality parameters, enzymes and yield parameters were assessed as an indicator of high temperature stress from three rice varieties namely Prathyasa, Hraswa and Manuratna. These varieties were collected from Regional Research Station (RRS), Moncombu and from Agricultural Research Station (ARS), Mannuthy respectively. The experiment was laid out in CRD with two treatments such as control and high temperature conditions and replicated thrice. The plants were kept in a temperature controlled polyhouse from seedling to maturity stage. Seed quality and enzyme parameters were analyzed at 30 and 50 days after 50 percent flowering and yield parameters were taken at harvest stage.

Seed filling parameters recorded at milky and dough stage had revealed that high temperature stress condition resulted an increase of reducing sugar (14.810 mg g^{-1}), carbohydrates (898.310 mg g^{-1}), starch (14.961 mg g^{-1}), and flavonoids (14.385 mg $100g^{-1}$) compared to control. However, amylose (17.692 %), seed protein (4.081 %), and anthocyanin (15.490 mg $100g^{-1}$) showed reduction under high temperature stress condition.

Activity of invertase was reduced under high temperature condition compared to control in all varieties from 15 to 30 days after 50 % flowering.

Under high temperature condition, spikelet fertility percentage and grain yield/plant were reduced compared to control condition. Among three varieties, highest grain yield/plant (22.790 g/plant) and spikelet fertility percentage (80.598 %) under high temperature was recorded by Prathyasa. Average reduction of grain yield/ plant and spikelet fertility percentage under high temperature was 18.733 g/plant and 74.245 % respectively. Percentage of yield reduction was maximum for Hraswa variety (27.57 %) when compared to varieties Prathyasa (21.1 %) and Manuratna (21.18%).

Pollen viability percentage was reduced under high temperature condition compared to control. Prathyasa recorded the highest pollen viability (86.358 %) and Hraswa recorded the lowest pollen viability (77.948 %). Average reduction in pollen viability under high temperature condition was 82.653 %.

There is significant difference for seed protein content among varieties. Highest seed protein content was recorded by Hraswa. There is no significant difference between Prathyasa and Manuratna for reducing sugar content, amylose, starch, invertase and yield.

High temperature stress in rice recorded an increase of reducing sugar, carbohydrates, starch and flavonoids. Grain yield/ plant, spikelet fertility percentage, amylose, anthocyanin, seed protein and invertase activity were reduced under high temperature condition in all the varieties. Flavonoids are the secondary metabolites that are increased in high temperature condition and it helped in defense. High temperature had adverse impact on yield, spikelet fertility and pollen viability and the impact were greater in Hraswa variety.

The correlation analysis revealed that under high temperature condition yield showed positive and significant correlation with total carbohydrate, starch, anthocyanin, spikelet fertility and pollen viability. Hence it is important to identify the rice varieties

which possess the major seed filling parameters which play a vital role in increasing thermotolerance that result in giving a satisfactory grain yield even under conditions of heat stress.