PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR STUDIES IN MEDICINAL RICE (*Oryza sativa* L.), NJAVARA, AS INFLUENCED BY ABIOTIC STRESSES

WAGH YOGESH SAHEBRAO

(2015-21-013)

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2020

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR STUDIES IN MEDICINAL RICE (*Oryza sativa* L.), NJAVARA, AS INFLUENCED BY ABIOTIC STRESSES

By

WAGH YOGESH SAHEBRAO

(2015-21-013)

THESIS

Submitted in partial fulfillment of the

requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR STUDIES IN MEDICINAL RICE (*Oryza sativa* L.), NJAVARA, AS INFLUENCED BY ABIOTIC STRESSES"** is a bonafide record of research work done by me during the course of research and that 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.

Place: Vellayani
Date:

Wagh Yogesh Sahebrao

(2015-21-013)

CERTIFICATE

Certified that this thesis **"PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR STUDIES IN MEDICINAL RICE (*Oryza sativa* L.), NJAVARA, AS INFLUENCED BY ABIOTIC STRESSES"** is a record of research work done independently by **Mr. Wagh Yogesh Sahebrao** 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 him.

Place: Vellayani
Date:

Dr. M. M. Viji

(Major Advisor, Advisory Committee)

Professor and Head

Dept. of Plant Physiology

College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Mr. Wagh Yogesh Sahebrao a candidate for the degree of **Doctor of Philosophy in Agriculture** with major field in Plant Physiology, agree that the thesis entitled **"PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR STUDIES IN MEDICINAL RICE (*Oryza sativa* L.), NJAVARA, AS INFLUENCED BY ABIOTIC STRESSES"** may be submitted by **Mr. Wagh Yogesh Sahebrao** in partial fulfilment of the requirement for the degree.

Dr. M. M. Viji
Major Advisor,
Professor and Head
Dept. of Plant Physiology,
College of Agriculture, Vellayani

Dr. Beena R.
Assistant Professor
Dept. of Plant Physiology,
College of Agriculture,
Vellayani

Dr. Roy Stephen
Professor
Dept. of Plant Physiology,
College of Agriculture,
Vellayani

Dr. K. B. Soni
Professor
Dept. of Plant Biotechnology,
College of Agriculture,
Vellayani

Dr. R. V. Manju
Professor
Dept. of Plant Physiology,
College of Agriculture,
Vellayani

Dr. Thomas George
Professor
Dep. of SS & AC
College of Agriculture,
Vellayani

External Examiner

Dr. P. JEYAKUMAR, M.Sc.(Ag.), Ph.D.,
Professor and Head
Department of Crop Physiology
Tamil Nadu Agricultural University
Coimbatore - 641 003.

ACKNOWLEDGEMENT

I bow to the lotus feet of ***Almighty,*** *whose grace had endowed me the inner strength and confidence and blessed me with a helping hand at each step during my long sojourn at Kerala.*

At this moment of accomplishment, my heart is overwhelmed with gratitude and I wish if these words could convey the subtle feelings.

I am ineffable in expressing my deep sense of gratitude to my Major Advisor ***Dr. M. M. Viji,*** *Professor and Head, Dept. of Plant Physiology, College of Agriculture, Vellayani, for her inspiring guidance, critical comments, constant supervision, support and encouragement throughout the course of my research work. And helping me to explore the field of Plant Physiology and my potentials. I am really grateful for the keen interest taken by her in the prompt correction of the manuscript.*

My sincere thanks are due to, ***Dr. Roy Stephen*** *Professor, Dept. of Plant Physiology, College of Agriculture, Vellayani, member of my advisory committee for the valuable guidance, timely suggestions and help rendered in the conduct of the experiment.*

I also owe my deep sense of gratitude and sincere thanks to ***Dr. R. V. Manju*** *Professor, Dept. of Plant Physiology, College of Agriculture, Vellayani, member of my advisory committee for the her timely help, critical scrutiny of the manuscript and valuable guidance for the successful completion of my research work.*

I am extremely thankful to ***Dr. K. B. Soni,*** *Professor and Head, Dept. of Plant Biotechnology, College of Agriculture, Vellayani, member of my advisory committee for her constant support, suggestions, valuable guidance and timely help rendered in the conduct of the experiment.*

I take this opportunity to express my indebtedness with veneration and devotion to ***Dr. Beena R.*** *Assistant Professor, Dept. of Plant Physiology, and* ***Dr. Thomas George*** *Professor, Dep. of SS & AC College of Agriculture, Vellayani College of Agriculture, Vellayani, member of my advisory committee for support, suggestions and guidance throughout the study.*

My heartfelt gratitude cannot be captured in words for the unflinching support, constant encouragement, warm concern, patience and valuable advice of my friends ***Dr. Pritam, Dr. Sachin, Dr. Gayathri, Prashant, Nilesh Dada, Shubham, Pratik, Preeti, Vipin, Dr. Smitha chichi, Dr. Maheshwari, Irfana and Anila chichi*** *whose prayers, love and affection rendered me a successful path which propped up my career all along. I wish to express my sincere gratitude to my senior friends* ***Dr. Deepa chichi, Dr. Pritin, Dr. Srikanth, Dr. Shiddhesh and Dr. Vinay*** *for their whole hearted support.*

My duty is incomplete if I forget my junior friends ***Dr. Nithya, Dr. Manasa, Afna, Ammu, Sayooj, Lekshmi, Amaritha, Bhavana, Sudha, Sreerag, Sreevardhan, Nagmani, Chanda, Manu, Vivek and Rameshwar*** *whose helping hands, love and affection fetched a remarkable place in my days in Kerala.*

I express my deep sense of gratitude to ***Kerala Agricultural University*** *for financial and technical support for persuasion of my study and research work.*

Above all, I am forever beholden to my beloved ***parents, brothers, sisters and family members*** *for their unfathomable love, boundless affection, personal sacrifice, incessant inspiration and constant prayers, which supported me to stay at tough tracks. Thank you to my mother,* ***Kamal,*** *and father,* ***Sahebrao*** *for guiding me as a person and teacher throughout this process and believed in me and wanted the best for me. Thank you to my Brothers,* ***Mahesh (Sonu), Ramesh, Ganesh and Rajendra*** *and Sisters,* ***Seema, Sarla and Mangal*** *for loving me unconditionally.*

Wagh Yogesh Sahebrao

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF PLATES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF APPENDICES

Introduction

1. INTRODUCTION

For centuries medicinal plants have been serving humanity as a source of traditional as well as herbal drugs. The medicinal plants are the "backbone" of traditional medicine and drugs. Globally more than 3.3 or 4.0% billion people in the less developed countries are dependent on medicinal plants on a regular basis to meet their primary health care needs (Davidson-Hunt, 2000; WHO, 2003). The WHO promotes plant medicine or traditional medicine in national health care programs because they are considered to be much safer than the modern synthetic drugs and having better compatibility with minor or no toxic effect and easily accessible at a price within the reach of a common people (Dar *et al.,* 2017). The use of traditional medicinal plants is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. China alone contributes about 40% of the total traditional medicines consumption whereas, in Japan more than mainstream pharmaceutical products, herbal medicinal preparations are more in demand.

In India, the collection and processing of medicinal plants and plant products contribute a major part to the national economy as well as both full and part-time employment and in enhancing export earnings (Holley and Cherla, 1998). In recognition of the significance of the sub-sector and the fact that it is largely undocumented, the World Bank and the IDRC Medicinal plants Network (IMPN) agreed to produce this state of the art report on the medicinal plants sector in India (Mazid *et al.,* 2012). There are about 17,000 species of higher plants, among which around 8,000 species are considered medicinal and has hosted the development of an ancient and traditional method of treatments namely Ayurveda, Siddha, Unani and the modern system of medication, allopathy (Singh, 2015).

Rice crop occupies around 23 per cent, 35 per cent and 44 per cent of an area under gross cropped, food grains and cereals respectively and grown in over a wide

geographical range and numerous cultural conditions (Umadevi *et al.,* 2012). Rice has immense nutritional and medicinal properties known from the earliest days. Certain rice varieties with therapeutic properties are cultivated and being used in the treatment of many ailments in several counties. Rice bran extract which is rich in vitamin-B is used for prevent and cure beriberi in Philippines. Rice used for treating eye and acute inflammation of the inner body and strengthening weak stomach, mature hulls are used for treating dysentery and rice water is prescribed to counteract inflamed surface in the countries Malaysia, Cambodia, china and India respectively (Kalaivani *et al.,* 2016).

Kerala has enormous number of rice varieties which have medicinal and therapeutic properties. Among all those varieties, only very few are still being cultivated in limited parts of Kerala on small scale for their medicinal values e.g. Njavara, Chennellu, Kunjinellu, Erumakkari and Karuthachembavu (Elsy *et al.,* 1992; Menon and Potty, 1999; Ashraf and Lokanadan, 2017). Out of these varieties mentioned above, Njavara is a unique grain plant in the *Oryza* genus and has been cultivated over 2500 years. The ancient Ayurvedic text Ashtanga Hridaya written by Vagabattain 400-500 AD, mentioned medicinal use of Njavara especially in Panchakarma treatment (Murthy, 2001). In Ashtanga Hridaya (Sootrsthanam Chapter 6 sloka 7-10 Annaswaroopavigyani) described there are two different types of Njavara *viz.,* yellow and black colored grain based on colour of the glume (Joseph *et al.,* 2007). Over the time studies on genetic diversity of njavara and commonly used rice varieties, it is evident that Njavara is genetically different from other rice varieties (Elsy *et al.,* 1992; Deepa *et al.,* 2009; Jose *et al.,* 2010; Kumar *et al.,* 2010). They are found predominantly in Wayanad, Palakkad, Kuttanad, and northern Kerala respectively. The farmers have maintained the genetic purity of Njavara and this was possible as this variety has very short duration than other commonly cultivated rice varieties and also has asynchronous flowering pattern and thus avoiding the cross pollination with other varieties (Sreejayan *et al.,* 2010).

Njavara has many medicinal properties to cure many diseases associated to digestive, respiratory and circulatory systems. Old time medicinal practitioners used njavara to cure various illness comprising rheumatism, arthritis, cerebral palsy, muscular dystrophy, blood pressure and also for the relaxation and rejuvenation of weak muscles in aged persons (Rani and Sukumari, 2016). For the treatment of paralysis, arthritis and neurological problems there is specialized therapy in Ayurveda called "Kizhi" in which njavara rice is cooked along with milk and used for massaging the body (Deepa *et al.*, 2008). This treatment increases the blood circulation and relieves stiffness of the joints and arthritis pain *via*, heat generation and extensively sweating. However there is no scientific data available for this treatment (Deepa *et al.*, 2008). The isolated flavonoid compound from njavara rice bran shows good antioxidant activity and brings cure to skin inflammation and other related skin infections (Bakiyalakshmi and Boominathan, 2014). Also antioxidant properties of njavara rice also help to maintain the sugar level of diabetic patients.

In plants, abiotic stresses are the major exogenous factors responsible for the production of various antioxidants and secondary metabolites in plants. There are many types of abiotic factors *viz.,* drought, high and low temperature, alkalinity, salinity and ultraviolet light stress which are potentially activate of several secondary metabolite pathways in plants. These abiotic factors are widely used as elicitors to increase the production or to induce de novo synthesis of secondary metabolites under *in vitro* systems and could increase the secondary metabolite production in cell, tissue and organ cultures of plants (Dicosmo and Misawa, 1985; Sudha and Ravishankar, 2003). Abiotic stresses found to enhance biosynthesis of almost all classes of secondary metabolites (Selmar and Maik, 2013). But depending on physiological and developmental stages of plants, the production of secondary metabolites varies greatly and usually they are produced at very low concentrations (Rao and Ravishankar, 2002).

Hence the present project was proposed with the objective, to elicit information on the physiological, biochemical and molecular attributes associated to secondary metabolites accumulation due to abiotic stresses *viz.*, shade, drought and UV-B stress in medicinal rice njavara.

Review of Literature

2. REVIEW OF LITERATURE

Medicinal and aromatic plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of medicinal qualities of certain plants has been transferred over centuries among the people. Majority of the world's population is dependent on medicinal plants as the most important source of life-saving drugs. The safety, quality and efficiency of therapeutic products from plants is an important concern both in industrialized as well as developing countries (Singh, 2015). According to World Health Organization, in developing countries more than 80 percent of the population primarily depends on herbal medicines for basic healthcare needs. Approximately only 10% of medicinal species are used commercially, out of the 50,000 different medicinal plant species collected from wild and the demand for herbal medicine is not only high but also increasing over years (Srivastava, 2000; Pourmohammad, 2013).

Rice is the basic food for more than a billion people all over the world because of its availability, nutritional value and medicinal properties. There are several different medicinal varieties of rice being cultivated and used in various parts of the world and none of them are being used for medicinal purposes as broadly as njavara (*Oryza sativa* L., var. 'Njavara'). Njavara is used in the treatment of various diseases related to circulatory, respiratory and digestive ailments in traditional medicine (Reshmi and Nandini, 2013). The detailed information about nutritional and medicinal qualities of njavara rice can be seen in the ancient ayurvedic text "Ashtanga Hridaya", circa 400-500 AD (Murthy, 2001). Njavara is the only cultivar conventionally used in ayurvedic system of medicine in some specific treatments such as Panchakarma in "Njavara Kizhi" and "Njavara Theppu".

The majority of human diseases in the current scenario is due to oxidative stress which results due to disproportionation between occurrence and neutralization of oxidants (Braca *et al.,* 2002; Hazra *et al.,* 2008). When the quantity of free radicals increase in cells, it seeks stability by pairing with macromolecules such as proteins, lipids and DNA which then results in cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Devi and Arumughan, 2007; Aswatha *et al.,* 2008; Lai *et al.,* 2009). Antioxidants have the function of scavenging the free radicals and it is very well documented that rice bran is a rich resource of natural antioxidants (Rao-Akiri *et al.,* 2010; Mohanlal *et al.,* 2011). A particular group of flavonoid i.e. flavones is the chemotherapeutic agent found at higher concentration in njavara rice. Also studies with flavones isolated from njavara rice have shown anti-inflammatory effect in carrageenan induced rat paw edema (Mohanlal *et al.,* 2011).

The antioxidant property of njavara rice also helps to maintain the sugar level of diabetic patients with an increase in vitamin-E and scavenging activity of diphenylpicrylhydrazyl (DPPH), hydroxyl radicals, and superoxide anion in the blood (Reshmi and Nandini, 2013). Recently, it was reported that njavara rice has got anti-cancer properties too. Also an anti-cancer gene associated with 'Bowman-Brisk trypsin inhibitor protein' has been identified in njavara rice (Shareesh, 2007). The anti-tumor effect of njavara rice is attributed to the rare flavonolignans, tricin 4′-O-(*erythro-β*-guaiacylglyceryl) ether and tricin 4′-O-(*threo-β*-guaiacylglyceryl) ether. Edaphic, hydrological and atmospheric factors are reported to play an important role in the quality and yield of njavara rice (Shalini *et al.,* 2012).

In plants, the accumulation of antioxidants and various kinds of flavonoids are largely influenced by various endogenous and exogenous factors also, though they are controlled by genetic means. Abiotic stresses are the major exogenous factors responsible for the production of various antioxidants and secondary metabolites in plants. The consequences of abiotic stresses is the activation of several secondary

metabolite pathways in plants. Secondary metabolites accumulated in the plants help them in protecting themselves against herbivores, pathogens as well as environmental stresses (Ramakrishna and Ravishankar, 2011). Under normal environmental conditions plants produce very low concentration of secondary metabolites, as the plants do not need much defense strategies. Secondary metabolites are responsible for odors, tastes and colour of plants (Bennett and Wallsgrove, 1994). Also depending on physiological and developmental stages of plants, the production of secondary metabolites varies greatly and usually they are produced at very low concentrations of less than 1% dry weight only (Rao and Ravishankar, 2002).

There are many types of abiotic factors *viz.*, drought, high and low temperature, alkalinity, salinity and ultraviolet light stress which are potentially damaging to plants. But these abiotic factors are widely used as elicitors to increase the production or to induce de novo synthesis of secondary metabolites under in *vitro* systems (Dicosmo and Misawa, 1985). Studies have shown that different kinds of elicitors could increase the secondary metabolite production in cell, tissue and organ cultures of plants (Sudha and Ravishankar, 2003; Karuppusamy, 2009). Abiotic stresses *viz.*, temperature, humidity, light intensity, water, CO_2 and minerals are found to enhance the accumulation of almost all classes of secondary metabolitessuch as simple and complex phenols, flavonoids as well as different kinds of terpenes and alkaloids (Akula and Gokare, 2011; Selmar and Maik, 2013).

In this chapter, an effort has been made to review the relevant literatureavailable at national and international level on various aspects relevant to thepresent study.

2.1 EFFECT OF SHADE ON VARIOUS CHARACTERS

Light is the main source of energy for plant growth and development andnormal plant growth requires optimum light. But extremely high or low light conditions would lead tophoto inhibition and light deficiency respectively and damage the plant growth

severely. It is well known that light can have impact on growth, morphology, anatomy, physiology, cellular biochemistry, flowering time and plant productivity of plants (Dai *et al.,* 2009; Favaretto *et al.,* 2011; Deng *et al.,* 2012). Light is one of the most importantenvironmental factors among all other factors affecting plant existence, reproduction and distribution (Keller *et al.,* 2005; Kumar *et al.,* 2011).

2.1.1 Effect of shade on Physiological characters

2.1.1.1 Effect of shade on plant height

Plant height is an important agronomic trait of crops that directly affect the yield, crop architecture, apical dominance, biomass, resistance to lodging and crowding. Poor agronomic traits such as smaller grains, excessive tillering, narrower or rolled leaves and poor disease resistance are often correlated with the dwarf trait of the crop and which in turn lead to insufficient growth and huge reduction in yield potential. (Ueguchi-Tanaka *et al.,* 2000; Tanabe *et al.,* 2005; Arite *et al.,* 2007; Li *et al.,* 2009).

Light intensity is one of the key environmental factors regulating the basic characteristics of rice development. Under shaded conditions, the height of rice plants increase whereas, other morphological traits as well as yield and yield attributes decrease (Ren *et al.*, 2002; Deng *et al.*, 2009; Liu *et al.*, 2009). However, the effect of shade on plant height varies greatly among different species. Gibson *et al.* (2004) carried out an experiment to determine the effect of reduced light on rice and late water grass (*Echinochloa phyllopogon*) during 1999 and 2000. The treatments were 50%, 18% shade and 100% sunlight and among them 18% shade showed higher plant height and the least was recorded at 100% sunlight during both the years

In a study by Sunilkumar and Geethakumari (2002) with three different shade conditions *viz.,* 0, 20 and 40% using four rice varieties (Swarnaprabha, A4-4-2, A4-1-3 and Mattatriveni) it was found that at 20 % shade, highest plant height was observed,

followed by 40% shade and open condition. Also, Alridiwirsah *et al.* (2018) tested eleven varieties of rice under shaded conditions and reported that the plant height increased in all the varieties under 25% shade followed by 50% shade compared to the open condition.

While studying inter cropping systems between corn and soybean, the plant height along with stem mass ratio was reported to increase when soybean was grown between two rows of corn than soybean crop grown alone in open field (Fan *et al.,* 2018). A medicinal plant sage (*Salvia officinalis* L.) also showed similar result of increase in plant height when exposed to intense shaded condition of 50 and 70% (Reza *et al.,* 2017).

Though there is increase in plant height, the plants look more etiolated under shaded condition (Taiz and Zeiger, 2002) and apical dominance is exhibited under lower shade levels (Mendes *et al.,* 2001; Moniruzzaman *et al.,* 2009). Also increase in the plant height with increase in shade levels mainly indicate a phototropic response taking place to modify the distribution of leaves so as to help plants to get enough light (Takemiya *et al.,* 2005; Yang *et al.,* 2007; Wang *et al.,* 2009; Mapes and Xu, 2014). Another reason attributed is that the low light stress, stimulate rapid cell division and cellular expansion which in turn lead to an increase in plant height and leaf length (Schoch, 1972).

On the contrary,a study conducted by Chauhan (2015) in wild type rice and cultivated rice indicated that the plant height did not increase with shade (50% and 25% of shade) and the reason attributed was that the threshold for the photomorphogenic response was not exactly reached by the shade treatments given in that study.

2.1.1.2 Effect of shade on leaf area index, specific leaf area and number of tillers

Leaves play an important role in a biogeochemical cycle in the environment and hence any changes in leaf characters lead to changes in plant growth and

development (Wright *et al.,* 2004; He *et al.,* 2006). Also it is well known that leaf surface is important to carry out processes like absorption of photosynthetic light, carbon dioxide (CO_2) uptake and fixation, transpiration of water and emission of organic volatile compounds (Patil *et al.,* 2018).

Leaf area index (LAI) is the main physical and structural property of vegetation and is defined as half of the total leaf per unit of the ground surface covered by the plant (Chen and Cihlar, 1996). Also specific leaf area (SLA) is the best trait to know the whole plant growth (Cornelissen *et al.,* 2003; Cheng *et al.,* 2016). SLA is described as the distribution of leaf biomass production in relation to water loss and referred as the water use efficiency at plant canopy level. Hence SLA has a crucial role in linking carbon and water cycle in plants (Pierce *et al.,* 1994; Gunn *et al.,* 1999).

Irrespective of varieties, shading is reported to increase leaf area index (LAI) and specific leaf area (SLA) in rice while delaying tillering and reducing the tiller number (Tsai and Lai, 1990). In a field experiment conducted with two different wheat cultivars; YM 158 and YM 11 (shade tolerant and shade sensitive respectively) at three different shade levels (8, 15, 23%) it was found that both the varieties exhibited higher LAI and SLA at 23% shade followed by 15% and 8% (La *et al.,* 2010). Similar results were obtained in barley crop when exposed to light intensity of 90 µmol m^{-2} s^{-1} compared to natural sun light (Gunn *et al.,* 1999). An experiment conducted in sage (*Salvia officinalis* L.) a medicinal plant showed an increase in LAI and leaf dry weight under shaded condition compared to full light grown plants. However, the leaf dry weight was found to decrease with the increase in shade level (Reza *et al*., 2017).

Aumonde *et al.* (2013) conducted a study by subjecting red rice seedlings to three different light intensities *viz.,* 35%, 65% and 100% for 21 days under greenhouse condition and reported that SLA, LAI and leaf area ratio were higher in seedlings grown under shaded condition than those grown in open condition. Also in that study, the number of tillers were reported to reduce with the decrease in light intensity. Fabre

et al. (2016) reported an increase in SLA in two high yielding rice varieties *viz.,* IR64 and IRRI 146 grown under 58% shade in greenhouse condition compared to control.

In an investigation carried out to study the effect of low light (50% light), red light and normal light on winter rice, it was found that LAI was significantly higher under low light than under red and normal lights especially during panicle initiation stage as well as flowering stage (Barmudoi and Bharali, 2016).

Another effect of shade on rice plants is reduction in tiller numbers and the possible reason is that some of the tiller buds might not grow into productive tillers due to insufficient photoassimilates which is very important for plant growth (Ginting *et al*., 2015; Sridevi and Chellamuthu, 2015). In an experiment conducted with four rice varieties (Si Kembiri, Situ Patenggang, Situ Bagendit and Towuti) grown under two different shade conditions (20% and 40%) it was found that the number of tillers significantly decreased as the shade levels increased (Ginting *et al*., 2015).

Emmanuel and Mary (2014) investigated a Nigerian local rice variety under different light intensities *viz.,* 75, 50 and 25% along with 100% light as control. The result indicated that the tiller numbers decreased as the light intensity decreased. Also, Muhidin *et al*. (2018) reported that productive tillers significantly reduced under shaded conditions in upland red rice when subjected to similar treatments as indicated in the above mentioned study.

Another study carried out with eleven superior rice varieties, when subjected to two different shade levels (25%, 50%) indicated that at 50% shade level, least number of tillers were produced compared to 25% shade level (Alridiwirsah *et al.,* 2018). Panda *et al*. (2019) also reported similar finding while studying the effect of low light on seven different genotypes of rice.

In order to capture sufficient light and to have better utilization of available light for the production of photoassimilates *via,* photosynthesis, plants change their leaf

morphology by way of increasing both leaf area and leaf biomass, under low light conditions (Cohen *et al.,* 1997; Evans and Poorter, 2001; Lima *et al.,* 2008).

2.1.1.3 Effect of shade on Leaf gas exchange parameters

Leaf gas exchange parameters like photosynthesis, stomatal conductance and transpiration are the basis of plant growth and development and they are highly sensitive to any changes in the environment. Even slight variation in light intensity during the day can cause changes in leaf gas parameters in plants (Yamori *et al.,* 2016). There are many reports indicating that the photosynthetic rate, transpiration rate and stomatal conductance decrease under low light condition in different crops like soybean (Yang *et al.,* 2014; Feng *et al.,* 2019), rice (Panda *et al.,* 2019), wheat (Acreche *et al.,* 2009) mustard (Zhu *et al.,* 2017), sorghum (Li *et al.,* 2014) medicinal plant *Anoectochilus roxburghii* (Shao *et al.,* 2014) and sage (*Salvia officinalis* L.) (Rezai *et al.,* 2017).

Panda *et al*. (2019) investigated seven rice genotypes (Nirajo, Purnendu, Malliksalli, Megharice1, ASD-14, Swarnaprabha and IR-8) grown under low light (25%) and normal light conditions and reported that the photosynthetic rate, stomatal conductance and transpiration rate were all found to decrease in low light treatment by an average of about 20% to 30% in all genotypes. Similar results were also reported in two wheat cultivars subjected to 22% and 33% of the incident natural light (Mu *et al.,* 2010).

Three rice varieties (IR20, CO43 and Swarnaprabha) were grown under 50 percent shade and the results indicated that the photosynthetic rate and photosynthetic efficiency were lesser under shaded condition compared to control in all three varieties (Viji *et al.,* 1997). Also Delouche *et al*. (2007) reported that at 50 percent shade, the rate of photosynthesis was found to decrease both in cultivated rice and weedy rice by 46% and 38% respectively.

In an investigation carried out in two cultivars of wheat under three shaded conditions (8%, 15% and 23%), it was found that the rate of photosynthesis decreased with shade (Li *et al.,* 2010). Ren *et al.* (2016) also reported similar results in maize when the crop was grown under 40% shade. Photosynthetic rate, stomatal conductance and transpiration rate were found to significantly reduce under low light intensity in mustard plants after 15 days of shade treatment (Zhu *et al.,* 2017).

A study with seedlings of sage, a medicinal plant when exposed to 25, 50 and 75 percent of shade conditions and the leaf gas exchange parameters recorded at 60^{th} and 75^{th} day after planting revealed that the photosynthesis rate, stomatal conductance and transpiration rate decreased as light intensity decreased (Zervoudakis *et al.,* 2012). Also in another similar study conducted by Reza *et al*. (2017) in sage, it was found that there was decrease in leaf gas exchange parameters along with water use efficiency under shade.

Photosynthesis is a very sensitive and complex physiological process. However the reason behind decrease in the rate of photosynthesis under low light condition is thought to be due to increase in stomatal closure and decrease in stomata per millimeter and which in turn lead to reduction in stomatal conductance and transpiration rate (Sato and Kim, 1980; Farquhar and Sharkey, 1982; Liu *et al.,* 2014). Shi *et al.* (2006) reported that under shade, the activity of ribulose bisphosphate carboxylase (Rubisco) which is an important enzyme to carry out the process of photosynthesis decrease significantly. Also, Jiao and Li (2001) demonstrated that low light condition changes the rate of electron transfer, quantum yield of PS II and non-photochemical quenching.

2.1.2 Effect of shade on biochemical characters

2.1.2.1 Effect of shade on total flavonoid and total phenol content

Flavonoids and phenols are the naturally occurring, most common and widely distributed polyphenolic compounds in plants (Carlo *et al.,* 1999). Phenolic compounds

have a three-ringed structure and are derived from aromatic amino acid, phenyalanine and tyrosine (Routray and Orsat, 2012) which give them upper hand in ion-metal chelation (Zielinski *et al.,* 2001; Khokhar and Owusu-Apenten, 2003; Babatundeand Oseni, 2016). Phenolic compounds are secondary metabolites and act as antioxidants. They can maintain cell redox status and also regulate cell signaling pathways *via,* gene regulation under stressed conditions (Maggi-Capeyron *et al.*, 2001; Yun *et al.*, 2008).

It is well documented that flavonoids have anti-inflammatory, antiallergic, antibacterial, anti-fungal and cancer protective properties. Also they are reported to give protection against cardiovascular diseases (Knekt *et al.,* 1996; Mohammed *et al.,* 2013). On the other hand, phenols in plants inhibit oxidative damage to macromolecules in the cells such as DNA, protein and lipids in the membrane and regulates oxidative status of the cell *via*, suppressing hydrogen donor and singlet oxygen and acting as reducing agent (Krishnaswamy and Raghuramulu, 1998; Nagah and Seal, 2005; Yun *et al.,* 2008).

Phenolic compounds in plants are mainly synthesized in response to adverse environmental conditions *viz.,* insect and pathogen attack, ultra violet-B (UV-B) radiation, wounding etc. (Chung *et al.,* 2003; Diaz-Napal *et al.,* 2010; Kennedy and Wightman, 2011). Specifically, flavonoids have got the role of protecting leaf tissues from potentially harmful lights such as ultra violet-B (UV-B) radiation and higher level of irradiation. However, photosynthetically active radiation (PAR) also has the capacity to change the concentration of flavonoids either directly or indirectly (Bergquist *et al.,* 2007). Cen and Bornman (1990) reported that higher levels of PAR may support UV-B-induced flavonoid synthesis to a greater extent than lower levels of PAR.

In a study with seven rice varieties grown under 75 percent shade, the accumulation of flavonoids was found significantly lower in four varieties (namely NH686, KMR3, 399 and Swarna) compared to control and at the same time three

varieties (Swarnaprabha, Nagina 22 and 192S) showed less variation between shade treated and control plants (Panigrahy *et al.*, 2019).

In another study with *Labisia pumila* (a herbal plant) locally known as Kacip Fatimah, native to Malaysia when subjected to two levels of light intensities (70% and 30%), the result showed that the levels of flavonoid and phenol content were higher under 70 percent light than 30 percent light (Karimi *et al.,* 2013). Similar results were reported in baby spinach also (Bergquist *et al.,* 2007).

In phenolic compound biosynthetic pathway, phenylalanine ammonialyase (PAL) is an important enzyme. But under shaded condition the activity of PAL enzyme decreases, because the activity of PAL enzyme gets induced by high light intensity conditions (Kumari *et al.,* 2009). Warren *et al.* (2003) demonstrated that the phenolic compound concentration in plants under higher light intensity increases due to more accumulation of primary photosynthates under that condition.

2.1.2.2 Effect of shade on chlorophyll content

Chlorophyll is an important pigment involved in capturing and transmission of solar light. Usually biosynthesis and degradation of chlorophyll take place in the presence of light. However, under high intensity of light, chlorophyll gets degraded drastically (Gonçalves *et al.,* 2005). On the contrary, under shade or low light situation plants go through compensatory mechanism such as an increase in photosynthetic pigments (Lichtenthaler *et al.,* 1981; Baig *et al.,* 2005; Rezai *et al.,* 2018).

However leaf chlorophyll content of rice grown under shaded condition is found to vary greatly among cultivars. Zhu *et al*. (2008) demonstrated that the rice varieties tolerant to low light intensity exhibited higher total chlorophyll content and chlorophyll ‘b’ content and at the same time lowest chlorophyll a/b ratio than the varieties susceptible to shade. In another similar study, rice crop exhibited high leaf chlorophyll content under low light condition at grain filling stage (Liu *et al.,* 2009)

Three rice varieties IR 20 (shade susceptible), CO 43 and Swarnaprabha (shade tolerant) were subjected to 50 percent shade and it was revealed that all the three varieties recorded highest level of chlorophyll contentand lowest chlorophyll a/b ratio under shaded condition whereas, the low light susceptible variety recorded lowest chlorophyll content than the tolerant varieties under shaded condition (Viji *et al.,* 1997). Restrepo and Garcés (2013) reported that chlorophyll content in two rice cultivars increased at panicle initiation and flowering stage of the crop under 50% shade when compared with no shade condition.

Muhidin *et al*. (2018) conducted a study using four different upland rice varieties with four shade treatments (less than 25% shade, 50% shade, 75% shade and more than 75% shade) and reported that the chlorophyll content was higher in plants subjected to 75% and 50% shade. But at lesser than 25% shade, the chlorophyll content of the plants was found relatively higher compared to other shade levels. Alridiwirsah *et al*. (2018) also observed similar results when eleven rice cultivars were subjected to 25% and 50% shade condition as compared to those grown in normal light.

In an investigation with seven genotypes of rice (Nirajo, Purnendu, Malliksalli, Megha rice1, ASD-14, Swarnaprabha and IR-8) grown under low light (25%) and normal light condition the shaded treatment recorded significantly higher content of total chlorophyll than normal light condition (Panda *et al.,* 2019).

Under low light conditions, plants have the ability to adapt and develop strategies such as increasing the chlorophyll content by three-four folds with large and thin leaves (Taiz and zeiger, 2002). This responses of plants allow them to capture sufficient light required for photosynthesis by maintaining enough number of photosynthetic antennae in the leaves (Czeczuga, 1987). Accumulation of more chlorophyll pigment in leaves increase the efficiency of light absorption of leaves and which finally lead to accomplishing carbon balance in plants under low light stress (Dai *et al.,* 2009).

2.1.2.3 Effect of shade on proline content

Under stress, plants synthesize many different classes of metabolites which are non-toxic organic compounds with low molecular weight and highly soluble and among them the most important one is the amino acids. Previously there were many studies reporting positive correlation between plant stresses and accumulation of proline. Proline is an amino acid and plays beneficial role in various stresses in plants. Proline is not only an excellent osmolyte but also acts as an antioxidant, metal chelator, a signaling molecule and stabilizes sub-cellular structures (Ashraf and Foolad, 2007; Hayat *et al.,* 2012).

Mo *et al*. (2015) conducted a study on two aromatic rice varieties exposing them (at grain filling stage) to 67 percent reduced light and found that the proline content increased significantly under shade compared to open condition. In another experiment with hybrid summer cucumber grown under four black shading nets (providing 25%, 50%, 65% and 75% shade) proline content was found to show an increasing trend as the shade levels decreased. In that study the highest proline content was recorded in 25 percent shade and the lowest proline content in 75 percent shade (Semida *et al.,* 2017).

In a study with one-year-old grape plants grown under four different shade levels (25%, 45%, 65% and 80% shade) it was found that the proline content increased with the increasing percentage of shade (Qiu *et al.,* 2018). Alagupalamuthirsolai *et al*. (2018) reported that small cardamom plants accumulated highest proline content under 50% shaded condition followed by 75% shade and the least value was recorded in open natural light condition.

2.2 EFFECT OF DROUGHT ON VARIOUS CHARACTERS

Plants face many biotic and abiotic stresses throughout their life cycle due to changes in surrounding environmental conditions. Drought or water deficit condition

is the major stress which adversely affect crop productivity and yield (Lambers *et al.,* 2008; Farahani *et al.,* 2009). Under such conditions plants show certain changes in their morphology as well as physiology which lead to reduction in plant growth and development (Rahdari and Hoseini, 2012). Also drought stress leads to harmful effects on plant growth characters *viz.,* germination rate, root-shoot length, number of tillers, fresh and dry weight, total biomass, floral initiation, panicle development, pollination, fertilization, seed development, seed yield and seed quality (Jaleel *et al.,* 2007; Bolat *et al.,* 2014). There are several reports indicating variation in plant height, leaf count, leaf diameter, reduction in tiller number and reduction in stem length in plants exposed to water deficit conditions (Sankar *et al.,* 2008; Khan and Kabir, 2014).

At physiological level, water stress leads to very poor photosynthetic rate, reduction in assimilate partitioning, reduction in stomatal conductance, decline in activity of Rubisco enzyme and increase in rate of respiration (Bota *et al.,* 2004; Shahzad *et al.,* 2016). Dehydration condition also is responsible for decrease in cell volume and subsequent cell shrinkage and viscous cellular contents. This viscous state of cytoplasm may develop toxicity which is harmful to enzymes to function normally including photosynthetic machinery (Hoekstra *et al.,* 2001).

Reactive oxygen species such as superoxide anion radicals (O^{-}_{2}), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and alkoxy radicals (RO) are predominantly produced in plants under water deficit condition (Munne-Bosch and Penuelas, 2003). Reactive oxygen species cause damage to the normal functioning of cells by reacting with proteins, lipids and DNA in the cells (Foyer and Fletcher, 2001). Under drought condition, secondary metabolites also get affected. Torras-Claveria *et al*. (2012) reported twenty different phenolic compounds getting upregulated in tobacco plants under water deficit condition. Ma *et al*. (2014) also reported an increase in total phenols, total flavonoids, anthocyanins, and schaftosides in wheat leaves in response to drought. Phenolic compounds are also reported to play

an important role in the drought tolerance mechanism in many plants. However, this response is reported to vary between plant species (Akula and Ravishankar, 2011).

2.2.1 Effect of drought on Physiological characters

2.2.1.1 Effect of drought on plant height

Water is the very important factor for plants and required for plant life processes like seed germination, cell division and cell elongation. Unavailability of water, alters plant growth *viz.,* change in plant architecture, plant height, leaf size and leaf number and also bring changes in reproductive phase of plants (Ajum *et al.,* 2011).

It was reported that a reduction in plant height of high yielding rice varieties (Nada, MT58 and MTA) was noticed when they were subjected to different irrigation intervals *viz.,* 1 day interval and 2 days interval (Ahmadikhah and Marufinia, 2016). Similarly a reduction in plant height was reported in cotton crop also when drought stress was given at different stages of the crop (Ahmad *et al.,* 2013).

In another study with five different rice genotypes (Swarna Sub1, Nagina 22, NDR102, NDR 97 and SuskSamrat) when subjected to drought stress at vegetative and flowering stage a reduction in plant height (49.31%, 17.56%, 28.55%, and 19.99%) was observed in all the different genotypes respectively (Singh *et al.,* 2018). Alghabari and Ihsan (2018) conducted a pot culture experiment to investigate the effect of water stress on barley plants, where the plants were grown at 100, 50 and 30% field capacities and reported that there was 30-50% reduction in plant height at 30% field capacity followed by 50% field capacity (40-50% reduction) compared to the control (100% field capacity).

In another field experiment under drought with five different rice genotypes *viz.,* drought susceptible (IR64), tolerant (Aeron1), moderately tolerant (MR219) and two mutant rice genotypes (MR219-4 and MR219-9), maximum reduction in plant height was reported in drought susceptible genotype followed by the mutant genotypes

and the drought tolerant genotype (Kamarudin *et al.,* 2018). Also greater reduction in plant height of rice plants was reported when drought was imposed at different phenological stages of rice plants, especially when the rice plants were imposed to drought right from vegetative stage onwards the reduction in plant height was much higher compared to control (Parfitt *et al.,* 2017).

Many other important crops such as soybean (Specht *et al.,* 2001), maize (Kamara *et al.,* 2003), okra (Bhatt and Rao, 2005), legumes (Fening *et al.,* 2009) and wheat (Zhang *et al.,* 2018) also showed reduction in plant height under drought. Reduction in plant height under drought lead to limited availability of water for normal cell metabolic processes. This limitation of water impair the process of mitosis and more senescence take place and there is reduction in cell turgor which lead to inhibition of cell division, cell elongation and expansion and thus the plant height gets reduced (Nonami, 1998; Bhatt and Rao, 2005, Kaya *et al.,* 2006; Hussain *et al.,* 2008; Henry *et al.,* 2016).

2.2.1.2 Effect of drought on leaf area index, specific leaf area and number of tillers

The study of leaf morphology such as leaf expansion, leaf elongation and leaf emergence are thought to be useful traits that are found directly associated with vigor and photosynthetic efficiency of plants. Leaves reflect the effect of drought more clearly than any other organs in the plants. Reduction in the number, size and area of the leaves are the initial signs of water shortage seen in plants and the conditions required for leaf expansion and leaf elongation *viz.,* leaf turgor, accumulation of assimilates, emergence rates and leaf temperature get modified under water deficit condition (Reddy *et al.,* 2003; Anjum *et al.,* 2016). Increased rate of leaf senescence along with decrease in the production of new leaves have been reported in long term severe water deficit conditions (DeSouza *et al.,* 1997).

Leaf characters like leaf area index (LAI) and specific leaf area (SLA) are the basic physiological tools to show growth and development of plants and are found to decrease under drought. For example, sunflower plants recorded decrease in leaf area index at maturity and flowering stage of the plants under water stress (Hussain *et al.,* 2008). Similarly, Ennajeh *et al*. (2010) observed significant reduction in leaf area and specific leaf area in two olive varieties when subjected to drought stress.

Dalirie *et al*. (2010) observed changing patterns of LAI in wheat genotypes by terminal drought stress. In that experiment, LAI recorded was maximum at around 240-245 days after planting and then the value decreased gradually till harvest due to terminal drought stress. SLA and LAI also were significantly affected under 75% and 40% field capacities compared to 100% field capacity in one year old seedling of Eucalyptus (*Eucalyptus camaldulensis* Dehnh) grown in lysimeters in an experiment carried out for over two years (Rad *et al.,* 2011). In another experiment conducted by Mathobo *et al*. (2017) in dry beans (*Phaseolus vulgaris* L.) it was shown that there was a significant reduction in leaf area index with increase in water stress. In an investigation to understand the effect of drought on two pinto bean cultivars, leaf area index was found to decrease by 55.96 and 30.57 percent when drought was imposed at two weeks after emergence and two weeks after flowering stage respectively (Sorkhi and Fateh, 2019).

Drought has very high adverse effect on growth and development which leads to reduction in yield of crops. Rice is a crop which is very susceptible to drought condition (Tao *et al.,* 2006; Yang *et al.,* 2008) and cause decrease in rice yield by affecting yield related parameters *viz.,* number of tillers, number of spikelets per panicle, spikelet sterility, filled grains percentage and thousand grain weight (Kamoshita *et al.,* 2004; Bouman *et al.,* 2005; Botwright-Acuna *et al.,* 2008; Moonmoon and Islam, 2017). Also, Singh *et al.* (2018) reported that when water stress

was given to five different rice varieties there was significant decrease in leaf area as well as tiller numbers compared to control.

Kamarudin *et al*. (2018) observed reduction in tiller number and leaf area of flag leaf in five different rice genotypes when subjected to drought (-30 kPa soil water tension) after 25 days of transplanting. Also another drought study, with 40% filed capacity imposed at different critical stages of six different genotypes of rice has shown significant reduction in effective tiller numbers compared to control plants (Moonmoon and Islam, 2017).

Water stress interrupt cell development by reducing cell division and further reducing mature cell size. This in turn cause reduction in number of cells per leaf in young leaves and result in reduction in leaf area as well as tiller numbers (Henry *et al.*, 2016). Moreover under drought, decrease in mortality of apical portion of leaf and increased leaf rolling cause significant reduction in leaf area (Anjum *et al.*, 2017).

2.2.1.3 Effect of drought on Leaf gas exchange parameters

The leaf gas exchange parameters show the very first response towards drought stress *viz.*, closing the leaf stomata, which affect diffusion of CO_2 to the leaves (Muller and Whitsitt 1996). The stomatal closure caused by water stress limit the uptake of CO_2 by leaves, and the restricted CO_2 availability further lead to increased susceptibility to photo-damage and inhibit photosynthesis (Cornic and Massacci, 1996). However, the major effect is the decrease in rate of photosynthesis due to impaired photosynthetic machinery, reduction in food production, diminished activities of Calvin cycle enzymes, reduction in leaf expansion and premature leaf senescence (Fu and Huang, 2001; Monakhova and Chernyadèv, 2002; Wahid and Rasul, 2005).

Gu *et al*. (2012) evaluated thirteen rice genotypes under water stress given at flowering and at grain filling stages of the crop and reported that the leaf gas exchange parameters i.e. photosynthetic rate, stomatal conductance and rate of transpiration

decreased significantly under water stress condition compared to the control. Similarly in another study also the photosynthetic rate, stomatal conductance and rate of transpiration were reported to be severely affected in drought condition in seven rice cultivars namely Apo, IR55419-04, IR64, IR71525-19-1-1, Moroberekan, PSBRc80 and Vandana (Lauteri *et al.*, 2014).

In a study with five genotypes of rice namely, Improved White Ponni (IWP), IWP-4-2, IWP-1-52, IWP-1-57 and Apo (moderately tolerant to drought stress) when subjected to drought at reproductive stage by withholding irrigation for 25 days before heading, it was found that all five genotypes recorded lesser values for leaf gas exchange parameters under water stress condition. However, the moderately tolerant cultivar (Apo) was found to perform well under drought (Dhivyapriya *et al.*, 2016). In another study, Kumar *et al.* (2020) also reported significant reduction in leaf gas exchange parameters and reduced water use efficiency at different growth stages of seven rice genotypes (IR 84899-B-179-16-1-1-1, IR 88964-24-2-1-4, IR 83387-B-B-27-4, IR84899-B-183-CRA-19-1, IR84894-143-CRA-17-1, Sahbhagi Dhan and IR64) under drought condition.

Hura *et al.* (2007) examined four crops (filed bean, spring triticale, maize hybrid and amaranthus) categorized as C_3 and C_4 plants grown in two different field capacities (70% and 30%) and reported that the leaf gas exchange parameters recorded highest value in maize followed by amaranthus, spring triticale and the least in field bean grown in 30% field capacity as compared to those grown under 70% field capacity. Similarly significant reduction in photosynthetic rate, stomatal conductance and transpiration rate were observed in dry bean (*Phaseolus vulgaris* L. cultivar DBS 360) under moisture stress compared to the control (fully irrigated) by Mathobo *et al.* (2017).

Stomatal limitation and non-stomatal limitation are the two main limiting factors which cause decrease in leaf gas exchange parameters like photosynthetic rate,

stomatal conductance and transpiration rate in plants under drought (Farooq *et al.,* 2009). Under drought situation excessive production of abscisic acid take place in the mesophyll cells and they get stored in mesophyll chloroplasts. The increased abscisic acid, then translocate to guard cells and promote the closure of stomata (Matysik *et al.,* 2002; Fathi and Tari, 2016). Closing of stomata decrease the rate of intake of CO_2 by leaves and due to which more free electrons are produced. These electrons in turn will be involved in production of harmful reactive oxygen species (Farooq *et al.,* 2009). The decrease in transpiration rate is mainly due to stomatal closure and increase in the dissipated heat (Yokota *et al.,* 2002).

Also the decrease in leaf gas exchange parameters during water deficit condition is mainly due to non-stomatal mechanisms like decreased activity of important photosynthetic enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), phosphoenol pyruvate carboxylase (PEPCase) and NADP-malic enzyme (NADP-ME) (Hoekstra *et al.,* 2001; Reddy *et al.,* 2004; Zhou *et al.,* 2007). Moreover, reduced water content in the tissues down regulates non-cyclic electron transport and thus reduces the ATP production (Farooq *et al.,* 2009). It is also well documented that there is increased activity of Rubisco binding inhibitors under reduced water content in the leaves (Bota *et al.,* 2004).

2.2.2 Effect of drought on biochemical characters

2.2.2.1 Effect of drought on total flavonoid and total phenol content

Flavonoids and phenol are the most distributed compounds among the groups of plants exhibiting antioxidant properties and they play role in defensive mechanism to cope with abiotic stresses (Romani *et al.,* 2002; Blokhina *et al.,* 2003; Cheynier *et al.,* 2013; Quan *et al.,* 2016). Also the phenolic compounds play important role in different physiological processes which are related to plant growth and development

like seed germination, cell division and synthesis of photosynthetic pigments (Tanase *et al.,* 2009).

Sarker and Oba (2018) reported increased rate of phenol and flavonoids accumulation during water stress, in *Amaranthus* (Accession VA3) genotype in a pot culture experiment with four different field capacities *viz.,* 100%, 90%, 60% and 30%. Habibi (2018) also reported, highest level of total phenol and flavonoids under mild drought (40% field capacity) followed by severe drought (20% field capacity) compared to control (80% field capacity) in *Aloe vera* leaves.

In another study with four tomato cultivars, total phenols, flavonoid and lycopene content of fruits were found to significantly increase under drought condition compared to well watered condition (Klunklin and Savage, 2017). Quan *et al.* (2016) studied the responses of 20 different rice cultivars to drought and reported that there was positive correlation between flavonoid and phenol content with increase in water deficit condition. In a laboratory experimental condition with rice plants, it was found that the phenol content increased with increase in stress levels given by polyethylene glycol (PEG) at different concentration 5%, 10%, 15% and 20% (Shehab *et al.*, 2010).

Drought stress enhances the accumulation of phenols and flavonoids in the plants by regulating their biosynthetic pathways (Li *et al.,* 2018; Rezayian *et al.,* 2018; Gharibi *et al.,* 2019) and protect them from the harmful effects of drought stress (Nichols *et al.,* 2015). A study conducted in Arabidopsis plants at transcriptomic and metabolomics level, revealed that increased flavonoid content under water deficit condition was very much helpful to provide resistance to plants under stressed conditions (Nakabayashi *et al.,* 2014). Flavonoids formed in cytoplasm during drought stress, detoxify the harmful effects of H_2O_2 molecules generated due to stress and it is also proposed that in presence of ascorbate, oxidized flavonoid recycle in flavonoids which is primary metabolite in the reaction detoxifying H_2O_2 (Yamasaki *et al.,* 1997; Hernandez *et al.,* 2009).

2.2.2.2 Effect of drought on chlorophyll content

Drought affect the plants in many ways by making changes in metabolic functions and one of those is the reduction or loss of photosynthetic pigment synthesis. Photosynthetic pigments are important for the process of photosynthesis and the changes such as reduction or loss of photosynthetic pigments lead to reduction in both light harvesting and generation of reducing powers and ultimately impair photosynthetic process. Any change in chlorophyll content is closely associated to plant biomass and yield (Jaleel *et al.,* 2009). Decrease in chlorophyll content during drought is dependent on the level or severity of drought. Pandey and Shukla, (2015) reported that drought stress lead to reduction in chlorophyll content along with the reduction in maximum quantum yield of PSII (F_v/F_m) in rice plants.

Pirdashti *et al.* (2009) subjected four rice cultivars (Tarom, Khazar, Fajr and Nemat) to interrupted irrigation to induce drought at vegetative stage, flowering stage and grain filling stage. The results indicated that there was maximum reduction in chlorophyll content when irrigation was interrupted at grain filling stage followed by interruption of irrigation at flowering and vegetative stages compared to the well irrigated condition.

In a study with four rice different cultivars (KDML105, PT1, NSG19 and IR20) grown under four different regimes of soil water content *viz.,* fully irrigated condition (control), mild water-deficit (7 days withholding irrigation), severe water-deficit (14 days withholding irrigation) and re-watering 3 days prior to grain harvesting, it was found that there was lowest chlorophyll content in severe water-deficit treatment followed by mild water-deficit treatment and re-watering treatment, whereas the control recorded highest chlorophyll content (Cha-um *et al.,* 2010).

In another study with five different rainfed rice varieties namely NERICA 1, 2, 3, 4 and 5 when subjected to three levels of water stress *viz.,* well watered throughout

the life cycle, water deficit at vegetative stage and water deficit at reproductive stage, it was found that the chlorophyll content was lesser in the treatment where the plants were subjected to water deficit condition at vegetative stage compared to the other two conditions (Sikuku *et al.,* 2012). Khayatnezhad and Gholamin (2012) also obtained similar results in ten maize cultivars when subjected to water deficit condition compared to fully watered condition. Similarly in crops such as wheat and sunflower also a decrease in chlorophyll content was reported under drought stress (Mannivannan *et al.,* 2007; Nikolaeva *et al.,* 2010).

The primary reasons in the reduction of chlorophyll content in plants during drought stress are the impaired chlorophyll biosynthetic pathway, chlorophyll degradation, loss of chloroplast membrane and increase in lipid peroxidation (Fu and Huang, 2001; Maisura *et al.,* 2014; Pandey and Shukla, 2015).

2.2.2.3 Effect of drought on proline content

In plants, many different types of compatible solutes such as proline, sucrose, polyols, trehalose, glycine betaine and proline betaine accumulate in large quantities in response of different stresses (Ashraf and Harris, 2004). Accumulation of proline is a common phenomenon in response to almost all environmental stresses such as drought, salinity, heavy metal, low temperature etc. in plants (Rhodes *et al.,* 2002; Munns, 2005; Sharma and Dietz, 2006). Intercellular concentration of proline has been found to increase under different stress conditions by more than 100-fold in plants (Verbruggen and Hermans, 2008; Liang *et al.,* 2013).

In a laboratory investigation with eight pigmented rice varieties, subjected to drought stress (imposed by 100 mM mannitol for four days) it was found that the accumulation of proline was more than 1.5 fold in mannitol-induced water stress condition compared to control (Chutipaijit *et al.,* 2012). Similarly, Lum *et al.* (2014) conducted another lab experiment with eight upland rice cultivars by using four

concentrations of PEG (-2, -4, -6 and -8 bars) along with 0 bar as control. The results indicated that in all the cultivars, proline content in leaves increased gradually with increase in drought stress compared to control. Another field study with five rice genotypes exposed to drought stress (-30 kPa soil water tension) indicated that under drought condition proline concentration increased in leaves (Kamarudin *et al.,* 2018).

It is well documented and reviewed that in plants, proline acts as an osmolyte for osmotic adjustment under different stress conditions (Hayat *et al.,* 2012; Liang *et al.,* 2013; Chun *et al.,* 2018). Apart from acting as an osmolyte, proline also plays important role in stabilizing cellular structures such as membranes and proteins, scavenging free radicles and maintaining redox potential under stressed conditions (Ashraf and Foolad, 2007). Also proline act as a compatible hydrotrope, eliminating cytoplasmic acidosis and maintaining suitable $NADP^+$/NADPH ratio for proper metabolism under stressed conditions (Hare and Cress, 1997; Strizhov *et al.,* 1997). Normally accumulation of proline is found in cytoplasm where, it acts as molecular chaperons for maintaining functional structure of proteins and maintain pH and redox status of cell (Hayat *et al.,* 2012).

2.3 EFFECT OF ULTRAVIOLET-B (UV-B) RADIATION ON VARIOUS CHARACTERS

The solar radiation is mainly divided into two major spectrums *viz.,* photosynthetically active radiation (PAR) (400-700 nm) and ultraviolet radiation (UV) (100-400 nm). Ultraviolet (UV) radiation constitute only 10% of the total solar radiation and the UV radiation reaching the earth surface depends on energy output of sun and transmission properties of ozone layer of atmosphere. Depending on the wavelength, UV radiation is further divided into four different groups of radiation such as, vacuum ultraviolet (100-200 nm), UV-C (200 to 280 nm), UV-B (280 to 320 nm) and UV-A (320 to 400 nm).

PAR and UV-A are not affected by atmosphere and they reach the earth's surface and they do not have any harmful biological effect on living organisms. UV-C and vacuum-UV are the most biologically damaging radiations but totally absorbed by ozone and other atmosphere components before penetrating the earth's surface and hence there is no significant effect on biological processes under natural conditions. But UV-B radiation is selectively absorbed by ozone layer (Green *et al.,* 1974; Vass *et al.,* 2005; Bhattacharya *et al.,* 2012). A decrease in ozone layer lead to an increase in incoming ultraviolet-B (UV-B) radiation significantly and could cause changes in the spectral UV composition reaching the surface of earth (UNEP, 2010; WMO, 2014). UV-B constitute only 0.5% of the total solar radiation but it has a very high potential to cause high biological damages due to its high energy potential (Zlatev *et al.,* 2012; Blaustein and Searle, 2013; Li *et al.,* 2013).

Overexposure to the UV-B radiation lead to reduction in productivity and quality of plants. According to United Nations Environment Programme (UNEP) report based on a wide range of filed experiments, it is evident that a decline in productivity up to 6% takes place in terrestrial areas (UNEP, 2010). Exposure to UV-B radiation leads to changes in morphological characters such as decrease in plant height, internode length, fresh biomass of leaves, shoot and roots, leaf area, plant dry weight, increased auxiliary branching as well as leaf curling (Furness *et al.,* 1999; Zuk-Golaszewska *et al.,* 2003; Krizek *et al.,* 2006; Bandurska *et al.,* 2012). UV-B radiation can affect important physiological processes like photosynthesis *viz.,* impairing the thylakoid membranes, enzymatic processes in the Calvin cycle and destruction of amino acid residues (Reddy *et al.,* 2003; Zhao *et al.,* 2004). UV-B radiation also affects the aperture of stomatal pore, number of stomata per leaves, stomatal movements and rate of stomatal opening which lead to changes in gas exchange properties in plants (Hetherington and Woodward, 2003; Tossi *et al.,* 2014). Also production of reactive oxygen species (ROS) and associated oxidative damage under high UV-B doses have been reported in plants (Hideg *et al.,* 2002). Recently many studies have revealed that

UV-B radiation is an important regulator of secondary metabolites. It is also documented that low concentration of UV-B radiation activate biosynthesis pathways of secondary metabolites such as phenolic compounds, carotenoids and glucosinolates (Schreiner *et al.,* 2014).

2.3.1 Effect of UV-B radiation on Physiological characters

2.3.1.1 Effect of UV-B radiation on plant height

It is well known that plant height is an important agronomic trait of crops that directly affects crop architecture, apical dominance, biomass, resistance to lodging and crowding and ultimately the yield. There are many reports in rice suggesting that elevated UV-B radiation has negative influence on plant height, productive tillers per unit ground area, spikelet sterility and grain weight (Teramura *et al.,* 1991, Hakala *et al.,* 2002, Hidema *et al.,* 2005).

It is reported that the plant height reduced in two rice cultivars 'Cocodrie and Clearfield 161' exposed to elevated UV-B radiation supplied by UV-B emitting fluorescent tubes from 8:00 am to 4:00 pm for 4 weeks (Mohammed *et al.,* 2007). Similarly in cotton also plant height was found to significantly reduce by 47% under high UV-B radiation than control plants (Kakani *et al.,* 2003).

In another study under greenhouse condition with rice variety 'Jyothi', exposed to UV-B radiation (with fluorescent tubes daily 4 hrs. from10 am to 2 pm), it was found that the plant height significantly reduced in UV-B radiation treated plants at tillering and flowering stage, compared to plants grown in the condition without UV-B (Wagh and Nandini, 2019). Kataria and Guruprasad (2012) conducted an experiment with three wheat varieties where in the plants were grown both in UV-B radiation blocked cages as well as in open natural solar condition and reported that the plant height was found to reduce in plants grown in open natural solar condition compared to the plants grown in UV-B blocked condition.

In order to understand the effect of enhanced UV-B radiation on soybean, three cultivars namely Hai339, Heinong35 and Kennong18 were grown in a field condition and exposed to elevated UV-B radiation. The study revealed that under enhanced UV-B radiation there was reduction in plant height, dry weight of stem and yield per plant of all the soybean cultivars studied compared to the control plants (Liu *et al.,* 2013). Also in barley, the plant height, leaf number, leaf area and biomass were reported to decrease in the plants grown under enhanced UV-B radiation condition compared to those grown in natural condition (Jun *et al.,* 2010).

There are two very important fundamental processes *viz.,* cell division and cell elongation which are required for the growth and development of plants. Many previous studies have indicated that the UV-B radiation negatively affects both cell division and cell elongation (Liu *et al.,* 1995; Logemann *et al.,* 1995; Hopkins *et al.,* 2002). Some researchers have opined that the reduction in morphological traits under UV-B radiation could be attributed to the reduction of IAA activity and imbalance of hormones *via*. photo oxidation (Rozema *et al.,* 2001; Jansen *et al.,* 2002; Wagh, 2015).

2.3.1.2 Effect of UV-B radiation on leaf area index, specific leaf area and number of tillers

Leaves are important in determining light interception as well as important to determine plant productivity. Leaves also are very sensitive towards any changes in their surrounding environment. In order to respond to UV-B radiation, plants induce photomorphogenic responses *viz.,* decrease in leaf enlargement, reduction leaf area and increase in foliar hair density (Manetas, 2003; Lake *et al.,* 2009). Along with leaf characters other parameters *viz.,* number of tillers, grain weight and spikelet sterility are also important in determining the yield in rice (Sheehy *et al.,* 2001). Also there are reports indicating that elevated UV-B radiation has got negative influence on the above mentioned parameters (Teramura *et al.,* 1991, Hakala *et al.,* 2002, Hidema *et al.,* 2005).

In order to understand the effect of elevated UV-B radiation on green gram (cv. KM-2) the plants were exposed to 12.2 kJ m^{-2} d^{-1} radiation (supplied by UV-B lamps) and the results indicated that UV-B had suppressed LAI, SLA, root length, shoot length and number of leaves (Rajendiran and Ramanujam, 2003). Similarly, Grammatikopoulos *et al.* (1998) also reported a significant reduction in leaf area, number of leaves, allocation of biomass to both above and below ground parts as well as appearance of thicker leaves in seedlings of *Laurusnobilis* L. and *Ceratoniasiliqua* L. when exposed to ambient solar UV-B radiation compared to non UV-B radiation condition.

In another study with seeds of *A. thaliana* were grown in controlled environmental chamber with UV-B radiation (6 kJ m^{-2} s^{-1}) treatment for 21 days, the results indicated that UV-B radiation treated plants exhibited lower leaf area and lower fresh and dry weight compared to the control plants (Boeger and Poulson, 2006).

To understand the effect of supplementary UV-B radiation (ranging from 0.18 to 0.32 W/m^2) an investigation was carried out in two low land Japanese rice cultivars (Sasanishiki and Norin 1). The study revealed that, under UV-B radiation both the varieties produced lesser number of tillers along with lesser number of panicles as compared to control (Kumagai *et al.*, 2001). In another study carried out in wheat grown under open natural solar UV-B radiation, it was found that there was reduction in the number of tillers and leaf area in the plants grown in open natural solar UV-B condition compared to control (Kataria and Guruprasad, 2012).

Also in a study by Mohammed and Tarpley, (2009) nine rice cultivars namely, Cheniere, CL161, Cocodrie, Cypress, Sierra, Presidio, XL8, XL723 and CLXL729 were exposed to UV-B radiation (supplied with the help of UV florescent lamps daily 9 hr) with three different concentration 0, 8, or 16 kJ m^2. The results revealed that in all the cultivars studied there was an overall decrease in number of tillers with increase in UV-B radiation concentration.

It is a well-known fact that high level of UV-B radiation is responsible for damages at the molecular level such as DNA, proteins and membranes in the cell. Also many studies have revealed that high level of UV-B radiation damage the DNA which further lead to delay in cell division or delay in cell expansion (Srivastava, 2002; De Lima-Bessa *et al.,* 2008; Hectors *et al.,* 2010). Delay in cell division and cell elongation might lead to further changes in leaf morphology, for example decreased leaf area, LAI and SLA. Sometimes this even lead to premature leaf senescence with consequences upon whole plant growth and development such as reduction in number of tillers (Caldwell *et al.,* 1998; Milchunas *et al.,* 2004). There are studies indicating reduction in leaf area and increase of leaf curling under high UV-B radiation as an adaptive mechanism to minimize harmful effects of UV-B radiation (Zlatev *et al.,* 2012).

2.3.1.3 Effect of UV-B radiation on Leaf gas exchange parameters

Primary source of energy for plants is sunlight and the plants are very sensitive towards any change in the quality and quantity of sunlight. Gas exchange parameters like photosynthesis, stomatal conductance and transpiration are affected by the quality and quantity of sunlight. Numerous studies have shown that UV-B radiation has negative effect on these leaf gas exchange parameters (Sullivan *et al.,* 2003; Surabhi *et al.,* 2009; Yu *et al.,* 2013; Wagh and Nandini, 2019). Also several reviews have summarized the effect of UV-B radiation (field experiments as well as laboratory experiments) on different plant species. Sensitivity of plants towards UV-B radiation have been reported to vary greatly depending on the species, cultivars and growth conditions (Kakani *et al.,* 2003; Caldwell *et al.,* 2007; Kataria *et al.,* 2014).

In a study conducted by Lidon and Ramalho (2011) in the rice variety ‘Safari’ grown in growth chamber with UV-B stress imposed by fluorescent lamps (UV-B radiation given for 1 h per day for seven days between 8 to 14 days of germination) the results indicated that the leaf gas exchange parameters like net photosynthesis and stomatal conductance decreased by 20% and 85% respectively compared to the control.

Reddy *et al.* (2013) carried out an experiment with two corn hybrids and four levels of UV-B radiation (0, 5, 10, and 15 kJ m^{-2} d^{-1}) and reported an inverse relationship between leaf gas exchange parameters and UV-B radiation dosage (given during the growing period of the crop).

In an experiment with rice cultivar Liangyoupeijiu (LYPJ) exposed to UV-B radiation for 8 h per day (with the help of UV-B lamps) during reproductive development, the result revealed a significant reduction in net photosynthetic rate, stomatal conductance, transpiration rate, photosynthetic pigment contents and photochemical efficiency of photosystem II in UV-B radiation treated plants compared to control plants (Yu *et al.*, 2013). Similarly in summer rape (*Brassica napus*, cv. 'Landmark') exposed to 1 kJ m^{-2} d^{-1} UV-B radiation it was found that, there was significant decrease in leaf gas exchange parameters compared to control (Januskaitiene, 2013). Surabhi *et al.* (2009) conducted an experiment with three cowpea varieties. They were subjected to four levels of UV-B radiation *viz.*, control (0), 5, 10 and 15 kJ m^{-2} d^{-1} in controlled environmental chamber and the result showed a decrease in net photosynthesis rate and transpiration rate with increase in the intensity of UV-B radiation. However stomatal conductance was reported non-significant in that study.

Over the past two decades, UV-B radiation induced inhibition of leaf photosynthesis have been demonstrated in many plant species. Also it is established, that UV-B radiation potentially impairs the performance of main processes of photosynthesis which are, photophosphorylation reactions of the thylakoid membrane, CO_2-fixation reactions of the Calvin cycle and stomatal control of CO_2 supply (Allen *et al.,* 1998; Kataria *et al.,* 2014). Some studies also have indicated that enhanced UV-B radiation damage the photosystem II (PS II) to a larger extent and lead to reduced photosynthesis (Tyystjarvi, 2008; Dobrikova *et al.,* 2013; Hassan *et al.,* 2013). The main targets of UV-B radiation in PS-II are water-oxidizing manganese (Mn) cluster,

the reaction centers of the D1 and D2 protein, quinine electron acceptors and tyrosine electron donors (Ihle, 1997; Jansen *et al.,* 1998; Vass *et al.,* 2005). Regulation of stomata is also another factor which regulate the processes of photosynthesis and transpiration in plants. Previous studies have shown that there was reduction in CO_2 assimilation due to UV-induced reduction in stomatal conductance (Nogues *et al*., 1999; Jansen and Van-Den-Noort, 2000; Lu, 2009; Reddy, 2013).

Another possible reason in the decrease in leaf gas exchange parameters may be the degradation of Rubisco enzyme under UV-B exposure (Wilson *et al.,* 1995; Yu *et al.,* 2013) because Rubisco contains aromatic amino acid, tryptophan which can absorb UV-B radiation strongly and leads to degradation of proteins (Hartman and Harpel, 1994; Kataria *et al.*, 2014).

2.3.2 Effect of UV-B radiation biochemical characters

2.3.2.1 Effect of UV-B radiation on total flavonoid and total phenol content

In plants, one of the most common response to high UV-B radiation is an increase in phenolic compounds in the leaves and shoots. UV light is reported to be a stimulus for the synthesis of phenolic compound in the plants (Searles *et al.,* 2001). Under UV-B radiation, accumulation of phenolic compounds increase due to induction of the general phenylpropanoid pathway (Logemann *et al.,* 1999). Phenolic compounds such as flavonoid and phenol accumulate in different cellular compartments, including cell walls, vacuoles, chloroplasts, nucleus, trichomes and epidermal cells (Jansen *et al.,* 2012; Schreiner *et al.,* 2014). In many cases, phenolic compounds act as antioxidant as a general stress response. However, there are several reports indicating that flavonoids function in plants to screen harmful radiation, bind phytotoxins and help in regulating stress responses by controlling auxin transport (Winkel-Shirley, 2002).

In a pot culture experiment with two rice varieties, Jyothi and Uma grown under three different levels of UV-B radiation (i.e. reduced UV-B radiation condition,

enhanced UV-B radiation provided with the help of UV-B lamps and natural solar UV-B condition) the result indicated that flavonoid and phenol content increased under natural solar UV-B condition followed by enhanced UV-B radiation condition compared to reduced UV-B radiation condition (Wagh, 2015). Also in another study conducted in *Betula pendula* L. with three UV-B radiation treatments (UV-B 100%, UV-B 50% and UV-B 0% of solar natural radiation) the HPLC-mass spectrometry (MS) analysis revealed that twenty different kinds of phenolic compounds were significantly induced under high UV-B treatment compared to zero UV-B radiation treatment (Morales *et al.,* 2010).

Yuan *et al.* (2010) studied the effect of UV-B (2.5, 5.0 and 7.5 kJm^{-2}) radiation on flavonoid contents in the seedlings of two rice cultivars (Huangkenuo and Hexi 41) in a pot culture experiment. Flavonoid content in both the varieties, recorded significantly higher values under 7.5 kJm^{-2} followed by 5.0 kJm^{-2} of UV-B radiation. In an experiment with *Trigonella foenum-graecum* L. (fenugreek) exposing to two treatments *viz.,* 3.0 kJs^{-1} of UV-B radiation for 4 hrs and 8 hrs a day and it was found that there was maximum accumulation of flavonoid and phenol content under 4 hrs of UV-B radiation treatment (Sebastian *et al.,* 2018).

Ambasht and Agrawal (1998) conducted a field experiment with rice under supplemental UV-B radiation (7.1 kJ m^{-2}) and reported highest flavonoid and phenol accumulation under elevated UV-B radiation. In an investigation by Rodriguez-Calzada *et al.* (2019) with *Capsicum annuum* exposed to UV-B radiation with the help of UV-B lamps daily 4 hrs. (from 10:30 h to 14:30 h) it was found that the accumulation of flavonoid and phenol content in leaves significantly increased under UV-B treatment.

On UV-B radiation exposure, accumulation of UV-B absorbing compounds like flavonoids and phenols act as the key components of acclimation response to increase the capabilities of photo repair in plants. The epidermal layer is the location

of accumulation of phenolic compounds in shoots and leaves exposed to UV-B radiation and protects internal cell layers by attenuating the impinging UV-B radiation at the epidermis (Tevini *et al.,* 1991; Braun *et al.,* 1993; Olsson *et al.,* 1998). Many studies have revealed that increase in the levels of phenolic compounds are due to increase in expression and activity of genes, such as *CHS* and *PAL* which encode enzymes chalcone synthase and phenylalanine ammonialyase, respectively of the phenylpropanoid pathway (Kubasek *et al.,* 1992; Mackerness, 2000; Brosche *et al.,* 2002; Hideg *et al.,* 2013). Casati and Walbot, (2003) carried out microarray hybridization assay in response to UV-B radiation and reported that genes involved in pathways associated with UV-absorbing pigments were dramatically up-regulated and photosynthesis-associated genes were down-regulated. It has also been reported that some pathways regulated by UV-B radiation are shared by other stresses such as salt, drought and oxidative stress for defense. Also UV-B radiation is found to activate some additional pathways which are not shared by other stresses.

2.3.2.2 Effect of UV-B radiation on chlorophyll content

Another major effect of UV-B radiation is reduction in photosynthetic pigments such as chlorophyll and carotenoid in plant leaves. There are two possible ways that UV-B radiation affect chlorophyll pigment and they are either inhibition of their synthesis or their effect on the enzymes involved in the chlorophyll biosynthetic pathway (Ranjbarfordoei *et al.,* 2011). Reduction in photosynthetic capacity under UV-B radiation can be due to degradation of chlorophyll under UV-B radiation (Jordan *et al.,* 1994).

In an experiment with rice cultivar '93-11' exposing to UV-B radiation for 6, 12 and 24 h wherein the accumulated levels of UV-B radiation were 14.4, 28.7 and 57.5 kJm^{-2} respectively, the results indicated significant reduction in chlorophyll content with increase in UV-B radiation compared with control (Du *et al.,* 2011). Similarly, Salama *et al.* (2010) conducted an experiment in three desert plants namely,

M. parviflora L., *P. major* L., *R. vesicarius* L. and *Sisymbrium erysimoides* Desf by exposing them to UV-B radiation for 6 h with the help of UV-B lamps and reported that under UV-B radiation all the three desert plants recorded reduction in chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content compared to control plants.

Kataria and Guruprasad (2014) conducted a study in four varieties of *Amaranthus tricolor* (Pusa kiran, Pusa lalchaulai, Arka arunima and Arka Suguna) with three treatments *viz.*, growing in natural solar radiation, growing in the cages covered with polythene filter that transmits all the ambient solar radiation and without UV-B radiation treatment. Results obtained indicated that there was maximum reduction in total chlorophyll content under natural solar radiation followed by polythene filter treatment and UV-B reduced treatment. Similar results were reported in rice cultivar 'Jyothi' also when exposed to natural solar radiation compared to the treatment with polythene filter that transmits all the ambient solar radiation (with UV-B lamps) and without UV-B radiation treatment (Wagh and Nandini, 2019).

Many studies have suggested that, depending on the plant species, chlorophyll content either increase or decrease in response to UV-B radiation (Sun and Payn, 1999; Barsig and Malz, 2000). Also UV-B radiation has been reported to inhibit the synthesis of chlorophyll and degrade the enzymes involved in chlorophyll biosynthetic pathways. However, there are some reports indicating that UV-B radiation lead to reduction of chlorophyll 'a' content compared to chlorophyll 'b' content, as UV-B radiation cause selective damage to chlorophyll 'a' biosynthesis or degradation of its precursors (Marwood and Greenberg, 1996). Under UV- B radiation, significant reduction of carotenoids also have been determined as carotenoids have role in protecting chlorophyll pigments from photo oxidative damage and the reduction in carotenoid could lead to degradation of chlorophyll content under UV-B radiation (Agrawal and Rathore, 2007; Mishra *et al.*, 2008; Cicek *et al.*, 2012).

2.3.2.3 Effect of UV-B radiation on proline content

It is well documented that, under stressed condition most of the crop species accumulate proline as an adaptive response to stress condition (Verbruggen and Hermans, 2008). Normally, proline accumulates in cytoplasm where it function as molecular chaperons and helps stabilizing the structure of proteins and maintains cell redox status through maintaining cytosolic pH (Hayat *et al.,* 2012).

In an experiment with three rice varieties (Norin 1, Sasanishiki and Surjamkhi) subjected to UV-B radiation supplied by UV-B fluorescent tubes for 5 hours a day, it was found that there was an increase in proline content under UV-B radiation in all the three varieties *viz.*, 78% in Norin 1, 54% in Surjamkhi and 28% in Sasanishiki compared to control (Fedina *et al.,* 2010). Also another experiment with three annual desert plants, exposing to UV-B radiation for 6 hrs. for 6 days (supplied by UV-B fluorescent tubes) showed increase in proline content compared to control (Salama *et al.,* 2011).

In a study to understand the effect of combined drought and UV-B radiation stress on lettuce cultivar 'Romaine' grown under three conditions *viz.,* drought stress (-2.0 MPa), UV-B radiation (5 kJ $m^{-2}d^{-1}$) and combined drought and UV-B stress it was found that the proline content was higher under drought stress followed by UV-B stress and combined stress condition (Basahi *et al.,* 2014). In another similar study with *Spilanthes acmella* Murr., plant also it was shown that the proline content increased by 376.51 percent under UV-B radiation and by 595.06 percent under drought stress compared to control (Reshmi and Rajalakshmi, 2012).

Accumulation of proline seen higher under UV-B radiation is mainly to protect the cells from peroxidative damage (Saradhi *et al.,* 1995). Also, Salama *et al.* (2011) suggested the accumulation of proline content as an important factor for providing tolerance to UV radiation in plants. In addition to that, the increase in proline content

is also considered as a protective mechanism against UV radiation due to the generation of reactive oxygen species.

2.4 GENE EXPRESSION STUDY

Plants produce large number of secondary metabolites *viz.,* two different pathways: the shikimate pathway and the malonate pathway. Among these two pathways, shikimate pathway produce most of the phenolic compound in the plants whereas, malonate pathway is less significant in the higher plants. However, malonate pathway is an important source of phenolic compounds in fungi and bacteria (Taiz and Zeiger, 2010).

There are two main pathways responsible for synthesis of phenolic compounds in plants: the shikimic acid pathway and phenylpropanoid metabolism pathway (Chen *et al.,* 2018; Wang *et al.,* 2019). Phenylpropanoid metabolism pathways mainly synthesize important secondary metabolites such as, flavonoids, phenols, anthocyanin, lignin, stilbenes and tannins in plants (Rio *et al.,* 2013; Kallscheuer *et al.,* 2017). Whereas the shikimate pathway start with coupling of two compounds phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate and give rise to 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) and at the final stage of shikimate pathway, chorismate is converted into phenylalanine (which is the primary substrate to phenylpropanoid pathway) with the help of enzyme chorismate mutase. In phenylpropanoid pathway, at the first stage phenylalanine gets converted into cinnamic acid with the help of enzyme phenylalanine ammonia-lyase (PAL) and then by hydroxylation, cinnamic acid gets converted into coumaric acid with the help of cinnamic acid 4-hydroxylase enzyme. Later coumaric acid gets hydroxylased to form different types of phenolic compounds and flavonoids.

2.4.1 Flavonoid pathway in rice

Over the periods many studies have suggested the flavonoid biosynthesis pathway involved in rice (Shih *et al.,* 2008; Brazier-Hicks *et al.,* 2009; Galland *et al.,* 2014). An overview of flavonoid biosynthesis pathway in rice grain is given in figure 1. Firstly coumaric acid from general phenylpropanoid pathway gets converted into a 4-coumaroyl-CoA by CoA ligases. Then by the action of chalcone synthase (CHS) it leads to the formation of chalcone and later it gets converted in to a naringenin with the help of enzyme chalcone isomerase (CHI). Naringenin is the primary substrate for biosynthesis of different types of flavonoids.

Biosynthesis of flavonoids such as, anthocyanin and proanthocyanidin are catalyzed by flavanone 3-hydroxylase (F3H) and dihydroflavonol 4-reductase (DFR) and in the later stage they are synthesized by the action of anthocyanidin synthase (ANS) and leucoanthocyanidin reductase (LAR) respectively. Biosynthesis of tricin start with generation of apigenin with the help of enzyme flavone synthase II (FSII). At the last stage of the pathway tricin forms by the action of *O*-methyltransferase (OMT) from selgin.

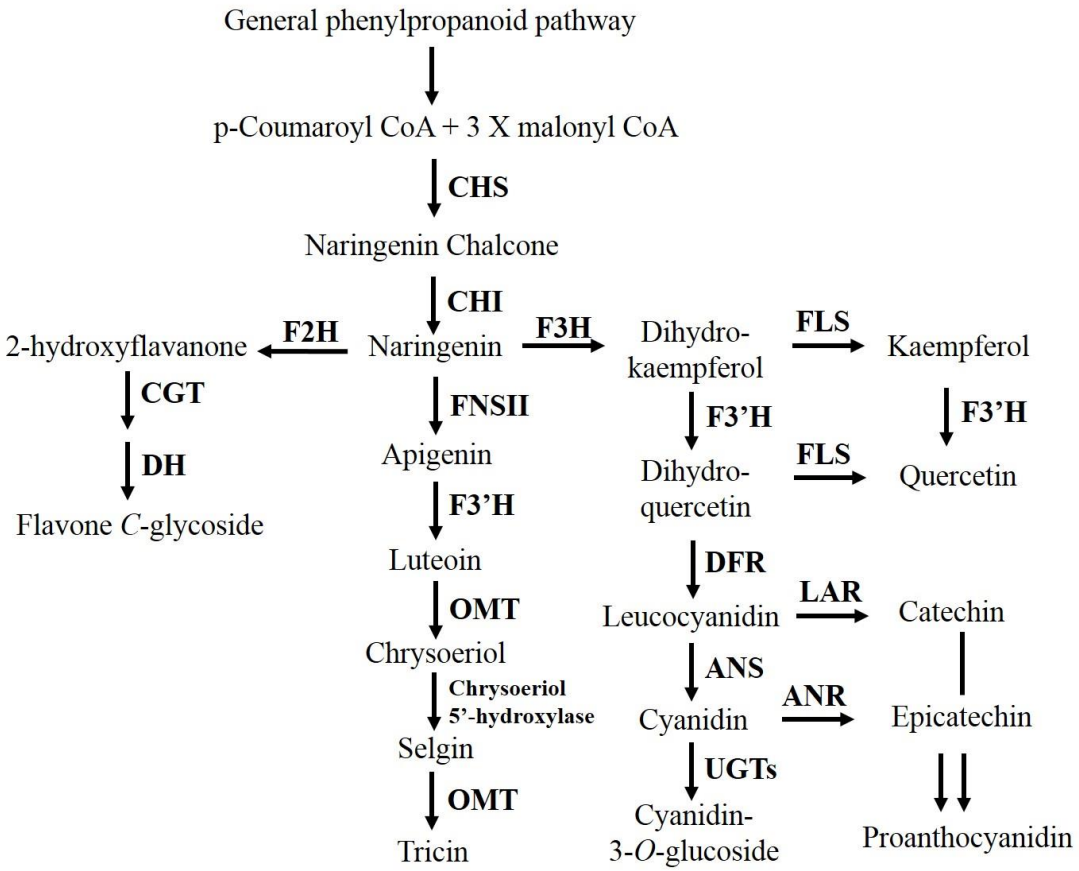


Figure 1: Proposed flavonoid biosynthesis pathway in rice grain (Park *et al.*, 2016). (The abbreviations of enzyme names are follows: ANS-Anthocyanidin synthase; ANR-Anthocyanidin reductase; CGT-*C*-glucosyl transferase; CHS-Chalcone synthase; CHI-Chalcone isomerase; DFR-Dihydroflavonol 4-reductase; DH-Dehydratase; F2H-Flavanone 2-hydroxylase; FLS-Flavonol synthase; F3H-Flavanone 3-hydroxylase; F3'H-Flavonoid 3'-hydroxylase; FNSII-Flavone synthase II; LAR-Leucoanthocyanidin reductase; OMT-*O*-methyltransferase; UGT-UDP-glucosyl transferase)

In pigmented rice grain, most of the flavonoids are derived from 3'4'-dihydroxylated leucocyanidin, whereas 3'4'-dihydroxylated leucocyanidin is derived from 4′-hydroxylated leucopelargonidin which is absent in pigmented rice (Park *et al.*,

2016). Enzyme Flavonoid 3'-hydroxylase (F3'H) catalyze B-ring hydroxylation of flavonoid and helps to add different types of flavonoids, which implies that activity of F3'H is prominent in pigmented rice grains. Two F3'H genes, *CYP75B3* and *CYP75B4* have been identified in rice belonging to cytochrome P450 family (Seitz *et al.,* 2006). In *Arabidopsis thaliana* transparent testa mutant 7 (*tt7*), *CYP75B3* has been identified as defective allele of F3'H, which catalyzes the 3'-hydoxylation of B-ring of flavonoids (Shih *et al.,* 2008). *CYP75B4* has been characterized very recently and it catalyzes not only 3'-hydroxylation but also 5'-hydroxylation of 3'-methoxylated flavone chrysoeriol to form selgin and later selgin gets converted into tricin with the help of *O*-methyltransferase (Lam *et al.,* 2015).

In response to several abiotic stresses, biosynthesis of secondary metabolites including some polyphenols and flavonoids increase in plants. These polyphenols and flavonoids provide tolerance to the plants under different stress conditions such as drought, temperature, salinity, UV radiation etc. (Ancillotti *et al.,* 2015; Smirnov *et al.,* 2015; Handa *et al.,* 2019; Naikoo *et al.,* 2019). Under stressful conditions, biosynthesis of phenolic compounds are regulated by the activity of some important enzymes such as PAL and CHS. These stresses also up-regulate transcript level of genes encoding key enzymes in biosynthesis of phenolic compound pathway such as, *PAL*, *CHS*, *F3H*, *F3'H*, *DFR* and *FLS* (Ma *et al.,* 2014; Zhou *et al.,* 2018; Gharibi *et al.,* 2019; Sharma *et al.,* 2019).

Materials and Methods

3. MATERIALS AND METHODS

The present study was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani, during December 2015 to December 2019. The details of the materials used and the methods adopted in the study are presented in this chapter.

3.1 GENERAL DETAILS

The experiment was conducted at the College of Agriculture, Vellayani. The geographical coordinates of the location of this college are 8°5' N latitude and 76°16' E longitude with an altitude of 29 m above mean sea level. The current study was planned with three different experiments. The first experiment was with medicinal rice varieties *viz.* black glumed njavara and yellow glumed njavara exposing to two shade levels and two different field capacities. In the second experiment, rice plants were raised in polyhouse and exposed to UV-B radiation at three different critical stages of plants. Then based on the results of the accumulation of secondary metabolite i.e. total flavanoids in experiment one and two, the third experiment was carried out for further molecular studies.

3.1.1 Varietal Details

3.1.1.1 Black glumed njavara

Black glumed njavara rice has red seeds with black-shaded grains. This is a type of njavara rice normally resistant to diseases and drought condition. Black glumed njavara rice has short duration with maturation time of 60-90 days and height of plant is more than 1 m. This rice is mostly cultivated in northern districts of Kerala.

Plate 1: Type of Njavara rice (A-Black glumed njavara; B-Yellow glumed njavara)

3.1.1.2 Yellow glumed njavara

Yellow glumed njavara rice also has red colour seeds, but their grains are golden yellow in colour. This type of rice takes 60-90 days to mature depending on the season and the land in which grown and the height of plant is more than 1 m. The crop is vulnerable to lodging and diseases upon maturity and also susceptible to drought. Usually, this variety is grown in the second cropping season.

3.2 EXPERIMENTAL DETAIL

3.2.1 Experiment number 1

This experiment was laid out in a Completely Randomized Design (CRD), in pots with 5 treatments and 4 replications and with 3 pots in each replication. The treatments comprised of two levels of shades and two levels of field capacity and carried out with both varieties of njavara rice.

The five treatments are:

T_1 - 20% shade

T_2 - 40% shade

T_3 - 50% field capacity

T_4 - 75% field capacity

T_5- Control

Two varieties of rice used are:

V_1- Black njavara

V_2- Yellow njavara

3.2.1.1 Details of treatment

The experiment was conducted in shade houses of size 65 m^2 and the shade levels of 20% and 40% of the radiation was provided by using polyethylene nets purchased from Kerala Agro Industries Corporation. The control pots were maintained

Plate 2: View of the experimental plot (A-Shade house with two different shade nets; B-Rain out shelter)

in natural open condition. For imposing water deficit stress, the dry weight and wet weight of pots with soil at 100% field capacity (FC) of each individual pots were recorded with the help of electronic digital top loading weighing balance. The water holding capacity of the soil was calculated gravimetrically and the drainage holes were closed with cement. The weight of pots to maintain field capacity (FC) at 50% and 75% were calculated by using following formula and the pots were irrigated and maintained at same weight throughout the experiment and the control plants were grown at 100% FC level.

Pot weight at 100% FC = A+Q100

Pot weight at 50% FC= A+Q50

Pot weight at 75% FC= A+Q75

Where,

A= Dry weight of soil and pot

Q100, Q50 and Q75= quantity of water at 100% FC, 50% FC and 75% FC respectively

3.2.2 Experiment number 2

The experiment was laid out in a Completely Randomized Design (CRD), in pots with 4 treatments and 3 replications and with 4 pots in each replication. Plants were subjected to UV-B (280-320 nm) radiation for 4 hours per day (10am - 2pm) at different critical stages of plants as mentioned below:

T_1 - UV-B treatment from vegetative stage

T_2 - UV-B treatment from panicle initiation stage

T_3 - UV-B treatment from flowering stage

T_4 - Control (without U-B radiation)

Two varieties of rice used are:

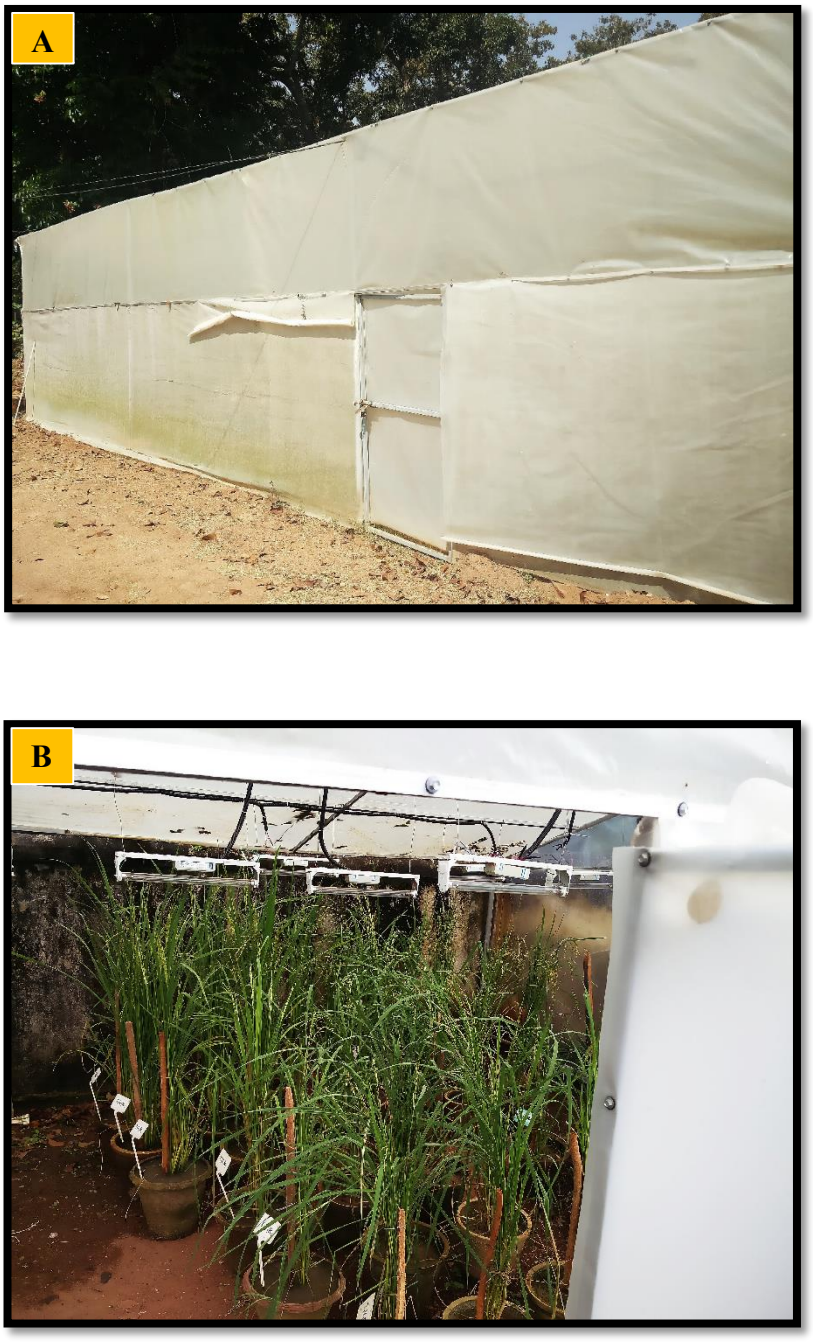


Plate 3: View of the experimental plot (A-Polyhouse; B-UV-B florescent tubes installed

V_1 - Black njavara

V_2 - Yellow njavara

3.2.2.1 Details of treatment

The experiment was conducted in ventilated polyhouse of a size 65 m^2 to give treatment with UV-B radiation. The cladding material used for polyhouse was thick polyester of 0.13 mm thickness, purchased from Kerala Agro Industries Corporation. The sides of the polyhouse were covered with insect proof net (40 mesh). Further, the polyhouse was compartmentalized in two parts. In the first chamber ten number of UV-B fluorescent tubes (TUV T8-15W SlV/25, Philips) were installed at the roof of the polyhouse, maintaining a distance from plant canopy by 50 cm and the tubes were switched on for 4 hrs. daily (from 10 am to 2 pm) which is considered as the biologically active photoperiod. The average UV-B radiation provided by the tubes was measured as 4 Wm^{-2} at canopy level of plants. The second compartment was maintained in a similar condition as that of compartment-I but without UV-B radiation to serve as control.

3.2.3 Experiment number 3

The experiment number 3 was carried out with selected treatments determined based on the higher accumulation of total flavanoids in grains of both experiment 1 and 2. The molecular analysis was carried out at the grain filling stage of the crop.

3.2.4 Raising of crop and management

The plants were raised in clay pots of size 12 inches which were filled with soil brought from Instructional Farm, College of Agriculture, Vellayani and mixed with farm yard manure as well as sand in the proportion of 1:1:1 ratio. The nursery for seedling were maintained at rice field under rat proof cage of department of plant physiology. The rice seedlings of 15 days old were transplanted at the rate of three

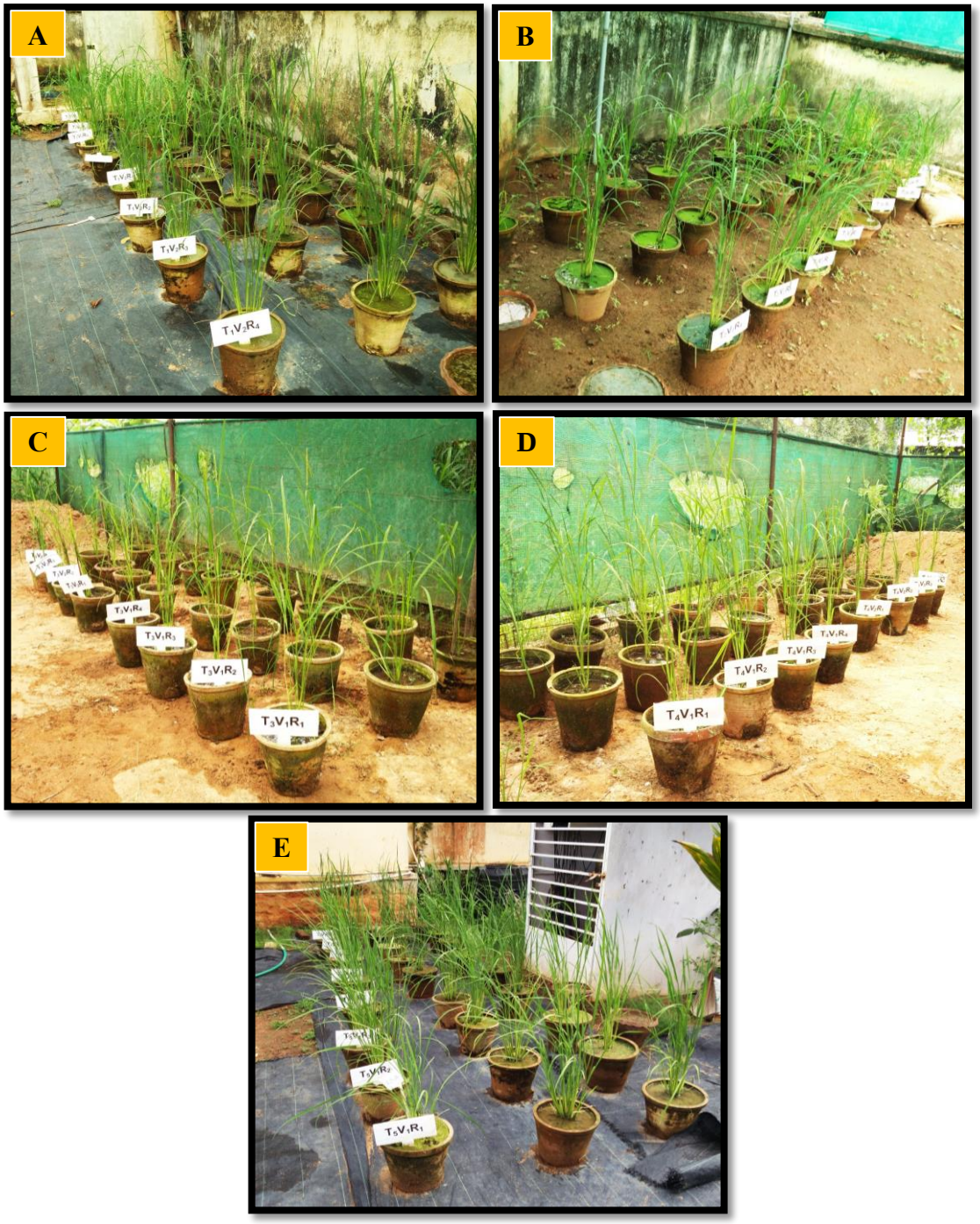


Plate 4: Growth of Njavara rice under different treatment at panicle initiation stage (A-20% shade; B -40% shade; C-50% field capacity; D-75% field capacity; E-Control)

Plate 5: Growth of black njavara under different treatment at maturity stage (T_1- UV-B treatment from vegetative stage; T_2- from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara)

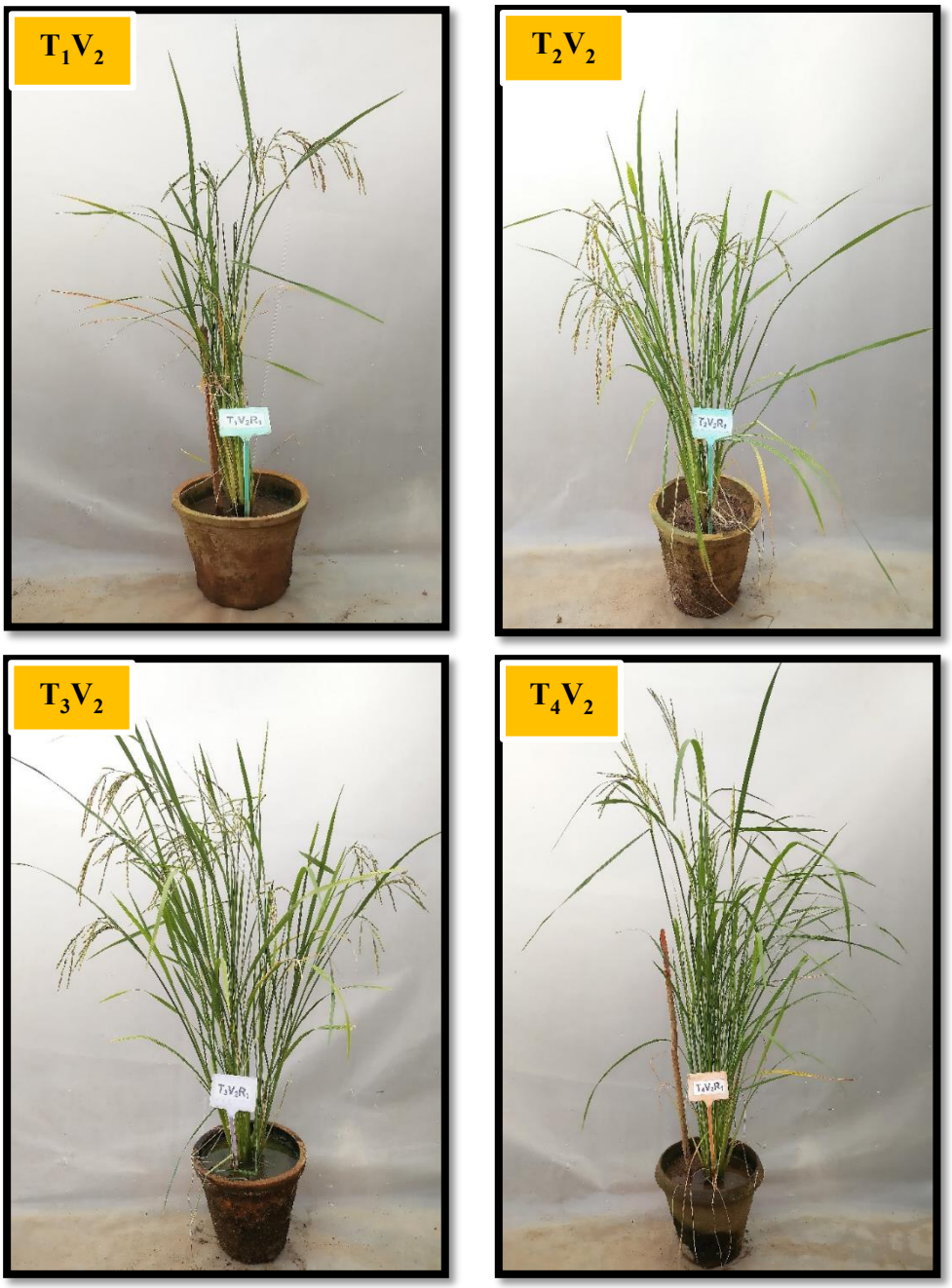


Plate 6: Growth of yellow njavara under different treatment at maturity stage (T_1- UV-B treatment from vegetative stage; T_2- from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_2- Black Njavara)

seedlings per hill and one hill per pot. The fertilizer management was adapted as per the package of practices recommendations, 2011 of Kerala Agricultural University. Each pot received the manurial schedule at the rate of 5 tones farm yard manure per hectare as basal which was incorporated at the time of filling pots. The crop was given N, P and K at the rate of 40:20:30 and out of which 33.33 per cent N, 100 per cent P and 50 per cent K were given as a basal dose. Later 33.33 per cent N was given at active tillering stage and remaining 33.33 per cent N and 50 per cent K were applied at the panicle initiation stage. Urea, rock phosphate and MOP were the fertilizers used for the soil application.

3.3 OBSERVATIONS RECORDED

3.3.1 Physiological characters

3.3.1.1 Plant height

The plant height was recorded at different critical stages of plants from ground level to tip of the longest leaf and expressed in centimeter.

3.3.1.2 Leaf area index (LAI)

The leaf area index was measured by adopting the method suggested by Yoshida et al. (1971). Leaf area of each pot was determined by measuring the length and width of fully matured leaves and by calculating the leaf area of the plant. The land area covered by the plant was determined by calculating the area of the pot used. Finally LAI was calculated by using the following formulae:

LAI= Sum of leaf area per pot (sq cm)/ Area of land covered by pot (sq cm)

3.3.1.3 Specific leaf area (SLA)

To determine the specific leaf area, fully expanded third leaf from each pot was collected. Leaf area of leaves were found out and dry weight of leaflets were taken by drying at 80°C for two days. SLA was calculated using the formula:

SLA (cm^2/g) = Leaf area/ dry weight

3.3.1.4 Number of tillers per pot

The number of tillerswere counted at respective stages from each pot and mean value was expressed in number per pot.

3.3.1.5 Leaf gas exchange parameters

Leaf gas exchange parameters namely, photosynthetic rate, stomatal conductance and transpirational rate were obtained by using portable photosynthetic system (CIRAS-3 Ver.1.06, Amesbury, USA). The observations were taken in each replication and the measurements were recorded three times in each replication and then the average value was worked out. Reading was taken between 9.00 to 10.30 am using this equipment. The following gas exchange parameters were recorded and the units are expressed in parenthesis.

a) Photosynthesis Rate (μmol CO_2 m^{-2} s^{-1})

b) Stomatal Conductance (mol H_2O m^{-2} s^{-1})

c) Transpirational Rate (mmol H_2O m^{-2} s^{-1})

3.3.2 Biochemical characters

3.3.2.1 Flavanoid content (from leaf)

The flavanoid content was determined by the well-known method suggested by Ordonez *et al.* (2006) using aluminium chloride ($AlCl_3$). Leaf samples of 0.5 g were homogenized in 80% methanol at 40°C. Then the samples were cooled down to room

temperature and centrifuged at 4,500 rpm for 15 min. The supernatant was collected and out of which 0.5 ml of plant extract was taken. It was followed by addition of 0.5 ml 80% methanol and 4 ml distilled water. Then 0.3 ml of 5% sodium nitrite ($NaNO_2$) was added and incubated for 5 min after that 0.3 ml of 10% $AlCl_3$ was added and the solution was allowed to stand for 6 min. The reaction was stopped by adding 2 ml of 1 M sodium hydroxide (NaOH) and the final volume was brought up to 10 ml by adding distilled water. The sample was allowed to stand for 15 min and the absorbance was measured at 510 nm by using spectrophotometer (Model-ELICO SL 218, Double Beam, UV-VIS, Spectrophotometer, India).

The total flavanoid content was calculated from the calibration curve using quercetin and expressed as mg quercetin (QE)/ g of plant sample.

3.3.2.2 Phenol content

Phenol content was estimated by the method suggested by Malick and Singh (1980). The plant sample of 0.5 g was homogenized in 10 ml of 80 per cent ethanol and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and evaporated to get dry and then to it, 5 ml of distilled water was added and dissolved the contents. Then from that 0.2 ml was taken in a test tube and made up the volume to 3 ml with distilled water. Later 0.5 ml of folin-ciocalteau reagent was added and kept for 3 minutes. The reaction was stopped by adding 2 ml of 20% sodium carbonate (Na_2CO_3) solution and the absorbance was measured at 650 nm using spectrophotometer (Model-ELICO SL 218, Double Beam, UV-VIS, Spectrophotometer, India). The phenol content was expressed as mg g^{-1} of fresh weight.

The standard solution was prepared with catechol and the absorbance was taken at 650 nm. Calculation was done by using the given formula.

$$\frac{\text{Test sample absorbance}}{\text{Standard solution absorbance}} \times \frac{\text{Concentration of standard solution}}{\text{Weight of sample}}$$

3.3.2.3 Chlorophyll content

The estimation of chlorophyll a, chlorophyll b and total chlorophyll were carried out using the method given by Hiscox and Israelstam (1979). For the estimation, 100 mg leaf sample was taken and to which 10 ml of dimethyl sulphoxide (DMSO): acetone (80:20) was added and left in dark overnight. Next day the leaf sample was filtered using whatman filter paper No. 2 and the final volume was made up to 25 ml and the chlorophyll content was determined spectrophotometrically (Model-ELICO SL 218, Double Beam, UV-VIS, Spectrophotometer, India) at two wavelengths(645 nm and 663 nm) and expressed as milligram per fresh weight of plant tissue.

The calculation was done by using the following formulae.

Chlorophyll "a" = [(12.7 x A663) – (2.69 x A645)] x V/1000 x W

Chlorophyll "b" = [(22.9 x A645) – (4.68 x A663)] x V/1000 x W

Total chlorophyll = [(20.2 x A645) + (8.02 x A663)] x V/1000 x W

Where,

A = Absorption at a given wavelength

V = Total volume of sample in extraction medium

W = Weight of sample

3.3.2.4 Proline content

Leaf sample of 0.5 g was homogenised in 10 ml of 3% aqueous sulphosalicylic acid and then filtered through whatman no.2 filter paper. From that 2 ml of filtrate was transferred in a test tube and 2 ml of glacial acetic acid and 2 ml of acid ninhydrin were added and kept for 1 hour in boiling water bath. Then 4 ml of toluene solution was added to the test tube and stirred well for 20-30 seconds and the toluene layer was separated and brought to room temperature. The colour intensity was measured at 520

nm in a spectrophotometer (Model-ELICO SL 218, Double Beam, UV-VIS, Spectrophotometer, India).

The proline content was calculated on a fresh weight basis using the following formula:

$$\text{Micro moles per g tissue} = \frac{\mu \text{ g proline/ ml X ml toluene}}{115.5} \text{ X } \frac{5}{\text{g of sample}}$$

3.3.2.5 Quantification of secondary metabolites (Total flavanoids from grains)

The flavanoid content in grains was estimated using the method mentioned above (flavanoid estimation in leaves) with slight modification using $AlCl_3$ (Ordonez et al., 2006). The grains were ground to fine powder and from which 0.5 g of grain powder was homogenized in 80% methanol and incubated at 40°C for 24 hours. Then the sample was cooled down to room temperature and centrifuged at 4,500 rpm for 15 min. The supernatant was collected and out of which 0.5 ml was taken and to which 0.5 ml 80% methanol and 4 ml distilled water were added. Then 0.3 ml of 5% sodium nitrite ($NaNO_2$) was added and incubated for 5 min. After that 0.3 ml of 10% $AlCl_3$ was added and the solutionwas allowed to stand for 6 min. The reaction was stopped by adding 2 ml of 1 M sodium hydroxide (NaOH) and the final volume was brought to 10 ml by adding distilled water. The sample was allowed to stand for 15 min and the absorbance was taken at 510 nm by using Spectrophotometer (Model-ELICO SL 218, Double Beam, UV-VIS, Spectrophotometer, India).

The total flavanoid content was calculated from a calibration curve using quercetin and expressed as mg quercetin (QE)/ g of plant sample.

3.3.3 Molecular analysis

3.3.3.1 Protein profiling (SDS-PAGE)

Protein profiling was done using sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE). Electrophoresis is the technique used to separate and characterize molecules based on the mobility of ions in an electric field and used to separate macromolecules such as DNA, RNA and proteins. Polyacrylamide Gel Electrophoresis (PAGE) is one of the foremost method used widely to separate proteins with sodium dodecyl sulphate (SDS) which is used to linearize protein by denaturing and giving negative charge to proteins. In the present study SDS-PAGE analysis was done by the protocol suggested by Sadasivam and Manickam (2016). All required stock solutions prepared shown in appendix I.

3.3.3.1.1 Gel preparation and casting of gel

The gel unit was cleaned thoroughly using distilled water then with ethanol and dried and then glass plates were assembled accurately. Resolving gel (12%) (Appendix II-A) was prepared using 30% acrylamide stock solution, 1.5 M trisHCl (pH 8.8), 10% SDS, distilled water, 10% ammonium per sulphate solution and TEMED (N,N,N',N'-tetramethylethylene-1-diamine). The solution was mixed gently and then carefully poured between the glass plates up to three-forth portion. It was then overlaid with distilled water to prevent contact with air for proper polymerization, since oxygen inhibits polymerization. Stacking gel (5%) (Appendix II-B) was prepared using 30% acrylamide stock solution, 0.5 M trisHCl (pH 6.8), 10% SDS, distilled water, 10% ammonium per sulphate (APS) solution and TEMED. The overlaid water was then removed very carefully and stacking gel was poured between the glass plates and comb was inserted properly and gel was allowed to set. Later placed the gel plate in Bio-Rad electrophoresis apparatus and poured electrode buffer containing tris base, glycine and SDS and the comb was removed carefully without damaging the wells.

3.3.3.1.2 Sample Preparation and Gel Loading

The sample buffer was prepared using trisHCl (pH 6.8), bromophenol blue, β-mercaptoethanol, glycerol and SDS (Appendix I-D). The sample protein concentrate

was adjusted to 50 µg and then mixed with sample buffer. Then the samples were heated at 93°C for 3 minutes before loading, to ensure complete interaction between proteins and SDS. Meanwhile the wells were flushed with buffer using a syringe to remove residual gel reagents before loading the samples. The protein samples were allowed to cool and the samples along with pre-stained protein ladder (5 µl) were loaded in the wells.

3.3.3.1.3 Electrophoresis

Electrode buffer (Appendix I-E) was poured into the electrophoresis apparatus and initially run at 55 V until all the protein samples stacked on the resolving gel moved uniformly. This was done for uniform run of all protein residuals and then it was run at 65 V for 1 to 1.5 hrs until the dye front reached the bottom.

3.2.4.1.5 Gel Staining and Destaining

After the run was complete, gel was removed carefully from the glass plates and submerged in the staining solution for three to four hours with occasional shaking. Staining solution (0.1%) (Appendix III-A) was prepared by mixing coomassie brilliant blue R 250, glacial acetic acid, methanol and distilled water. The proteins absorbed the coomassie brilliant blue present in the staining solution. After staining, the gel was transferred to destaining solution (Appendix III-B) containing all the components except coomassie brilliant blue R 250 dye and allowed to destain till the bands were visible and the dye that was not bound to proteins was removed. Destaining was stopped at the right stage to visualize the protein bands. The gel was photographed after proper destaining.

3.3.3.2 Gene expression study using RT-PCR

3.3.3.2.1 Isolation of RNA

The RNA was isolated using TRIzol reagent from grains at the grain filling stage. The reagents, glassware, mortar and pestle, forceps and plastic wares such as microtips, microfuge tubes were autoclaved. All the reagents used were prepared using DEPC (Diethyl pyrocabonate) treated water. The DEPC treated water was prepared by adding 1 ml of DEPC to 1 liter of water (0.1%) and keeping overnight on magnetic stirrer and then autoclaving twice to remove DEPC completely.

Chilled mortar and pestle was wiped with RNAase zap to remove any traces of RNAase and then used for grinding the grain samples. 100 mg of samples were ground into fine powder using liquid nitrogen. 1 ml of TRIzol reagent was added to the powdered samples and mixed gently to homogenize the mixture and incubated at ambient temperature for 5 minutes. Then, the mixture was transferred to pre-chilled microfuge tube. Later, 0.2 ml chloroform was added and shaken vigorously for about 15 seconds and incubated for 5 minutes at room temperature. The microfuge tubes were kept in ice for 10 minutes and centrifuged at 12,000 g for 15 minutes at 4 °C. The aqueous phase from the tubes were transferred to fresh microfuge tubes. Hundred percent ice cold isopropanol of 0.5 ml was added to each tube and kept for incubation at room temperature for 10 minutes. Then the contents in the tubes were mixed by inverting the tube slowly and again centrifuged at 12,000 g for 10 minutes at 4 °C. After that the supernatant was discarded carefully and pellet was washed with 1 ml of 75% ethanol prepared in DEPC treated water and spun at 7,500 g for 5 minutes at 4 °C. Then the supernatant was removed and the pellet was air dried at 30-40 °C in the laminar air flow chamber. The pellet was then dissolved in 30 µl of RNAase free water and kept for incubation at about 55-60°C for 10 minutes. The isolated RNA was stored at -80 °C for further use.

3.3.3.2.2 Qualitative analysis and quantification of RNA

The quality of RNA was determined by using agarose get electrophoresis. The gel was prepared using 1.5 g of agarose powder dissolved in 100 ml of 1 X TBE

(Appendix IV) in the microwave oven. The gel was cooled down to about 60-65 °C and ethidium bromide (EtBr) was added to it. Then the gel was poured into a casting tray, the comb was kept and the gel was allowed to solidify. After removing the comb, the solidified gel was transferred to the electrophoresis tank which contained 1 X TBE buffer. Then, RNA sample of 5 µl added to 2 µl of loading dye (bromo phenol blue) and 5 µl DEPC treated water was mixed gently and poured in the wells carefully. The voltage was maintained at 5 V/cm and the gel was allowed to run till the RNA reached three fourth of gel. The bands were visualized and documented in Gel Doc Unit (Bio-Rad) using Quantity One software.

The quantification of RNA was determined by taking absorbance in UV-visible spectrophotometer (Model-ELICO SL 218, Double Beam, UV-VIS, Spectrophotometer, India) at the wavelength of 260 and 280 nm. The absorbance value of 1.0 at 260 nm indicated that 40 ng μl^{-1} of RNA was present in the samples. The concentration of RNA in the sample was determined by the formula:

$$\text{Concentration of RNA (ng } \mu l^{-1}) = A_{260} \times 40 \times \text{Dilution factor}$$

Whereas, A_{260}= Absorbance at 260 nm

The quality of the RNA samples was known from the ratio of the OD values recorded at 260 and 280 nm. The best quality of RNA was referred by A_{260}/A_{280} value between 1.8 and 2.

3.3.3.2.3 Preparation of cDNA

RNA isolated using the above protocol was then used for synthesis of cDNA. The cDNA was synthesized using "Thermo Scientific Verso cDNA Synthesis Kit" according to protocol provided by manufacturers. The kit contained verso reverse transcriptase which could generate long cDNA strands, RNAase inhibitor to protect

RNA templates from degradation and RT enhancer which help to remove DNA contamination. The kit also contained oligo dT primer and Random hexamer.

The composition of reaction mixture (20 µl) for cDNA synthesis is as follows:

5X cDNA synthesis buffer	4 µl
dNTP mix	2µl
Oligo-dT primer	1µl
RT enhancer	1µl
Verso Reverse transcriptase enzyme	1µl
RNA sample	4µl
Nuclease free water	7µl
Total volume	**20 µl**

The contents were mixed gently and incubated at 42°C for 30 minutes followed by another incubation at 92°C for 2 minutes and then the cDNA samples were stored at -80°C for further analysis.

3.3.3.2.4 Quality check of cDNA

The cDNA synthesis was confirmed by standard PCR technique using housekeeping gene *"Ubiquitine (UBQ5)"* with gene specific primers. The standard PCR mix was prepared as follow:

10X reaction buffer (1X)	2 µl
dNTP mix (100 µM)	1 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
Taq DNA polymerase	1 µl
Template DNA	1 µl
Nuclease free water	13 µl
Total volume	**20 µl**

The following conditions were given for the amplification of the *UBQ5* gene in the PCR cycler:

Initial denaturation	94°C for 3 min	
Denaturation	94°C for 30 sec	X 30 cycles
Annealing	55°C for 30 sec	
Extension	72°C for 30 sec	
Final extension	72°C for 5 min	

The final amplified PCR product was separated on agarose gel (1.2%) and observed and documented using Gel Doc Unit (Bio-Rad) using Quantity One software.

3.3.3.2.5 Gene expression analysis using quantitative real-time PCR

To study the expression of flavonoid pathway genes *viz. chalcone synthase (CHS) and CYP75B4* in the seeds of both the njavara varieties at grain filling stage, quantitative real-time PCR (RT-qPCR) was carried out. *Ubiquitine (UBQ5)* gene was used as internal reference and non-template (without cDNA) were kept as control. The primers used are as follows (Park *et al.*, 2016):

Genes	**Locus ID**	**Forward (5' to 3')**	**Reverse (5' to 3')**
CHS	Os11g0530600	GGGCTCATCTCGAAGAACAT	CCTCATCCTCTCCTTGTCCA
CYP75B4	Os10g0317900	TCTCCCATCCGCTTACAATA	ACCAATCTACCAACATACAACAA
UBQ5	Os01g0328400	GAAGTAAGGAAGGAGGAGGA	AAGGTGTTCAGTTCCAAGG

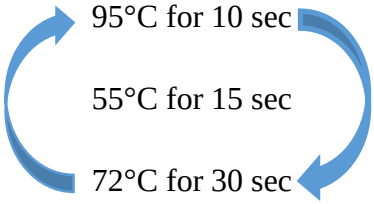
RT-qPCR was done using BIO-RAD CFXTM Touch Real-Time Detection System and data retrieved using BIO-RAD CFX Maestro 1.0 software. To carry out

RT-qPCR, SYBR Green Master mix provided by Origin Diagnostics & Research, India was used. Reaction mixture (20 µl) was prepared as follows:

SYBR Green Master mix (2X)	10 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
cDNA Template	1 µl
Nuclease free water	7 µl
Total volume	**20 µl**

The thermal profile for all RT-qPCR reactions were performed as follows:

Initial denaturation: 95°C for 3 min



X 40 cycles

Final extension 72°C for 2 min

The result obtained are expressed as fold changes *i.e.* increase or decrease in expression of genes. *UBQ5* gene was used as internal reference gene for normalization of RT-qPCR data. The fold changes in expression was calculated by using ΔΔCq method (Rao *et al.,* 2013). The difference between Cq value of query gene and reference gene was considered as ΔCq.

$$\Delta Cq = Cq\ (Query\ gene) - Cq\ (Reference\ gene)$$

Then the difference between ΔCq value of treatment and ΔCq value of control was considered as ΔΔCq value. The fold change in expression of genes were calculated by $2^{-\Delta\Delta Cq}$.

$$\Delta\Delta C_q = \Delta C_q(\text{Treatment}) - \Delta C_q(\text{Control})$$

3.3.4 Statistical analysis

The data were analyzed using statistical software SPSS and the treatments were compared using the design CRD.

Results

4. RESULTS

The results of the present study titled "Physiological, biochemical and molecular studies in medicinal rice (*Oryza sativa* L.), Njavara, as influenced by abiotic stresses" carried out at Department of Plant Physiology, Vellayani, during 2015 to 2019 are presented in this chapter. Results of observations on different physiological, biochemical parameters and molecular aspects at four different growth stages of two medicinal rice *viz.*, black njavara and yellow njavara are given here. The data recorded were analyzed statistically and presented in the relevant tables and appropriate figures.

4.1 EXPERIMENT-I

4.1.1 Physiological characters

4.1.1.1 Plant height

The data on plant height observed at four different stages of the crop showed significant variation between treatments and varieties (Table 1). In black njavara, treatment T_2 recorded maximum plant height at all the four growth stages studied (69.50, 128.87, 134.62 and 132.13 cm respectively) and T_5 recorded the minimum (57.66, 99.03, 116.39 and 113.31 cm respectively). Similarly, in yellow njavara variety also maximum plant height was observed in the treatment T_2 at all the growth stages studied (63.74, 120.49, 129.41 and 126.91 cm respectively). However, at vegetative stage, the treatment T_3 (58.31 cm) recorded the minimum value and at panicle initiation stage treatment T_1 (102.79 cm) recorded the minimum of plant height. Also at flowering stage (112.71 cm) and harvesting stage (109.88 cm), T_3 recorded the lowest plant height.

Plant height (cm)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	68.28	121.38	131.81	130.74	113.05	17.03
V_1T_2	69.50	128.87	134.62	132.13	116.28	20.37
V_1T_3	65.98	121.90	129.38	128.40	111.42	15.34
V_1T_4	68.41	123.11	131.54	129.78	113.21	17.19
V_1T_5	57.66	99.03	116.39	113.31	96.60	0.00
V_2T_1	62.19	102.79	128.47	125.91	104.84	-3.23
V_2T_2	63.74	120.49	129.41	126.91	110.14	1.66
V_2T_3	58.31	106.90	112.71	109.88	96.95	-10.51
V_2T_4	61.49	119.51	128.63	126.74	109.09	0.69
V_2T_5	62.21	110.59	131.18	129.39	108.34	0.00
SEm	1.509	2.684	1.557	1.505		
CD (0.05)	4.379	7.789	4.519	4.368		
V_1	65.96	118.86	128.74	126.87	110.11	
V_2	61.59	112.058	126.08	123.764	105.87	
SE(m)	0.675	1.200	0.696	0.673		
CD (0.05)	1.958	3.484	2.021	1.953		
T_1	64.08	115.05	130.04	127.85	109.26	
T_2	66.62	124.19	132.01	129.43	113.06	
T_3	63.36	112.09	122.26	120.31	104.51	
T_4	64.88	121.19	129.04	127.65	110.69	
T_5	59.93	104.81	123.78	121.35	102.47	
SEm	1.067	1.898	1.101	1.064		
CD (0.05)	3.096	5.508	3.196	3.089		

Table 1: Effect of different shade levels and field capacity levels on plant height (cm) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4- 75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

There was significant variation found in both the varieties black njavara (V_1) and yellow njavara (V_2) at all growth stages studied (Table 1) and in which, black njavara recorded the maximum plant height than yellow njavara at all four growth stages.

Also the plant height significantly varied under different treatments at all growth stages. The highest plant height was observed under T_2 (40% shade) treatment (66.62, 124.19, 132.01 and 129.43 cm respectively) at all four growth stages. However, the control (T_5) recorded the least plant height at vegetative stage (59.93 cm) and panicle initiation stage (104.81 cm) whereas, treatment T_3 (50% field capacity) recorded least plant height at flowering stage and harvesting stage (122.26 cm and 120.31 cm respectively).

4.1.1.2 Leaf Area Index (LAI)

The data on LAI was found to significantly vary between the different treatments and varieties studied during vegetative, panicle initiation and harvesting stages and at flowering stage LAI was found non-significant (Table 2). Black njavara showed highest LAI in T_5 (0.81) at vegetative stage but at panicle initiation stage and harvesting stage, T_2 showed highest LAI (5.44 and 6.39 respectively). The lowest LAI was recorded in T_3 at all the stages mentioned above (0.52, 1.16 and 2.87 respectively). In yellow njavara, T_2 recoded highest LAI at vegetative, panicle initiation and harvesting stage (1.39, 3.57 and 4.66 respectively) whereas, lowest LAI was recorded in T_3 (0.62, 1.39 and 3.12 respectively) at the stages mentioned above.

There was significant variation in LAI at vegetative and panicle initiation stages, but at flowering and harvesting stages it was found non-significant among the varieties (Table 2). At vegetative stage, yellow njavara (V_2) recorded highest (0.84) LAI than black njavara (V_1) (0.62) whereas, at panicle initiation stage V_1 (3.03) recorded higher LAI than V_2 (2.27).

Leaf Area Index (LAI)						
	Vegetative stage	**Panicle Initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	0.59	2.92	4.50	4.12	3.03	-20.05
V_1T_2	0.65	5.44	6.72	6.39	4.80	26.65
V_1T_3	0.52	1.16	3.24	2.87	1.95	-48.55
V_1T_4	0.55	1.76	4.32	3.66	2.57	-32.19
V_1T_5	0.81	3.87	5.60	4.89	3.79	0.00
V_2T_1	0.63	2.09	4.00	3.38	2.53	-19.43
V_2T_2	1.39	3.57	5.44	4.66	3.77	20.06
V_2T_3	0.62	1.39	3.32	3.12	2.11	-32.80
V_2T_4	0.70	2.10	4.62	4.29	2.93	-6.69
V_2T_5	0.84	2.19	5.02	4.50	3.14	0.00
SE(m)	**0.087**	**0.243**	**0.331**	**0.318**		
CD (0.05)	**0.254**	**0.707**	**N/A**	**0.923**		
V_1	0.62	3.03	4.88	4.39	3.23	
V_2	0.84	2.27	4.48	3.99	2.90	
SE(m)	**0.039**	**0.109**	**0.148**	**0.142**		
CD (0.05)	**0.113**	**0.316**	**N/A**	**N/A**		
T_1	0.61	2.51	4.25	3.75	2.78	
T_2	1.02	4.51	6.08	5.52	4.28	
T_3	0.57	1.27	3.28	3.00	2.03	
T_4	0.62	1.93	4.47	3.98	2.75	
T_5	0.82	3.03	5.31	4.70	3.47	
SE(m)	**0.062**	**0.172**	**0.234**	**0.225**		
CD (0.05)	**0.179**	**0.500**	**0.678**	**0.652**		

Table 2: Effect of different shade levels and field capacity levels on leaf area index of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

The LAI recorded among the treatments significantly varied at all the four growth stages (Table 2). Highest LAI was recorded under treatment 40% shade (T_2) at all the four stages (1.02, 4.51, 6.08 and 5.52 respectively) and the least was recorded under treatment 50% field capacity (T_3) (0.57, 1.27, 3.28 and 3.00 respectively) at different growth stages studied.

4.1.1.3 Specific Leaf Area (SLA)

Specific leaf area (SLA) was found significantly different among the different treatments and varieties at all the growth stages studied (Table 3). The variety black njavara (V_1) recorded maximum SLA (391.33, 377.12, 382.79 and 375.79 $cm^2\ g^{-1}$) under 40% shade (T_2) at vegetative, panicle initiation, flowering and harvesting stages respectively and the lowest SLA was recoded under 20% shade (T_1) at vegetative stage (239.71 $cm^2\ g^{-1}$). But at panicle initiation, flowering and harvesting stages the treatment 50% field capacity (T_3) recorded lower SLA (239.71, 303.93 and 296.93 $cm^2\ g^{-1}$ respectively) in black njavara. In the yellow njavara (V_2), treatment 40% shade (T_2) recorded maximum SLA at vegetative, panicle initiation and flowering stage (376.81, 364.04 and 373.53 $cm^2\ g^{-1}$ respectively), however at harvesting stage, the treatment 75% field capacity (T_4) recorded maximum SLA (368.53 $cm^2\ g^{-1}$). Whereas, lower SLA was recoded under 20% shade (T_1) at vegetative stage (248.46 $cm^2\ g^{-1}$) and at panicle initiation stage, flowering stage and harvesting stages, the treatment 50% field capacity (T_3) recorded the lowest SLA (196.59, 241.11 and 236.11 $cm^2\ g^{-1}$ respectively) in yellow njavara.

SLA was observed non-significant at vegetative stage in both varieties whereas, at panicle initiation, flowering and harvesting stages it was found to significantly vary (Table 3). The variety black njavara (V_1) recorded higher SLA at panicle initiation stage (309.39 $cm^2\ g^{-1}$) followed by flowering stage (346.17 $cm^2\ g^{-1}$) and harvesting stage (339.17 $cm^2\ g^{-1}$) compared to the yellow njavara (V_2) at the corresponding growth stages (291.22, 316.18 and 311.53 $cm^2\ g^{-1}$ respectively).

Specific Leaf Area (cm^2/g)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	239.71	279.88	314.71	367.72	300.51	-9.33
V_1T_2	391.33	377.12	382.79	375.79	381.76	15.18
V_1T_3	247.79	239.71	303.93	296.93	272.09	-17.91
V_1T_4	301.03	318.56	374.72	307.71	325.51	-1.79
V_1T_5	291.75	331.67	354.68	347.68	331.45	0.00
V_2T_1	248.46	265.22	301.29	297.04	278.00	-11.57
V_2T_2	376.81	364.04	373.53	314.92	357.33	13.67
V_2T_3	258.96	196.59	241.11	236.11	233.19	-25.82
V_2T_4	330.61	335.62	318.92	368.53	338.42	7.65
V_2T_5	275.75	294.63	346.04	341.04	314.37	0.00
SE(m)	**6.142**	**2.093**	**6.725**	**6.759**		
CD (0.05)	**17.826**	**6.075**	**19.517**	**19.615**		
V_1	294.32	309.39	346.17	339.17	322.26	
V_2	298.12	291.22	316.18	311.53	304.26	
SE(m)	**2.747**	**0.936**	**3.007**	**3.023**		
CD (0.05)	**N/A**	**2.717**	**8.728**	**8.772**		
T_1	244.09	272.55	308.00	332.38	289.26	
T_2	384.07	370.58	378.16	372.16	376.24	
T_3	253.37	218.15	272.52	266.52	252.64	
T_4	315.82	327.09	346.82	311.32	325.26	
T_5	283.75	313.15	350.36	344.36	322.91	
SE(m)	**4.343**	**1.480**	**4.755**	**4.779**		
CD (0.05)	**12.605**	**4.296**	**13.800**	**13.870**		

Table 3: Effect of different shade levels and field capacity levels on specific leaf area (cm^2/g) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara).

The SLA was observed to significantly vary at all the growth stages studied among treatments (Table 3). Highest SLA was observed in the treatment T_2 at vegetative stage (384.07 $cm^2 g^{-1}$), panicle initiation stage (370.58 $cm^2 g^{-1}$), flowering stage (378.16 $cm^2 g^{-1}$) and harvesting stage (372.16 $cm^2 g^{-1}$). The lowest SLA was recorded in the treatment T_1 at vegetative stage. However at panicle initiation, flowering and harvesting stages (218.15, 272.52 and 266.52 $cm^2 g^{-1}$ respectively), the treatment T_3 recorded the minimum values.

4.1.1.4 Number of Tillers

Number of tillers per plants was observed significantly different between treatments and varieties at only flowering stage while non-significant at vegetative stage, panicle initiation stage and harvesting stage (Table 4). At flowering stage, both the varieties (V_1-23.83; V_2-22.67) recorded highest number of tillers per plant under control (T_5) and the lowest number of tillers per plants was observed in the treatment 50% field capacity (T_3) (V_1-12.42; V_2-13.08) at the flowering stage.

Between the varieties, the number of tillers per plants was observed significantly different at only panicle initiation stage and non-significant at vegetative stage, flowering stage and harvesting stage (Table 4). Variety black njavara (V_1) recorded most number of tillers per plant (10.83) than the yellow njavara (V_2) variety (9.68) at panicle initiation stage.

The number of tillers per plant was found to significantly vary among the different treatments and at all growth stages studied (Table 4). Among the treatments, the control (T_5) showed the highest number of tillers per plant (8.54, 13.04, 23.25 and 20.29) whereas the treatment 50% field capacity (T_3) showed the lowest number of tillers per plant (3.67, 7.21, 12.75 and 10.99) respectively at different growth stages studied (Table 4).

Number of Tillers (per plant)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Means**	**% change over control**
V_1T_1	4.03	10.92	14.42	14.50	10.97	-32.28
V_1T_2	5.09	9.50	15.99	14.25	11.21	-30.80
V_1T_3	3.58	7.42	12.42	11.50	8.73	-46.11
V_1T_4	7.08	13.49	20.83	17.83	14.81	-8.58
V_1T_5	7.99	12.96	23.83	20.03	16.20	0.00
V_2T_1	4.08	8.83	13.67	14.00	10.15	-38.03
V_2T_2	4.45	7.75	16.33	14.25	10.70	-34.68
V_2T_3	3.75	7.00	13.08	10.49	8.58	-47.62
V_2T_4	6.75	11.67	22.50	17.49	14.60	-10.87
V_2T_5	9.08	13.17	22.67	20.58	16.38	0.00
SE(m)	**0.35**	**0.49**	**0.44**	**0.45**		
CD (0.05)	**N/A**	**N/A**	**1.29**	**N/A**		
V_1	5.55	10.83	17.49	15.69	12.39	
V_2	5.62	9.68	17.65	15.35	12.08	
SE(m)	**0.16**	**0.22**	**0.19**	**0.20**		
CD (0.05)	**N/A**	**0.64**	**N/A**	**N/A**		
T_1	4.04	9.88	14.04	14.25	10.55	
T_2	4.75	8.63	16.17	14.15	10.93	
T_3	3.67	7.21	12.75	10.99	8.66	
T_4	6.92	12.54	21.67	17.63	14.69	
T_5	8.54	13.04	23.25	20.29	16.28	
SE(m)	**0.25**	**0.35**	**0.31**	**0.39**		
CD (0.05)	**0.71**	**1.01**	**0.91**	**0.92**		

Table 4: Effect of different shade levels and field capacity levels on number of tillers per plant of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

4.1.1.5 Photosynthetic rate

The data on photosynthetic rate is given in table 5. Photosynthetic rate between varieties and treatments was found significant at only flowering stage but non-significant at vegetative stage, panicle initiation stage and harvesting stage (Table 5). At the flowering stage, both varieties *viz.*, black njavara (V_1) and yellow njavara (V_2) showed highest photosynthetic rate under treatment T_5 (19.40 and 18.95 μmol CO_2 $m^{-2}s^{-1}$ respectively). However, the lowest photosynthetic rate was recorded under treatment T_2 in the black njavara (11.15 μmol CO_2 $m^{-2}s^{-1}$) and in treatment T_3 (10.43 μmol CO_2 $m^{-2}s^{-1}$) in the case of yellow njavara (Table 5).

Photosynthetic rate was found significantly different at vegetative stage, flowering stage and harvesting stage but no significant variation was found at panicle initiation stage between the varieties. Black njavara (V_1) variety recorded highest photosynthetic rate at vegetative stage (13.93 μmol CO_2 $m^{-2}s^{-1}$), flowering stage (14.64 μmol CO_2 $m^{-2}s^{-1}$) and harvesting stage (12.42 μmol CO_2 $m^{-2}s^{-1}$). But the yellow njavara (V_2) variety recorded the lowest values (13.38, 13.98, and 11.64 μmol CO_2 $m^{-2}s^{-1}$ respectively) at all the above mentioned growth stages.

The photosynthetic rate recorded among the treatments significantly varied at all growth stages studied (Table 5). Highest rate of photosynthesis was recorded in control (T_5) at all the four growth stages of the study (18.05, 18.91, 19.18 and 15.10 μmol CO_2 $m^{-2}s^{-1}$ respectively) and the lowest was recorded in the treatment 50% field capacity (T_3) at vegetative stage (10.11 μmol CO_2 $m^{-2}s^{-1}$), panicle initiation stage (10.54 μmol CO_2 $m^{-2}s^{-1}$) and harvesting stage (9.64 μmol CO_2 $m^{-2}s^{-1}$). But at flowering stage, the treatment 40% shade (T_2) recorded the lowest photosynthetic rate (10.91 μmol $CO_2m^{-2}s^{-1}$).

Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	13.83	14.23	14.90	13.28	14.06	-22.45
V_1T_2	10.95	11.15	11.15	9.80	10.76	-40.65
V_1T_3	10.30	11.03	11.70	10.08	10.78	-40.54
V_1T_4	16.28	15.45	16.03	13.35	15.28	-15.72
V_1T_5	18.28	19.25	19.40	15.60	18.13	0.00
V_2T_1	13.45	13.45	14.23	12.18	13.33	-23.79
V_2T_2	10.45	11.10	10.68	9.53	10.44	-40.31
V_2T_3	9.93	10.05	10.43	9.20	9.90	-43.40
V_2T_4	15.23	16.23	15.63	12.68	14.94	-14.58
V_2T_5	17.83	18.58	18.95	14.60	17.49	0.00
SE(m)	**0.170**	**0.346**	**0.154**	**0.159**		
CD (0.05)	**N/A**	**N/A**	**0.448**	**N/A**		
V_1	13.93	14.22	14.64	12.42	13.80	
V_2	13.38	13.88	13.98	11.64	13.22	
SE(m)	**0.076**	**0.155**	**0.069**	**0.071**		
CD (0.05)	**0.221**	**N/A**	**0.200**	**0.206**		
T_1	13.64	13.84	14.56	12.73	13.69	
T_2	10.70	11.13	10.91	9.66	10.60	
T_3	10.11	10.54	11.06	9.64	10.34	
T_4	15.75	15.84	15.83	13.01	15.11	
T_5	18.05	18.91	19.18	15.10	17.81	
SE(m)	**0.120**	**0.245**	**0.109**	**0.112**		
CD (0.05)	**0.349**	**0.710**	**0.317**	**0.326**		

Table 5: Effect of different shade levels and field capacity levels on photosynthesis rate (µmol CO_2 m^{-2} s^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

4.1.1.6 Stomatal conductance

Data on stomatal conductance is presented in table 6. The data on stomatal conductance was found significantly vary between the different treatments and varieties studied during vegetative, panicle initiation and harvesting stages but at flowering stage it was found non-significant (Table 6).

Stomatal conductance within varieties was observed to significantly vary at vegetative stage, panicle initiation stage and flowering stage whereas, no significant difference was found at harvesting stages. Variety black njavara (V_1) (285.95, 336.84 and 348.80 mol H_2O $m^{-2}s^{-1}$ respectively) recorded higher stomatal conductance at above mentioned stages than the yellow njavara (V_2) (277.75, 316.73 and 325.35 mol H_2O $m^{-2}s^{-1}$ respectively).

There was significant variation in stomatal conductance among different treatments at all the growth stages studied. At vegetative stage, the treatment 20% shade (T_1) recorded highest stomatal conductance (327.36 H_2O m^{-2} s^{-1}) whereas, at panicle initiation stage, flowering and harvesting stage, the control (T_5) recorded the highest value of stomatal conductance (376.75, 384.89 and 340.75 mol H_2O m^{-2} s^{-1} respectively). However, lowest stomatal conductance was recorded in treatment 50% field capacity (T_3) at all the growth stages studied (225.88, 260.85, 271.50 and 260.38 mol H_2O m^{-2} s^{-1} respectively).

4.1.1.7 Transpiration rate

The data on transpiration rate is given in table 7. Transpiration rate between treatments and varieties was found to significantly vary at vegetative stage, panicle initiation stage and flowering stage but non-significant at harvesting stage (Table 7). In black njavara (V_1), the control (T_5) recorded the highest transpiration rate at vegetative stage (3.58 mmol H_2O m^{-2} s^{-1}), panicle initiation (4.34 mmol H_2O m^{-2} s^{-1}) stage and flowering stage (4.38 mmol H_2O m^{-2} s^{-1}). But the lowest transpiration rate

Stomatal conductance (mol H_2O m^{-2} s^{-1})						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	304.00	370.51	376.50	298.00	337.25	-8.38
V_1T_2	273.75	294.70	302.74	262.75	283.49	-22.98
V_1T_3	221.50	278.74	291.25	251.00	260.62	-29.19
V_1T_4	300.55	357.20	372.19	321.00	337.74	-8.24
V_1T_5	330.00	382.70	401.28	358.35	368.08	0.00
V_2T_1	350.74	359.50	366.26	304.25	345.19	0.60
V_2T_2	216.01	273.08	297.25	292.56	269.73	-21.39
V_2T_3	230.25	243.00	251.75	269.75	248.69	-27.52
V_2T_4	282.00	337.50	343.00	283.00	311.38	-9.25
V_2T_5	309.75	370.78	368.50	323.50	343.13	0.00
SE(m)	**3.767**	**2.737**	**7.760**	**8.660**		
CD (0.05)	**10.933**	**7.942**	**N/A**	**25.132**		
V_1	285.95	336.84	348.80	298.20	317.45	
V_2	277.75	316.73	325.35	294.60	303.61	
SE(m)	**1.685**	**1.224**	**3.470**	**3.873**		
CD (0.05)	**4.889**	**3.552**	**10.071**	**N/A**		
T_1	327.36	365.00	371.35	301.13	341.21	
T_2	244.88	283.87	300.05	277.63	276.61	
T_3	225.88	260.85	271.50	260.38	254.65	
T_4	291.25	347.35	357.63	302.00	324.56	
T_5	319.87	376.75	384.89	340.75	355.57	
SE(m)	**2.664**	**1.935**	**5.487**	**6.123**		
CD (0.05)	**7.731**	**5.616**	**15.924**	**17.771**		

Table 6: Effect of different shade levels and field capacity levels on stomatal conductance (mol H_2O m^{-2} s^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

Transpiration rate (mmol H_2O m^{-2} s^{-1})						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	3.23	3.45	3.59	1.55	2.96	-20.86
V_1T_2	1.54	1.66	1.93	1.26	1.60	-57.22
V_1T_3	1.61	2.00	1.94	1.05	1.65	-55.88
V_1T_4	1.77	2.57	2.51	1.49	2.09	-44.12
V_1T_5	3.58	4.34	4.38	2.67	3.74	0.00
V_2T_1	1.96	1.91	1.99	1.12	1.75	-39.66
V_2T_2	1.37	1.33	1.70	0.98	1.35	-53.45
V_2T_3	1.43	0.93	1.34	0.95	1.16	-60.00
V_2T_4	1.67	1.58	1.48	1.22	1.49	-48.62
V_2T_5	1.87	3.76	3.78	2.17	2.90	0.00
SE(m)	**0.197**	**0.137**	**0.170**	**0.134**		
CD (0.05)	**0.572**	**0.397**	**0.493**	**N/A**		
V_1	2.34	2.80	2.87	1.60	2.40	
V_2	1.66	1.90	2.06	1.29	1.73	
SE(m)	**0.088**	**0.061**	**0.076**	**0.060**		
CD (0.05)	**0.256**	**0.178**	**0.221**	**0.173**		
T_1	2.60	2.68	2.79	1.33	2.35	
T_2	1.45	1.49	1.81	1.12	1.47	
T_3	1.52	1.47	1.64	1.00	1.41	
T_4	1.72	2.08	1.99	1.35	1.79	
T_5	2.72	4.05	4.08	2.42	3.32	
SE(m)	**0.139**	**0.097**	**0.120**	**0.094**		
CD (0.05)	**0.405**	**0.281**	**0.349**	**0.274**		

Table 7: Effect of different shade levels and field capacity levels on transpiration rate (mmol H_2O m^{-2} s^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

was recorded in treatment T_2 at the above mentioned growth stages (1.54, 1.66 and 1.93 mmol H_2O m^{-2} s^{-1} respectively). In case of yellow njavara (V_2) the highest transpiration rate was recorded in T_1 at vegetative stage (1.96 mmol H_2O m^{-2} s^{-1}) and at T_5 in panicle initiation (3.76 mmol H_2O m^{-2} s^{-1}) and flowering stage (3.78 mmol H_2O m^{-2} s^{-1}). The lowest rate of transpiration was recorded in treatment T_2 at vegetative stage (1.37 mmol H_2O m^{-2} s^{-1}) and T_3 at panicle initiation (0.93 mmol H_2O m^{-2} s^{-1}) and flowering stage (1.34 mmol H_2O m^{-2} s^{-1}).

Both varieties showed significant variation at all the growth stages studied. Black njavara (V_1) recorded the higher rate of transpiration at all the growth stages (2.34, 2.80, 2.87 and 1.60 mmol H_2O m^{-2} s^{-1} respectively). However, the transpiration rate in yellow njavara (V_2) was found lesser at all the stages mentioned above (1.66, 1.90, 2.06 and 1.29 mmol H_2O m^{-2} s^{-1} respectively).

Transpiration rate recorded among the treatments significantly varied at all growth stages studied. Highest transpiration rate was recorded under control (T_5) at all the stages studied (2.72, 4.05, 4.08 and 2.42 mmol H_2O m^{-2} s^{-1} respectively). But the lowest transpiration rate was recoded in treatment 40% shade (T_2) at vegetative stage (1.45 mmol H_2O m^{-2} s^{-1}) and in treatment 50% field capacity (T_3) at panicle initiation, flowering stage and harvesting stage (1.47, 1.64 and 1.00 mmol H_2O $m^{-2}s^{-1}$ respectively).

4.1.2 Biochemical characters

4.1.2.1 Flavonoid content in leaves

The data on total flavonoid content is presented in table 8. Flavonoid content was found to vary significantly at only flowering stage and non-significant at vegetative stage, panicle initiation stage and harvesting stage between varieties and treatments (Table 8). At flowering stage, both black njavara (V_1) and yellow njavara (V_2) showed highest flavonoid content under treatment T_3 (18.95 and 15.58 mg g^{-1} FW

Flavonoid content (mg g^{-1}FW)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	11.45	12.77	13.18	14.82	13.06	-4.88
V_1T_2	10.97	11.66	12.07	13.23	11.98	-12.75
V_1T_3	14.84	16.01	18.95	16.03	16.46	19.88
V_1T_4	12.82	13.71	17.45	15.72	14.93	8.74
V_1T_5	12.50	12.49	16.62	13.29	13.73	0.00
V_2T_1	11.09	11.79	12.62	13.8	12.33	-2.76
V_2T_2	10.73	11.61	11.91	10.67	11.23	-11.44
V_2T_3	13.29	15.53	15.58	13.69	14.52	14.51
V_2T_4	11.72	12.79	15.02	13.57	13.28	4.73
V_2T_5	12.24	12.11	13.79	12.59	12.68	0.00
SE(m)	**0.402**	**0.572**	**0.315**	**0.502**		
CD (0.05)	**N/A**	**N/A**	**0.914**	**N/A**		
V_1	12.52	13.33	15.65	14.62	14.03	
V_2	11.81	12.77	13.78	12.88	12.81	
SE(m)	**0.180**	**0.256**	**0.141**	**0.224**		
CD (0.05)	**0.521**	**N/A**	**0.409**	**0.651**		
T_1	11.27	12.28	12.90	14.31	12.69	
T_2	10.85	11.64	11.99	11.95	11.61	
T_3	14.06	15.77	17.27	14.86	15.49	
T_4	12.27	13.25	16.23	14.69	14.11	
T_5	12.37	12.30	15.20	12.94	13.20	
SE(m)	**0.284**	**0.404**	**0.223**	**0.355**		
CD (0.05)	**0.824**	**1.173**	**0.646**	**1.030**		

Table 8: Effect of different shade levels and field capacity levels on flavonoid content (mg g^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

respectively) and the least content of flavonoid was recorded under treatment T_2 in the both the varieties (12.07 and 11.91 mg g^{-1} FW respectively).

The flavonoid content within varieties was observed to significantly vary at vegetative stage, flowering stage and harvesting stage. However at panicle initiation stage, flavonoid content was found non-significant among the varieties. Variety black njavara (V_1) recorded highest content of flavonoid at vegetative stage (12.52 mg g^{-1} FW), flowering stage (15.65 mg g^{-1} FW) and harvesting stage (14.62 mg g^{-1} FW) and the lowest values were recorded for yellow njavara (V_2) (11.81, 13.78 and 12.88 mg g^{-1} FW respectively).

Flavonoid content under different treatments was found to significantly vary at all the growth stages studied. The treatment 50% field capacity (T_3) recorded highest flavonoid content (14.06, 15.77, 17.27 and 14.86 mg g^{-1} FW respectively) at all the four stages studied. The least flavonoid content was observed in 40% shaded condition (T_2) (10.85, 11.64, 11.99 and 11.95 mg g^{-1} FW respectively) at all the stages studied.

4.1.2.2 Phenol content

The data on phenol content is presented in table 9. Phenol content between varieties and treatments was found significantly different at panicle initiation stage and harvesting stage and both the varieties showed highest content of phenol in the treatment T_3 at panicle initiation stage (V_1-2.83 mg g^{-1} FW; V_2-1.85 mg g^{-1} FW) and at harvesting stage (V_1-2.11 mg g^{-1} FW; V_2-1.76 mg g^{-1} FW). The lowest phenol content was recorded in the treatment T_2 both at panicle initiation stage and harvesting stage (Table 9).

Both varieties showed significant variation in phenol content at all the stages of growth. Black njavara (V_1) recorded higher phenol content (1.87, 1.88, 2.07 and 1.30 mg g^{-1} FW respectively) respectively at all the growth stages. But the

Phenol content (mg g^{-1} FW)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	1.39	1.42	1.64	0.96	1.35	-14.56
V_1T_2	1.34	1.22	1.59	0.87	1.26	-20.25
V_1T_3	2.64	2.83	2.88	2.11	2.62	65.82
V_1T_4	2.20	2.13	2.42	1.64	2.10	32.91
V_1T_5	1.76	1.81	1.82	0.91	1.58	0.00
V_2T_1	1.52	1.44	1.37	0.82	1.29	-11.64
V_2T_2	1.34	1.26	1.12	0.72	1.11	-23.97
V_2T_3	1.89	1.85	2.86	1.76	2.09	43.15
V_2T_4	1.85	1.70	2.24	1.01	1.70	16.44
V_2T_5	1.47	1.63	1.73	0.99	1.46	0.00
SE(m)	**0.149**	**0.131**	**0.132**	**0.044**		
CD (0.05)	**N/A**	**0.381**	**N/A**	**0.128**		
V_1	1.87	1.88	2.07	1.30	1.78	
V_2	1.61	1.57	1.87	1.06	1.53	
SE(m)	**0.067**	**0.059**	**0.058**	**0.020**		
CD (0.05)	**0.194**	**0.171**	**0.172**	**0.057**		
T_1	1.46	1.43	1.51	0.89	1.32	
T_2	1.34	1.24	1.35	0.80	1.18	
T_3	2.27	2.34	2.87	1.93	2.35	
T_4	2.02	1.91	2.33	1.33	1.90	
T_5	1.61	1.72	1.78	0.95	1.52	
SE(m)	**0.105**	**0.093**	**0.094**	**0.031**		
CD (0.05)	**0.306**	**0.270**	**0.272**	**0.090**		

Table 9: Effect of different shade levels and field capacity levels on phenol content (mg g^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

corresponding phenol content in yellow njavara (V_2) was lesser at the stages mentioned above (1.61, 1.57, 1.87 and 1.06 mg g^{-1} FW respectively).

However, the crop grown at 50% field capacity (T_3) recorded significantly higher content of phenol at all the four stages (2.27, 2.34, 2.87 and 1.93 mg g^{-1} FW respectively) than other treatments. But the crop grown in 40% shaded condition (T_2) recorded least content of phenol at all the four stages studied (1.34, 1.24, 1.35 and 0.80 mg g^{-1} FW respectively).

4.1.2.3 Chlorophyll content

a) Chlorophyll 'a'

The data on chlorophyll 'a' observed among different varieties and treatments was significant at vegetative and panicle initiation stage and non-significant at flowering and harvesting stage (Table 10). At vegetative stage, treatment T_4 (1.54 mg g^{-1} FW) in the black njavara (V_1) and T_3 (1.67 mg g^{-1} FW) in yellow njavara (V_2) recorded highest content of chlorophyll 'a' whereas the lowest value was recorded in T_5 (V_1-1.44 mg g^{-1} FW; V_2-1.01 mg g^{-1} FW) in both the varieties. Treatment T_2 recorded the highest content of chlorophyll 'a' at panicle initiation stage in both the varieties V_1 (1.46 mg g^{-1} FW) and V_2 (1.71 mg g^{-1} FW). However lowest values were recorded in T_3 (1.29 and 1.43 mg g^{-1} FW) in both the varieties respectively.

Chlorophyll 'a' was found significantly different during two growth stages *viz.*, panicle initiation stage and harvesting stage. The variety yellow njavara (V_2) recorded highest value of chlorophyll 'a' at panicle initiation stage (1.55 mg g^{-1} FW) and harvesting stage (1.25 mg g^{-1} FW). The corresponding values of chlorophyll 'a' for black njavara (V_1) are found lower (1.41 mg g^{-1} FW and 1.13 mg g^{-1} FW).

There was significant variation in chlorophyll 'a' content among different treatments at all the growth stages studied. At vegetative stage, the treatment 75% field

capacity (T_4) showed highest content of chlorophyll 'a' (1.59 mg g^{-1} FW) whereas, at all other three stages the treatment 40% shade (T_2) recorded highest value (1.58, 1.47 and 1.35 mg g^{-1} FW respectively) and the lowest value was recorded in control (T_5) at the vegetative stage (1.23 mg g^{-1} FW). But at panicle initiation stage, flowering stage and harvesting stage the treatment 50% field capacity (T_3) recorded lowest content of chlorophyll 'a' (1.38, 1.12 and 0.98 mg g^{-1} FW respectively).

b) Chlorophyll 'b'

Between varieties and treatments, chlorophyll 'b' content was found significant only at vegetative and harvesting stages (Table 10). Black njavara recorded highest content of chlorophyll 'b' in treatment T_1 (1.68 mg g^{-1} FW) and the lowest content was recorded in treatment in T_3 (0.59 mg g^{-1} FW). But in yellow njavara, the treatment T_2 (1.31 mg g^{-1} FW) recorded the highest content and the lowest was recorded in treatment T_1 (0.66 mg g^{-1} FW) at vegetative stage. But at harvesting stage, T_2 recorded highest chlorophyll 'b' content in both black njavara (1.15 mg g^{-1} FW) and yellow njavara (2.07 mg g^{-1} FW) varieties. However the least value was recorded in the treatment T_3 (0.45 mg g^{-1} FW) in case of black njavara and in T_4 (0.41 mg g^{-1} FW) in case of yellow njavara variety.

Chlorophyll 'b' content was found significant at only harvesting stage in both varieties wherein the highest value was recorded in yellow njavara (1.07 mg g^{-1} FW) and lowest in black njavara (0.82 mg g^{-1} FW).

Chlorophyll 'b' content was found significantly different at all the growth stages studied. However, 40% shaded treatment (T_2) recorded highest content of chlorophyll 'b' at all the four stages studied (1.45, 1.86, 1.59 and 1.61 mg g^{-1} FW respectively). But the lowest content of chlorophyll 'b' was found at 50% field capacity (T_3) (0.70, 0.59, 0.41 and 0.44 mg g^{-1} FW) respectively at all the stages of study.

Chlorophyll content (mg g^{-1} FW)												
	Vegetative stage			**Panicle initiation stage**			**Flowering stage**			**Harvesting stage**		
	Chl 'a'	**Chl 'b'**	**Total chl**	**Chl 'a'**	**Chl 'b'**	**Total chl**	**Chl 'a'**	**Chl 'b'**	**Total chl**	**Chl 'a'**	**Chl 'b'**	**Total chl**
V_1T_1	1.49	1.68	3.16	1.44	1.70	3.13	1.43	1.64	3.07	1.25	0.90	2.15
V_1T_2	1.52	1.59	3.12	1.46	1.62	3.07	1.46	1.40	2.86	1.28	1.15	2.43
V_1T_3	1.48	0.59	2.07	1.29	0.55	1.83	1.10	0.41	1.51	0.98	0.45	2.05
V_1T_4	1.54	0.69	2.23	1.44	0.63	2.09	1.34	0.78	2.12	0.99	0.68	1.67
V_1T_5	1.44	0.61	2.05	1.44	0.65	2.09	1.49	1.49	2.97	1.17	0.90	2.07
V_2T_1	1.55	0.66	2.21	1.67	2.02	3.45	1.43	1.41	2.84	1.45	1.65	3.09
V_2T_2	1.44	1.31	2.75	1.71	1.62	3.07	1.44	1.78	3.22	1.41	2.07	3.47
V_2T_3	1.67	0.81	2.47	1.43	0.86	2.54	1.14	0.42	1.56	0.98	0.42	1.40
V_2T_4	1.64	0.86	2.50	1.45	0.84	2.55	1.42	0.90	2.32	1.10	0.41	1.51
V_2T_5	1.01	1.15	2.16	1.47	0.63	2.10	1.45	1.65	3.09	1.34	0.83	2.17
SE (m)	**0.056**	**0.120**	**0.143**	**0.053**	**0.141**	**0.156**	**0.056**	**0.156**	**0.184**	**0.050**	**0.108**	**0.121**
CD (0.05)	**0.163**	**0.350**	**0.416**	**0.153**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**0.312**	**0.351**
V_1	1.49	1.03	2.52	1.41	1.03	2.44	1.37	1.14	2.51	1.13	0.82	1.95
V_2	1.46	0.96	2.42	1.55	1.20	2.74	1.37	1.23	2.60	1.25	1.07	2.33
SE (m)	**0.025**	**0.054**	**0.064**	**0.024**	**0.063**	**0.070**	**0.025**	**0.070**	**0.082**	**0.022**	**0.048**	**0.054**
CD (0.05)	**N/A**	**N/A**	**N/A**	**0.069**	**N/A**	**0.202**	**N/A**	**N/A**	**N/A**	**0.065**	**0.140**	**0.157**
T_1	1.52	1.17	2.69	1.56	1.62	3.18	1.43	1.53	2.96	1.32	1.27	2.59
T_2	1.48	1.45	2.93	1.58	1.86	3.44	1.47	1.59	3.05	1.35	1.61	2.95
T_3	1.57	0.70	2.27	1.38	0.59	1.97	1.12	0.41	1.53	0.98	0.44	1.42
T_4	1.59	0.77	2.36	1.45	0.74	2.32	1.38	0.84	2.22	1.05	0.54	1.59
T_5	1.23	0.88	2.11	1.44	0.76	2.31	1.45	1.57	3.02	1.25	0.87	2.12
SE (m)	**0.040**	**0.085**	**0.101**	**0.037**	**0.100**	**0.110**	**0.039**	**0.110**	**0.130**	**0.035**	**0.076**	**0.085**
CD (0.05)	**0.115**	**0.247**	**0.294**	**0.108**	**0.290**	**0.320**	**0.114**	**0.320**	**0.378**	**0.103**	**0.221**	**0.248**

Table 10: Effect of different shade levels and field capacity levels on chlorophyll content (mg g^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

c) Total chlorophyll

The data on total chlorophyll is given in table 10. Total chlorophyll content was observed significantly different between the varieties and treatments only at vegetative and harvesting stage. Black njavara (V_1) showed highest value for total chlorophyll content under T_1 (3.16 mg g^{-1} FW) at vegetative stage and T_2 (2.43 mg g^{-1} FW) at harvesting stage. However at vegetative stage, the lowest value was recorded under treatment T_5 (2.05 mg g^{-1} FW) and at harvesting stage the lowest value was recorded under treatment T_4 (1.67 mg g^{-1} FW). But in yellow njavara (V_2) variety, highest content of total chlorophyll was recorded in the treatment T_2 at vegetative and harvesting stage (2.75 and 3.47 mg g^{-1} FW respectively). At vegetative stage, the treatment T_5 (2.16 mg g^{-1} FW) and at harvesting stage the treatment T_3 (1.40 mg g^{-1} FW) recorded the lowest value of total chlorophyll content.

At varietal level, total chlorophyll content was found significant only at panicle initiation and harvesting stages. However at vegetative and at flowering stages, no significant variation was observed. At panicle initiation stage (2.74 mg g^{-1} FW) and harvesting stage (2.33 mg g^{-1} FW), the variety yellow njavara (V_2) recorded highest content of total chlorophyll and black njavara recorded the lowest value (2.44 mg g^{-1} FW and 1.95 mg g^{-1} FW) at panicle initiation and harvesting stage respectively.

Total chlorophyll was found to vary significantly among the treatments at all growth stages. Treatment T_2 (40% shaded) showed the highest values for total chlorophyll content at all the four growth stages (2.93, 3.44, 3.05 and 2.95 mg g^{-1} FW respectively). However, control (T_5) recorded the least total chlorophyll content at vegetative stage (2.11 mg g^{-1} FW) and T_3 (50% field capacity) recorded least values of total chlorophyll content at all other growth stages *viz.*, panicle initiation stage (1.97 mg g^{-1} FW), flowering stage (1.53 mg g^{-1} FW) and harvesting sage (1.42 mg g^{-1} FW).

4.1.2.4 Proline content

The proline content between varieties and treatments was found significantly different at vegetative stage, panicle initiation stage and at harvesting stage. However, no significant difference was found at flowering stage (Table 11). Black njavara (V_1) recorded highest proline content in the treatment T_3 (0.241, 0.419 and 0.348 μg^{-1} FW at the three above mentioned growth stages respectively). However the least proline content was recorded at vegetative stage in the treatment T_5 (0.151 μg^{-1} FW). But treatment T_1 showed least proline content at panicle initiation stage (0.287 μg^{-1} FW) and harvesting stage (0.258 μg^{-1} FW). In the yellow njavara variety, highest proline content was recorded in the treatment T_3 at vegetative stage (0.215 μg^{-1} FW) and at panicle initiation stage (0.357 μg^{-1} FW) but at harvesting stage the treatment T_2 (0.350 μg^{-1} FW) recorded the highest value. Least proline content was recorded in the treatment T_5 at all the three above mentioned growth stages (0.147, 0.238 and 0.278 μg^{-1} FW respectively).

The data on proline content is found significantly different between the varieties both at vegetative and panicle initiation stages. But it was found non-significant at flowering and harvesting stages. Among the different rice varieties, black njavara (V_1) recorded highest proline content at vegetative and panicle initiation stage (0.198 and 0.348 μg^{-1} FW respectively) and the corresponding values for yellow njavara (V_2) were found lower (0.183 and 0.300 μg^{-1} FW respectively).

Plants grown at 50% field capacity (T_3) recorded significantly higher content of proline at vegetative stage, panicle initiation stage and flowering stage (0.228, 0.388 and 0.488 μg^{-1} FW respectively). But at harvesting stage, the treatment 40% shade (T_2) recorded highest proline content (0.347 μg^{-1} FW) than other treatments. The plants grown in controlled condition (T_5) recorded least content of proline at vegetative stage (0.149 μg^{-1} FW) and flowering stage (0.289 μg^{-1} FW). However, at panicle initiation stage and harvesting stage, the treatment 20% shade (T_1) recorded the least proline content (0.262 and 0.280 μg^{-1} FW respectively).

Proline content (µg^{-1} FW)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	0.207	0.287	0.342	0.258	0.27	0.00
V_1T_2	0.225	0.350	0.468	0.345	0.35	29.63
V_1T_3	0.241	0.419	0.540	0.348	0.39	44.44
V_1T_4	0.168	0.346	0.327	0.323	0.29	7.41
V_1T_5	0.151	0.337	0.320	0.287	0.27	0.00
V_2T_1	0.175	0.250	0.317	0.302	0.26	13.04
V_2T_2	0.214	0.335	0.438	0.350	0.33	43.48
V_2T_3	0.215	0.357	0.436	0.338	0.34	47.83
V_2T_4	0.164	0.320	0.392	0.321	0.30	30.43
V_2T_5	0.147	0.238	0.258	0.278	0.23	0.00
SE(m)	**0.005**	**0.012**	**0.033**	**0.014**		
CD (0.05)	**0.014**	**0.035**	**N/A**	**0.040**		
V_1	0.198	0.348	0.399	0.312	0.31	
V_2	0.183	0.300	0.368	0.318	0.29	
SE(m)	**0.002**	**0.005**	**0.015**	**0.006**		
CD (0.05)	**0.006**	**0.016**	**N/A**	**N/A**		
T_1	0.191	0.262	0.329	0.280	0.27	
T_2	0.219	0.342	0.453	0.347	0.34	
T_3	0.228	0.388	0.488	0.339	0.36	
T_4	0.166	0.333	0.359	0.322	0.30	
T_5	0.149	0.294	0.289	0.313	0.26	
SE(m)	**0.003**	**0.009**	**0.023**	**0.010**		
CD (0.05)	**0.010**	**0.025**	**0.067**	**0.028**		

Table 11: Effect of different shade levels and field capacity levels on proline content (µg^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

4.1.2.5 Flavonoid content in Grains

The data on flavonoid content in grains is shown in table 12. Flavonoid content in grains between varieties and treatments was found significantly different. In both the varieties *viz.,* black njavara (V_1) and yellow njavara (V_2) the treatment 75% field capacity (T_4) recorded highest flavonoid content in grains (V_1-19.48 mg g^{-1} grain and V_2-18.56 mg g^{-1} grain), followed by treatment 20% shade (V_1-17.40 mg g^{-1} grain and V_2-16.78 mg g^{-1} grain). However, least flavonoid content in grains was recorded under treatment 50% field capacity (T_3) in black njavara (16.02 mg g^{-1} grain) and under treatment 40% shade (T_2) in yellow njavara (12.79 mg g^{-1} grain).

Flavonoid content in grain (mg g^{-1} grain)					
	V_1 (Black Njavara)	**% change over control**		**V_2 (Yellow Njavara)**	**% change over control**
T_1	17.40	3.82	**T_1**	16.78	26.36
T_2	16.11	-3.88	**T_2**	12.79	-3.69
T_3	16.02	-4.42	**T_3**	14.76	11.14
T_4	19.48	16.23	**T_4**	18.56	39.76
T_5	16.76	0.00	**T_5**	13.28	0.00
Mean	17.15		**Mean**	15.23	
	Variety		**Treatment**	**Var X Treat**	
SE(m)	0.297		0.470	0.665	
CD (0.05)	0.884		1.397	1.976	

Table 12: Effect of different shade levels and field capacity levels on total flavonoid content in grain (mg g^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara)

Flavonoid content in grains was found to significantly vary among the varieties and in which the variety black njavara (17.15 mg g^{-1} grain) recorded highest flavonoid content of grains than yellow njavara (15.23 mg g^{-1} grain).

4.2 EXPERIMENT-II

4.2.1 Physiological characters

4.2.1.1 Plant height

The data on plant height observed at four different stages of the crop showed no significant variation between the varieties and treatments (Table 13).

There was significant variation found between both the varieties; black njavara (V_1) and yellow njavara (V_2) at panicle initiation stage and harvesting stage. But at vegetative and flowering stages, plant height was found non-significant between varieties. At panicle initiation stage and harvesting stage, variety V_1 (107.16 and 120.58 cm respectively) recorded highest plant height than V_2 (103.10 and 115.29 cm respectively).

The plant height significantly varied under different treatments at all growth stages studied. The highest plant height was observed under control (T_4) (65.03, 109.53, 128.35 and 125.29 cm respectively) at all four growth stages studied. At vegetative stage, treatment T_2 (UV-B radiation from panicle initiation stage) recorded the lowest plant height (57.88 cm) whereas, at panicle initiation, flowering and harvesting stages, the treatment T_1 (UV-B radiation from vegetative stage) recorded the lowest plant height (95.84, 117.99 and 112.07 cm respectively).

4.2.1.2 Leaf Area Index (LAI)

The data on LAI was found to significantly vary between the different varieties and treatments only at panicle initiation stage. But at vegetative, flowering and

Plant height (cm)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	60.72	97.86	118.34	113.88	97.70	-10.85
V_1T_2	57.95	107.99	121.80	118.18	101.48	-7.40
V_1T_3	59.67	111.24	126.65	121.50	104.77	-4.40
V_1T_4	66.21	111.56	131.82	128.77	109.59	0.00
V_2T_1	56.69	93.83	117.48	110.27	94.57	-9.52
V_2T_2	57.81	106.42	120.76	113.91	99.73	-4.58
V_2T_3	58.04	104.63	126.12	115.15	100.99	-3.38
V_2T_4	63.857	107.51	124.88	121.82	104.52	0.00
SEm	**1.873**	**2.254**	**1.643**	**1.383**		
CD (0.05)	**N/A**	**N/A**	**N/A**	**N/A**		
V_1	61.14	107.16	124.65	120.58	103.38	
V_2	59.10	103.10	122.31	115.29	99.95	
SE(m)	**0.936**	**1.127**	**0.822**	**0.692**		
CD (0.05)	**N/A**	**3.407**	**N/A**	**2.091**		
T_1	58.71	95.84	117.99	112.07	96.15	
T_2	57.88	107.20	121.28	116.04	100.60	
T_3	58.85	107.93	126.39	118.32	102.87	
T_4	65.03	109.53	128.35	125.29	107.05	
SEm	**1.324**	**1.594**	**1.162**	**0.978**		
CD (0.05)	**4.004**	**4.819**	**3.514**	**2.958**		

Table 13: Effect of UV-B radiation on plant height (cm) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

Leaf Area Index (LAI)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	1.04	1.66	1.93	1.37	1.50	-57.98
V_1T_2	1.04	1.47	2.62	1.94	1.77	-50.42
V_1T_3	1.08	2.37	3.86	2.58	2.47	-30.81
V_1T_4	1.26	3.54	5.34	4.14	3.57	0.00
V_2T_1	0.72	0.98	1.25	1.09	1.01	-66.78
V_2T_2	0.62	1.60	2.19	1.59	1.50	-50.66
V_2T_3	0.76	1.87	3.76	2.11	2.13	-29.93
V_2T_4	0.69	2.55	5.13	3.79	3.04	0.00
SE(m)	**0.133**	**0.163**	**0.194**	**0.163**		
CD (0.05)	**N/A**	**0.494**	**N/A**	**N/A**		
V_1	1.11	2.26	3.44	2.51	2.33	
V_2	0.70	1.75	3.08	2.14	1.92	
SE(m)	**0.066**	**0.082**	**0.097**	**0.081**		
CD (0.05)	**0.201**	**0.247**	**0.293**	**0.246**		
T_1	0.88	1.32	1.59	1.23	1.26	
T_2	0.83	1.54	2.41	1.76	1.64	
T_3	0.92	2.12	3.81	2.34	2.30	
T_4	0.97	3.04	5.23	3.96	3.30	
SE(m)	**0.094**	**0.115**	**0.137**	**0.115**		
CD (0.05)	**N/A**	**0.349**	**0.414**	**0.348**		

Table 14: Effect of UV-B radiation on leaf area index at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

harvesting stages, LAI was found non-significant (Table 14). Both black njavara (3.54) and yellow njavara (2.55) recorded highest LAI under the control condition (T_4) mainly at the panicle initiation stage. However the lowest LAI was recorded under T_2 (UV-B treatment from panicle initiation stage) in black njavara (1.47) and under T_1 (UV-B treatment from vegetative stage) in yellow njavara (0.98) at the panicle initiation stage.

There was significant variation found among the varieties at vegetative, panicle initiation, flowering and harvesting stages (Table 14). Black njavara (V_1) recorded higher LAI (1.11, 2.26, 3.44 and 2.51) than the yellow njavara (V_2) (0.70, 1.75, 3.08 and 2.14 respectively) at all the four growth stages studied.

The LAI recorded among the treatments significantly varied at panicle initiation stage, flowering stage and harvesting stage but non-significant at vegetative stage. The control (T_4) recorded highest LAI at panicle initiation stage (3.04), flowering stage (5.23) and harvesting stage (3.96). Whereas, treatment T_1 (UV-B radiation from vegetative stage) recorded the lowest LAI (1.32, 1.59 and 1.23) at the above mentioned growth stages respectively.

4.2.1.3 Specific Leaf Area (SLA)

Specific leaf area (SLA) was found significantly different among the different treatments and varieties at all the growth stages studied (Table 15). The variety black njavara (V_1) recorded maximum SLA (364.29, 382.83, 347.77 and 407.47 $cm^2\ g^{-1}$) under control (T_4) at vegetative, panicle initiation, flowering and harvesting stage respectively. Whereas, the lowest SLA was recoded under treatment T_3 (UV-B treatment from flowering stage) at vegetative stage (273.38 $cm^2\ g^{-1}$). But at panicle initiation stage (255.18 $cm^2\ g^{-1}$) and harvesting stage (254.44 $cm^2\ g^{-1}$) the treatment T_1 (UV-B treatment from vegetative stage) recorded the least SLA and the treatment T_2 (UV-B treatment from panicle initiation stage) at flowering stage (229.63 $cm^2\ g^{-1}$) in black njavara. In yellow njavara (V_2), the control (T_4) recorded the highest SLA at

Specific Leaf Area (cm^2/g)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	298.31	255.18	234.94	254.44	260.72	-30.58
V_1T_2	276.43	272.11	229.63	270.59	262.19	-30.19
V_1T_3	273.38	302.19	280.42	315.39	292.85	-22.03
V_1T_4	364.29	382.83	347.77	407.47	375.59	0.00
V_2T_1	307.52	242.85	211.28	212.60	243.56	-26.47
V_2T_2	257.58	264.87	250.39	227.58	250.11	-24.50
V_2T_3	281.38	274.22	260.47	240.44	264.13	-20.27
V_2T_4	312.11	319.38	305.10	388.44	331.26	0.00
SE(m)	**3.371**	**2.061**	**9.200**	**2.064**		
CD (0.05)	**10.193**	**6.231**	**27.819**	**6.241**		
V_1	303.10	303.08	273.19	311.97	297.84	
V_2	289.65	275.33	256.81	267.27	272.27	
SE(m)	**1.686**	**1.030**	**4.600**	**1.032**		
CD (0.05)	**5.097**	**3.116**	**13.910**	**3.120**		
T_1	302.92	249.02	223.11	233.52	252.14	
T_2	267.01	268.49	240.01	249.09	256.15	
T_3	277.38	288.21	270.44	277.92	278.49	
T_4	338.20	351.10	326.44	397.95	353.42	
SE(m)	**2.384**	**1.457**	**6.505**	**1.459**		
CD (0.05)	**7.208**	**4.406**	**19.671**	**4.413**		

Table 15: Effect of UV-B radiation on specific leaf area (cm^2/g) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

vegetative, panicle initiation, flowering stage and harvesting stage (312.11, 319.38, 305.10 and 388.44 $cm^2 g^{-1}$respectively) and the lowest SLA was recorded under treatment T_2 at vegetative stage (257.58 $cm^2 g^{-1}$). But at panicle initiation, flowering and harvesting stages, the treatment T_1 recorded the lowest SLA (242.85, 211.28 and 212.60 $cm^2 g^{-1}$ respectively) in yellow njavara.

SLA was found to significantly vary between the varieties at vegetative, panicle initiation, flowering and harvesting stages (Table 15). The variety black njavara (V_1) recorded higher SLA (303.10, 303.08, 273.19 and 311.97 $cm^2 g^{-1}$) than yellow njavara (V_2) (289.65, 275.33, 256.81 and 267.27 $cm^2 g^{-1}$) at all the above mentioned growth stages respectively.

The SLA was observed to significantly vary among the different treatments studied (Table 15). Highest value for SLA was observed under control (T_4) at vegetative stage (338.20 $cm^2 g^{-1}$), panicle initiation stage (351.10 $cm^2 g^{-1}$), flowering stage (326.44 $cm^2 g^{-1}$) and harvesting stage (397.95 $cm^2 g^{-1}$). However the lowest value for SLA at vegetative stage, was recorded by the treatment T_2 (267.01 $cm^2 g^{-1}$) and at panicle initiation, flowering and harvesting stages, by the treatment T_1 (249.02, 223.11 and 233.52 $cm^2 g^{-1}$ respectively).

4.2.1.4 Number of Tillers

The data on number of tillers per plant observed at four different stages of the crop showed no significant variation between different varieties and treatments (Table 16).

Among the varieties, the number of tillers per plant was found to significantly vary at panicle initiation stage, flowering stage and harvesting stage and non-significant at vegetative stage (Table 16). Variety black njavara (V_1) recorded more number of tillers per plant (10.94, 18.22 and 16.69) at panicle initiation, flowering and

Number of Tillers (per plant)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	7.44	9.00	12.44	11.78	10.17	-39.17
V_1T_2	6.67	10.65	15.78	15.22	12.08	-27.75
V_1T_3	7.00	10.78	20.55	19.11	14.36	-14.11
V_1T_4	8.78	13.33	24.11	20.67	16.72	0.00
V_2T_1	7.00	8.67	10.78	9.78	9.06	-41.74
V_2T_2	6.67	9.64	14.22	12.89	10.86	-30.16
V_2T_3	6.78	9.67	20.67	19.00	14.03	-9.77
V_2T_4	7.55	12.553	22.00	20.11	15.55	0.00
SE(m)	**0.350**	**0.512**	**0.620**	**0.608**		
CD (0.05)	**N/A**	**N/A**	**N/A**	**N/A**		
V_1	7.47	10.94	18.22	16.69	13.33	
V_2	6.99	10.14	16.98	15.45	12.39	
SE(m)	**0.175**	**0.256**	**0.310**	**0.304**		
CD (0.05)	**N/A**	**0.774**	**0.937**	**0.920**		
T_1	7.22	8.83	11.61	10.78	9.61	
T_2	6.67	10.17	15.00	14.06	11.48	
T_3	6.89	10.22	20.61	19.06	14.20	
T_4	8.17	12.94	23.06	20.39	16.14	
SE(m)	**0.247**	**0.362**	**0.438**	**0.430**		
CD (0.05)	**0.748**	**1.095**	**1.325**	**1.301**		

Table 16: Effect of UV-B radiation on number of tillers per plant at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

harvesting stages than the yellow njavara (V_2) variety (14, 16.98 and 15.45) at the above mentioned growth stages respectively.

The number of tillers per plant was found to significantly vary among different treatments at all the growth stages studied (Table 16). Among the treatments, control (T_4) showed the highest number of tillers per plant (8.17, 12.94, 23.06 and 20.39 respectively) at all the growth stages studied. But the lowest number of tillers per plant (6.67) was recorded by the treatment T_2 at vegetative stage and by the treatment T_1 (8.83, 11.61 and 10.78) at panicle initiation, flowering and harvesting stages respectively.

4.2.1.5 Photosynthetic rate

The data on photosynthetic rate is given in table 17. Photosynthetic rate between varieties and treatments was found significant at panicle initiation stage and harvesting stage but non-significant at vegetative stage and flowering stage (Table 17). At panicle initiation stage and harvesting stage, both varieties *viz.*, black njavara (V_1) (21.80 and 20.73 μmol CO^2 $m^{-2}s^{-1}$ respectively) and yellow njavara (V_2) (19.27 and 14.97 μmol CO^2 $m^{-2}s^{-1}$ respectively) showed highest photosynthetic rate under control condition (T_5). However, the lowest photosynthetic rate was recorded under the treatment T_1 (UV-B treatment from vegetative stage) both in black njavara (12.17 and 11.63 μmol CO^2 $m^{-2}s^{-1}$) and in yellow njavara (10.80 and 10.67 μmol CO^2 $m^{-2}s^{-1}$) at the above mentioned growth stages respectively.

Photosynthetic rate was found significantly different at panicle initiation stage, flowering stage and harvesting stage but no significant variation was found at vegetative stage between the varieties. Black njavara (V_1) variety recorded higher photosynthetic rate at panicle initiation stage (16.22 μmol CO^2 $m^{-2}s^{-1}$), flowering stage (16.80 μmol CO^2 $m^{-2}s^{-1}$)and harvesting stage (14.38 μmol CO^2 $m^{-2}s^{-1}$) compared to the

Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	15.00	12.17	11.60	11.63	12.60	-38.75
V_1T_2	14.57	15.57	14.63	12.43	14.30	-30.48
V_1T_3	15.30	15.33	18.80	12.70	15.53	-24.50
V_1T_4	17.57	21.80	22.17	20.73	20.57	0.00
V_2T_1	19.57	10.80	10.57	10.67	12.90	-30.72
V_2T_2	15.87	16.43	12.23	12.40	14.23	-23.58
V_2T_3	17.00	14.93	17.13	12.80	15.47	-16.92
V_2T_4	19.30	19.27	20.93	14.97	18.62	0.00
SE(m)	**1.602**	**0.524**	**0.701**	**0.616**		
CD (0.05)	**N/A**	**1.586**	**N/A**	**1.863**		
V_1	15.61	16.22	16.80	14.38	15.75	
V_2	17.93	15.36	15.22	12.71	15.31	
SE(m)	**0.801**	**0.262**	**0.351**	**0.308**		
CD (0.05)	**N/A**	**0.793**	**1.060**	**0.931**		
T_1	17.28	11.48	11.08	11.15	12.75	
T_2	15.22	16.00	13.43	12.42	14.27	
T_3	16.15	15.13	17.97	12.75	15.50	
T_4	18.43	20.53	21.55	17.85	19.59	
SE(m)	**1.133**	**0.371**	**0.496**	**0.436**		
CD (0.05)	**N/A**	**1.121**	**1.499**	**1.317**		

Table 17: Effect of UV-B radiation on photosynthesis rate (µmol CO_2 m^{-2} s^{-1}) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

yellow njavara (V_2) variety (15.36, 15.22 and 12.71 µmol CO^2 $m^{-2}s^{-1}$) at the above mentioned growth stages respectively.

Photosynthetic rate between the treatments was found significantly different at panicle initiation stage, flowering stage and harvesting stage but no significant variation was found at vegetative stage (Table 17). Highest rate of photosynthesis was recorded in control (T_4) at different stages mentioned above (20.53, 21.55 and 17.85 µmol CO^2 $m^{-2}s^{-1}$ respectively) and the lowest was recorded in treatment T_1 (UV-B treatment from vegetative stage) at panicle initiation stage (11.48 µmol CO^2 $m^{-2}s^{-1}$), flowering stage (11.08 µmol CO^2 $m^{-2}s^{-1}$) and at harvesting stage (11.15 µmol CO^2 $m^{-2}s^{-1}$).

4.2.1.6 Stomatal conductance

Data on stomatal conductance is presented in table 18 and was found to significantly vary between different treatments and varieties studied during flowering and harvesting stages. But at vegetative and panicle initiation stages, stomatal conductance was found non-significant (Table 18). At flowering stage (V_1-420.33 mol H_2O $m^{-2}s^{-1}$; V_2-373.00 mol H_2O $m^{-2}s^{-1}$) and at harvesting stage (V_1-391.67 mol H_2O $m^{-2}s^{-1}$; V_2-371.33 mol H_2O $m^{-2}s^{-1}$) the control (T_4) recorded highest stomatal conductance in both the varieties. However the lowest stomatal conductance was recorded in treatment T_1 (UV-B radiation from vegetative stage) both in black njavara (V_1) and yellow njavara (V_2) at the flowering stage (259.67 and 243.00 mol H_2O $m^{-2}s^{-1}$respectively). But at harvesting stage, black njavara (V_1) recorded the lowest value under treatment T_1 (237.33 mol H_2O $m^{-2}s^{-1}$) and yellow njavara (V_2) under treatment T_2 (213.67 mol H_2O $m^{-2}s^{-1}$).

Stomatal conductance within varieties was observed to significantly vary at all the growth stages studied. Variety black njavara (V_1) (259.42, 338.83, 328.17 and 292.42 mol H_2O $m^{-2}s^{-1}$ respectively) recorded higher stomatal conductance at all the

Stomatal conductance (mol H_2O m^{-2} s^{-1})						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	239.67	291.67	259.67	237.33	257.09	-31.97
V_1T_2	238.33	325.00	265.00	266.67	273.75	-27.56
V_1T_3	272.00	326.67	367.67	274.00	310.09	-17.95
V_1T_4	287.67	412.00	420.33	391.67	377.92	0.00
V_2T_1	225.33	271.33	243.00	260.33	250.00	-27.73
V_2T_2	216.67	315.67	251.00	213.67	249.25	-27.95
V_2T_3	230.00	305.33	347.67	285.67	292.17	-15.54
V_2T_4	259.00	380.33	373.00	371.33	345.92	0.00
SE(m)	**8.038**	**3.851**	**2.183**	**1.940**		
CD (0.05)	**N/A**	**N/A**	**6.600**	**5.866**		
V_1	259.42	338.83	328.17	292.42	304.71	
V_2	232.75	318.17	303.67	282.75	284.34	
SE(m)	**4.019**	**1.926**	**1.091**	**0.970**		
CD (0.05)	**12.153**	**5.823**	**3.300**	**2.933**		
T_1	232.52	281.50	251.33	248.83	253.55	
T_2	227.54	320.33	258.00	240.17	261.51	
T_3	251.00	316.00	357.67	279.83	301.13	
T_4	273.37	396.17	396.67	381.50	361.93	
SE(m)	**5.684**	**2.723**	**1.543**	**1.372**		
CD (0.05)	**17.187**	**8.235**	**4.667**	**4.148**		

Table 18: Effect of UV-B radiation on stomatal conductance (mol H_2O m^{-2} s^{-1}) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

growth stages than the yellow njavara (V_2) (232.75, 318.17, 303.67 and 282.75 mol H_2O $m^{-2}s^{-1}$ respectively) at the above mentioned growth stages.

There was significant variation in stomatal conductance among different treatments at all the growth stages studied. At vegetative stage, flowering stage, panicle initiation stage and harvesting stages, the control (T_4) recorded highest stomatal conductance (273.37, 396.17, 396.67 and 381.50 mol H_2O $m^{-2}s^{-1}$ respectively). However, at vegetative stage and harvesting stage, the treatment T_2 recorded the lowest value of stomatal conductance (227.54 and 240.17 mol H_2O $m^{-2}s^{-1}$ respectively) whereas, at panicle initiation stage and flowering stage, lowest stomatal conductance was recorded under treatment T_1 (281.50 and 251.33 mol H_2O $m^{-2}s^{-1}$ respectively).

4.2.1.7 Transpiration rate

The data on transpiration rate is given in table 19. Transpiration rate between treatments and varieties was found to significantly vary at vegetative stage, panicle initiation stage and harvesting stage but non-significant at flowering stage (Table 19). In black njavara (V_1), control (T_4) recorded the highest transpiration rate at vegetative stage (4.55 mmol H_2O $m^{-2}s^{-1}$), panicle initiation stage (4.38 mmol H_2O $m^{-2}s^{-1}$) and harvesting stage (3.96 mmol H_2O $m^{-2}s^{-1}$). But the lowest transpiration rate (4.04, 2.32 and 1.46 mmol H_2O $m^{-2}s^{-1}$) was recorded in treatment T_1 (UV-B treatment from vegetative stage) at the above mentioned growth stages respectively. In case of yellow njavara (V_2) the highest transpiration rate was recorded in treatment T_2 (UV-B treatment from panicle initiation stage) at vegetative stage (4.01 mmol H_2O $m^{-2}s^{-1}$) and at panicle initiation stage (4.12 mmol H_2O $m^{-2}s^{-1}$) but in the control (T_4) at harvesting stage (3.90 mmol H_2O $m^{-2}s^{-1}$). However the lowest rate of transpiration was recorded under control (T_4) at vegetative stage (3.81 mmol H_2O $m^{-2}s^{-1}$) and under the treatment T_1 at panicle initiation stage (2.20mmol H_2O $m^{-2}s^{-1}$) and at harvesting stage (1.45 mmol H_2O $m^{-2}s^{-1}$).

Transpiration rate (mmol H_2O m^{-2} s^{-1})						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	4.04	2.32	2.41	1.46	2.56	-41.95
V_1T_2	4.39	3.70	2.52	1.98	3.15	-28.57
V_1T_3	4.11	3.52	3.85	2.07	3.39	-23.13
V_1T_4	4.55	4.38	4.74	3.96	4.41	0.00
V_2T_1	3.97	2.20	2.36	1.45	2.50	-38.57
V_2T_2	4.01	3.32	2.40	1.57	2.83	-30.47
V_2T_3	4.01	3.87	3.71	2.07	3.42	-15.97
V_2T_4	3.81	4.12	4.43	3.90	4.07	0.00
SE(m)	**0.113**	**0.094**	**0.079**	**0.037**		
CD (0.05)	**0.340**	**0.284**	**N/A**	**0.111**		
V_1	4.27	3.48	3.38	2.37	3.38	
V_2	3.95	3.38	3.22	2.25	3.20	
SE(m)	**0.056**	**0.047**	**0.039**	**0.018**		
CD (0.05)	**0.170**	**N/A**	**0.119**	**0.056**		
T_1	4.00	2.26	2.39	1.46	2.53	
T_2	4.20	3.51	2.46	1.77	2.99	
T_3	4.06	3.69	3.78	2.07	3.40	
T_4	4.18	4.25	4.59	3.93	4.24	
SE(m)	**0.080**	**0.066**	**0.056**	**0.026**		
CD (0.05)	**N/A**	**0.201**	**0.169**	**0.079**		

Table 19: Effect of UV-B radiation on transpiration rate (mmol H_2O m^{-2} s^{-1}) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

Both the varieties showed significant variation at vegetative, flowering and harvesting stage but was non-significant at panicle initiation stage. Black njavara (V_1) recorded higher rate of transpiration (4.27, 3.38 and 2.37 mmol H_2O $m^{-2}s^{-1}$) than the yellow njavara (V_2) (3.95, 3.22 and 2.25mmol H_2O $m^{-2}s^{-1}$) at the above mentioned growth stages respectively.

Transpiration rate recorded among the treatments significantly varied at panicle initiation stage, flowering stage and harvesting stage. However, at vegetative stage it was found non-significant. Highest transpiration rate was recorded under control (T_4) at panicle initiation, flowering and harvesting stage (4.25, 4.59 and 3.93 mmol H_2O $m^{-2}s^{-1}$ respectively). But the lowest transpiration rate (2.26, 2.39 and 1.46 mmol H_2O $m^{-2}s^{-1}$) was recorded in treatment T_1 (UV-B treatment from vegetative stage) at the above mentioned growth stages respectively.

4.2.2 Biochemical characters

4.2.2.1 Flavonoid content in leaves

The data on total flavonoid content in leaves is presented in table 20. Flavonoid content was observed to significantly vary at vegetative stage, flowering stage and harvesting stage, but non-significant at panicle initiation stage both between varieties and treatments (Table 20). In black njavara (V_1), the treatment T_3 (UV-B treatment from flowering stage) recorded the highest flavonoid content at vegetative stage (14.90 mg g^{-1} FW) whereas, at flowering and harvesting stages, treatment T_1 (UV-B treatment from vegetative stage) recorded the highest flavonoid content (19.97 and 19.27 mg g^{-1} FW respectively). But the lowest flavonoid content was recorded in control (T_4) at vegetative stage (11.31 mg g^{-1} FW), flowering stage (13.41 mg g^{-1} FW) and harvesting stage (15.47 mg g^{-1} FW) in black njavara (V_1). In the case of yellow njavara (V_2), highest flavonoid content was recorded by the treatment T_3 at vegetative stage (13.07 mg g^{-1} FW), by the treatment T_1 at flowering stage (18.08 mg g^{-1} FW) and by the

Flavonoid content (mg g^{-1}FW)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	13.62	17.13	19.97	19.27	17.50	31.68
V_1T_2	14.48	14.42	17.46	18.75	16.28	22.50
V_1T_3	14.90	14.55	13.59	17.75	15.20	14.37
V_1T_4	11.31	12.95	13.41	15.47	13.29	0.00
V_2T_1	13.01	16.90	18.08	18.68	16.67	36.75
V_2T_2	12.56	13.27	17.30	18.80	15.48	26.99
V_2T_3	13.07	13.66	14.41	17.54	14.67	20.34
V_2T_4	11.47	12.21	12.44	12.65	12.19	0.00
SE(m)	**0.346**	**0.289**	**0.348**	**0.287**		
CD (0.05)	**1.047**	**N/A**	**1.053**	**0.868**		
V_1	13.58	14.76	16.11	17.81	15.57	
V_2	12.53	14.01	15.56	16.92	14.76	
SE(m)	**0.173**	**0.145**	**0.174**	**0.144**		
CD (0.05)	**0.523**	**0.437**	**0.526**	**0.434**		
T_1	13.31	17.02	17.38	18.98	16.67	
T_2	13.52	13.85	19.03	18.78	16.30	
T_3	13.98	14.11	14.00	17.64	14.93	
T_4	11.39	12.58	12.92	14.06	12.74	
SE(m)	**0.245**	**0.205**	**0.246**	**0.203**		
CD (0.05)	**0.740**	**0.618**	**0.744**	**0.614**		

Table 20: Effect of UV-B radiation on flavonoid content (mg g^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

treatment T_2 at harvesting stage (18.80 mg g^{-1} FW). However the lowest flavonoid content (11.47, 12.44 and 12.65 mg g^{-1} FW) was recorded in the control (T_4) at the above mentioned growth stages respectively.

The flavonoid content within the varieties was found to significantly vary at all the growth stages studied. Black njavara (V_1) recorded highest content of flavonoid at vegetative stage (13.58 mg g^{-1} FW), panicle initiation stage (14.76 mg g^{-1} FW), flowering stage (16.11 mg g^{-1} FW) and at harvesting stage (17.81 mg g^{-1} FW). However the lowest values were recorded for yellow njavara (V_2) at the above mentioned growth stages (12.53, 14.01, 15.56 and 16.92 mg g^{-1} FW respectively).

Flavonoid content under different treatments were found to significantly vary at all the growth stages studied. Treatment T_3, recorded highest flavonoid content at the vegetative stage (13.98 mg g^{-1} FW) whereas, at panicle initiation stage and harvesting stage the treatment T_1 (17.02 and 18.98 mg g^{-1} FW respectively) recorded the highest flavonoid content. However, at flowering stage, the treatment T_2 (19.03 mg g^{-1} FW) recorded the highest flavonoid content. But the lowest flavonoid content was observed in treatment T_4 (11.39, 12.58, 12.92 and 14.06 mg g^{-1} FW respectively) at the different growth stages studied.

4.2.2.2 Phenol content

The data on phenol content is presented in table 21. Phenol content between varieties and treatments was significantly different only at panicle initiation stage. Both the varieties showed highest content of phenol in the treatment T_1 (UV-B treatment from vegetative stage) (V_1-1.89 mg g^{-1} FW; V_2-1.55 mg g^{-1} FW) and the lowest phenol content was observed in the control (T_4) (V_1-0.69 mg g^{-1} FW; V_2-0.76 mg g^{-1} FW) at panicle initiation stage.

Both the varieties showed significant variation in phenol content at all the stages of growth studied. Black njavara (V_1) recorded higher phenol content (0.86,

Phenol content (mg g^{-1} FW)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	0.92	1.89	2.92	3.42	2.29	146.24
V_1T_2	0.84	0.96	1.87	3.09	1.69	81.72
V_1T_3	0.86	1.02	1.17	2.61	1.42	52.69
V_1T_4	0.83	0.69	1.00	1.20	0.93	0.00
V_2T_1	0.83	1.55	2.52	3.14	2.01	125.84
V_2T_2	0.79	1.01	1.45	2.78	1.51	69.66
V_2T_3	0.78	0.91	1.11	2.47	1.32	48.31
V_2T_4	0.68	0.76	0.97	1.13	0.89	0.00
SE(m)	**0.053**	**0.052**	**0.102**	**0.098**		
CD (0.05)	**N/A**	**0.158**	**N/A**	**N/A**		
V_1	0.86	1.14	1.74	2.58	1.58	
V_2	0.77	1.06	1.51	2.38	1.43	
SE(m)	**0.026**	**0.026**	**0.051**	**0.049**		
CD (0.05)	**0.079**	**0.079**	**0.154**	**0.148**		
T_1	0.87	1.72	1.66	3.28	1.88	
T_2	0.82	0.99	2.72	2.94	1.87	
T_3	0.82	0.96	1.14	2.54	1.37	
T_4	0.76	0.73	0.99	1.17	0.91	
SE(m)	**0.037**	**0.037**	**0.072**	**0.069**		
CD (0.05)	**N/A**	**0.112**	**0.218**	**0.209**		

Table 21: Effect of UV-B radiation on phenol content (mg g^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

1.14, 1.74 and 2.58 mg g^{-1} FW respectively) than the yellow njavara (V_2) (0.77, 1.06, 1.51 and 2.38 mg g^{-1} FW respectively) at all the growth stages studied.

Phenol content under different treatments were found to significantly vary at panicle initiation, flowering and harvesting stage. But at vegetative stage, the phenol content was found non-significant among the treatments. Treatment T_1 recorded highest phenol content at panicle initiation stage (1.72 mg g^{-1} FW) and harvesting stage (3.28 mg g^{-1} FW). However at flowering stage, treatment T_2 (2.72 mg g^{-1} FW) recorded highest content of phenol. The lowest phenol content was observed under treatment T_4 at panicle initiation, flowering and harvesting stages (0.73, 0.99 and 1.17 mg g^{-1} FW respectively).

4.2.2.3 Chlorophyll content

a) Chlorophyll 'a'

The data on chlorophyll 'a' observed among different varieties and treatments was found significant at panicle initiation stage, flowering stage and harvesting stage but non-significant at vegetative stage (Table 22). The control (T_4) recorded highest chlorophyll 'a' in both the varieties at panicle initiation stage (V_1-2.70 mg g^{-1} FW; V_2-2.68 mg g^{-1} FW), flowering stage (V_1-2.69 mg g^{-1} FW; V_2-2.05 mg g^{-1} FW) and harvesting stage (V_1-2.57 mg g^{-1} FW; V_2-1.77 mg g^{-1} FW). Black njavara (V_1) recorded lowest chlorophyll 'a' content in treatment T_1 (UV-B treatment from vegetative stage) at panicle initiation stage (2.28 mg g^{-1} FW), flowering stage (1.30 mg g^{-1} FW) and harvesting stage (1.12 mg g^{-1} FW). Similarly yellow njavara (V_2) also recorded lowest chlorophyll 'a' content in the treatment T_1 (1.76, 1.13 and 1.21 mg g^{-1} FW respectively) at the above mentioned growth stages.

Chlorophyll 'a' was found significantly different during panicle initiation stage, flowering stage and harvesting stage whereas, at vegetative stage it was found non-significant between varieties. The variety black njavara (V_1) recorded higher value of

chlorophyll ‘a’ at panicle initiation stage (2.55 mg g^{-1} FW), flowering stage (1.80 mg g^{-1} FW) and harvesting stage (1.56 mg g^{-1} FW) than the yellow njavara (V_2) (2.14, 1.61 and 1.38 mg g^{-1} FW) at those growth stages.

There was significant variation in chlorophyll ‘a’ content among different treatments at panicle initiation stage, flowering stage and harvesting stage whereas, at vegetative stage it was found non-significant. Treatment T_4 recorded highest chlorophyll ‘a’ content at the above mentioned growth stages (2.69, 2.37 and 2.17 mg g^{-1} FW respectively). But the lowest chlorophyll ‘a’ content was recorded in the treatment T_1 (2.02, 1.22 and 1.16 mg g^{-1} FW respectively) at those growth stages.

b) Chlorophyll ‘b’

Among the varieties and treatments, chlorophyll ‘b’ was found significant only at flowering stage (Table 22). The control (T_4) recorded highest content of chlorophyll ‘b’ (1.50 mg g^{-1} FW) and the treatment T_1 (UV-B treatment from vegetative stage) recorded the lowest chlorophyll ‘b’ content (0.78 mg g^{-1} FW) in black njavara at the flowering stage. But, the treatment T_1 recorded the highest chlorophyll ‘b’ content (1.14 mg g^{-1} FW) and the treatment T_3 (UV-B treatment from flowering stage) recorded the lowest chlorophyll ‘b’ content (0.89 mg g^{-1} FW) in yellow njavara at flowering stage.

There was no significant variation observed between both varieties with regard to chlorophyll ‘b’ content

Chlorophyll ‘b’ content was found to significantly vary at panicle initiation stage, flowering stage and harvesting stage and at vegetative stage it was found non-significant. At panicle initiation stage, the treatment T_2 recorded highest chlorophyll ‘b’ content (1.02 mg g^{-1} FW) whereas, at flowering and harvesting stages the treatment T_4 recorded the highest chlorophyll ‘b’ content (1.29 and 1.03 mg g^{-1} FW respectively). However, lowest chlorophyll ‘b’ content was recorded under treatment T_1 at panicle

Chlorophyll content (mg g^{-1} FW)												
	Vegetative stage			**Panicle initiation stage**			**Flowering stage**			**Harvesting stage**		
	Chl 'a'	**Chl 'b'**	**Total chl**	**Chl 'a'**	**Chl 'b'**	**Total chl**	**Chl 'a'**	**Chl 'b'**	**Total chl**	**Chl 'a'**	**Chl 'b'**	**Total chl**
V_1T_1	0.93	0.53	1.46	2.28	0.57	2.85	1.30	0.78	2.08	1.12	0.27	1.39
V_1T_2	0.91	0.49	1.40	2.62	1.11	3.72	1.42	0.90	2.32	1.21	0.31	1.52
V_1T_3	1.01	0.57	1.58	2.61	0.74	3.35	1.80	0.95	2.75	1.34	0.35	1.69
V_1T_4	0.98	0.45	1.43	2.70	0.87	3.57	2.69	1.50	4.18	2.57	1.14	3.71
V_2T_1	0.96	0.55	1.51	1.76	0.65	2.41	1.13	1.14	2.27	1.21	0.34	1.55
V_2T_2	0.96	0.57	1.53	2.37	0.94	3.30	1.68	0.96	2.64	1.23	0.31	1.54
V_2T_3	0.86	0.50	1.36	1.77	0.56	2.33	1.58	0.89	2.47	1.31	0.47	1.78
V_2T_4	1.03	0.64	1.67	2.68	0.82	3.50	2.05	1.09	3.13	1.77	0.93	2.69
SE (m)	**0.098**	**0.080**	**0.171**	**0.134**	**0.148**	**0.220**	**0.078**	**0.115**	**0.160**	**0.097**	**0.077**	**0.101**
CD (0.05)	**N/A**	**N/A**	**N/A**	**0.406**	**N/A**	**N/A**	**0.236**	**0.347**	**0.483**	**0.293**	**N/A**	**0.306**
V_1	0.96	0.51	1.47	2.55	0.82	3.37	1.80	1.03	2.83	1.56	0.52	2.08
V_2	0.95	0.57	1.52	2.14	0.74	2.88	1.61	1.02	2.63	1.38	0.51	1.89
SE (m)	**0.049**	**0.040**	**0.085**	**0.067**	**0.074**	**0.110**	**0.039**	**0.057**	**0.080**	**0.048**	**0.038**	**0.051**
CD (0.05)	**N/A**	**N/A**	**N/A**	**0.203**	**N/A**	**0.332**	**0.118**	**N/A**	**N/A**	**0.146**	**N/A**	**0.153**
T_1	0.95	0.54	1.49	2.02	0.61	2.63	1.22	0.96	2.18	1.16	0.31	1.47
T_2	0.94	0.53	1.47	2.49	1.02	3.51	1.55	0.93	2.48	1.22	0.32	1.53
T_3	0.93	0.54	1.47	2.19	0.65	2.84	1.69	0.92	2.61	1.33	0.41	1.74
T_4	1.01	0.54	1.55	2.69	0.84	3.53	2.37	1.29	3.66	2.17	1.03	3.20
SE (m)	**0.070**	**0.057**	**0.121**	**0.095**	**0.104**	**0.155**	**0.055**	**0.081**	**0.113**	**0.069**	**0.054**	**0.072**
CD (0.05)	**N/A**	**N/A**	**N/A**	**0.287**	**0.316**	**0.470**	**0.167**	**0.245**	**0.342**	**0.207**	**0.165**	**0.216**

Table 22: Effect of UV-B radiation on chlorophyll content (mg g^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

initiation stage (0.61 mg g^{-1} FW) and at harvesting stage (0.31 mg g^{-1} FW). But at flowering stage, the treatment T_3 (0.92 mg g^{-1} FW) recorded lowest chlorophyll 'b' content.

c) Total chlorophyll

The data on total chlorophyll is given in table 22. Total chlorophyll content was observed significantly different between the varieties and treatments only at flowering and harvesting stage. Black njavara (V_1) showed highest value for total chlorophyll content under control (T_4) at flowering stage (4.18 mg g^{-1} FW) and at harvesting stage (3.71 mg g^{-1} FW). However in black njavara, lowest total chlorophyll content (2.08 and 1.39 mg g^{-1}) was recorded under treatment T_1 (UV-B treatment from vegetative stage) at the above mentioned growth stages respectively. But yellow njavara (V_2) recorded the highest total chlorophyll content in control (T_4) at flowering and harvesting stage (3.13 and 2.69 mg g^{-1} FW respectively). But the lowest total chlorophyll content was recorded under the treatment T_1 at flowering stage (2.27 mg g^{-1} FW) and under T_2 at harvesting stage (1.54 mg g^{-1} FW).

At varietal level, total chlorophyll content was found significant only at panicle initiation and harvesting stage. However at vegetative and flowering stages, no significant variation was observed. At panicle initiation stage (3.37 mg g^{-1} FW) and harvesting stage (2.08 mg g^{-1} FW), the variety black njavara (V_2) recorded highest content of total chlorophyll and yellow njavara (V_2) recorded the lowest value (2.88 mg g^{-1} FW and 1.89 mg g^{-1} FW) at panicle initiation and harvesting stage respectively.

Total chlorophyll was found to vary significantly among the treatments at panicle initiation stage, flowering stage and harvesting stage. Treatment T_4 (control) showed the highest values for total chlorophyll content at the above mentioned growth stages (3.53, 3.66 and 3.20 mg g^{-1} FW respectively). However, treatment T_1 (UV-B treatment from vegetative stage) recorded the least total chlorophyll content at panicle

initiation stage (2.63 mg g^{-1} FW), flowering stage (2.18 mg g^{-1} FW) and at harvesting stage (1.47 mg g^{-1} FW).

4.2.2.4 Proline content

The proline content between varieties and treatments was found significantly different only at flowering stage. However, no significant difference was found at vegetative stage, panicle initiation stage and at harvesting stage (Table 23). At flowering stage, both black njavara (V_1) and yellow njavara (V_2) recorded highest proline content in the treatment T_1 (UV-B treatment from vegetative stage) (V_1-0.433 μg^{-1} FW; V_2-0.481 μg^{-1} FW). But the control (T_4) recorded lowest proline content at flowering stage in both the varieties (V_1-0.194 μg^{-1} FW; V_2-0.207 μg^{-1} FW).

The data on proline content is found to significantly vary between different varieties at all the growth stages studied. Among the different rice varieties, yellow njavara recorded higher proline content at vegetative, panicle initiation stage, flowering stage and harvesting stage (0.136, 0.230, 0.354 and 0.341 μg^{-1} FW respectively) than the black njavara (0.122, 0.211, 0.325 and 0.331 μg^{-1} FW respectively) at the above mentioned growth stages.

Between the different treatments, T_4 (0.138 μg^{-1} FW) recorded significantly higher proline content than other treatments at vegetative stage. Whereas, treatment T_1 recorded higher proline content at panicle initiation stage, flowering stage and harvesting stage (0.319, 0.457 and 0.415 μg^{-1} FW respectively). Lowest proline content was recorded under treatment T_1 at vegetative stage (0.119 μg^{-1} FW). But at panicle initiation, flowering and harvesting stages, lowest proline content was recorded under treatment T_4 (0.174, 0.201 and 0.192 μg^{-1} FW respectively).

Proline content (µg^{-1} FW)						
	Vegetative stage	**Panicle initiation stage**	**Flowering stage**	**Harvesting stage**	**Mean**	**% change over control**
V_1T_1	0.114	0.305	0.433	0.412	0.316	115.71
V_1T_2	0.126	0.188	0.390	0.366	0.268	91.62
V_1T_3	0.123	0.190	0.285	0.357	0.239	86.91
V_1T_4	0.124	0.160	0.194	0.191	0.167	0.00
V_2T_1	0.125	0.332	0.481	0.418	0.339	117.71
V_2T_2	0.146	0.201	0.431	0.383	0.290	99.48
V_2T_3	0.119	0.199	0.296	0.369	0.246	92.19
V_2T_4	0.152	0.188	0.207	0.192	0.185	0.00
SE(m)	**0.007**	**0.012**	**0.003**	**0.006**		
CD (0.05)	**N/A**	**N/A**	**0.011**	**N/A**		
V_1	0.122	0.211	0.325	0.331	0.247	
V_2	0.136	0.230	0.354	0.341	0.265	
SE(m)	**0.011**	**0.006**	**0.002**	**0.003**		
CD (0.05)	**0.004**	**0.018**	**0.005**	**0.009**		
T_1	0.119	0.319	0.457	0.415	0.328	
T_2	0.136	0.195	0.411	0.374	0.279	
T_3	0.121	0.195	0.291	0.363	0.243	
T_4	0.138	0.174	0.201	0.192	0.176	
SE(m)	**0.005**	**0.008**	**0.002**	**0.004**		
CD (0.05)	**0.015**	**0.025**	**0.007**	**0.013**		

Table 23: Effect of UV-B radiation on proline content (µg^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

4.2.2.5 Flavonoid content in Grains

The data on flavonoid content in grains is shown in table 24. Flavonoid content in grains between varieties and treatments was found to vary significantly. In both the varieties *viz.*, black njavara and yellow njavara, highest flavonoid content in grains was recorded under treatment T_2 (UV-B treatment from panicle initiation stage) (V_1-28.74 mg g^{-1} grain and V_2-26.91 mg g^{-1} grain). However, the least flavonoid content in grains was recorded under control (T_4) in V_1 (22.63 mg g^{-1} grain) and V_2 (21.96 mg g^{-1} grain).

Flavonoid content in grain (mg g^{-1})					
	V_1 (Black Njavara)	**% change over control**		**V_2 (Yellow Njavara)**	**% change over control**
T_1	23.04	1.81	**T_1**	23.70	7.92
T_2	28.74	27.00	**T_2**	26.91	22.54
T_3	25.09	10.87	**T_3**	23.70	7.92
T_4	22.63	0.00	**T_4**	21.96	0.00
Mean	24.88		**Mean**	24.07	
	Variety		**Treatment**	**Var X Treat**	
SE(m)	0.244		0.345	0.488	
CD (0.05)	0.738		1.044	1.477	

Table 24: Effect of UV-B radiation on total flavonoid content in grain (mg g^{-1}) of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

Flavonoid content in grains was found to significantly vary among the varieties, in which variety black njavara (24.88 mg g^{-1} grain) recorded higher flavonoid content in grains than yellow njavara (24.07 mg g^{-1} grain).

4.3 EXPERIMENT-III

The treatments which resulted in the highest accumulation of flavonoid content in grains were selected from experiment 1 (20% shade and 75% field capacity) and experiment 2 (UV-B radiation treatment from panicle initiation stage) for molecular analysis of both the varieties in experiment 3 (Table 12 and 24). The protein profiling and gene expression studies were carried out during grain filling stage of the crop.

4.3.1 Protein profiling

Protein profiling was done by sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) using leaves of both varieties at grain filling stage of the crop. SDS-PAGE mediated protein profiling showed differential expression of proteins under different treatments as well as varieties (Plate 7).

The protein profile showed variation in the intensity of 55 kDa and 16 kDa polypeptides between different treatments and varieties. Those polypeptides corresponding to large subunit and small subunit of RuBisCO were present in all the treatments in both the varieties. However, the intensity of those bands were found higher in 20% shade (T_1), 75% field capacity (T_2) and control (T_4) whereas, under UV-B radiation treatment from panicle initiation stage (T_3) relatively lesser intensity was exhibited in both the varieties. Among the varieties, black njavara (V_1) exhibited more intense bands of 55 kDa and 16 kDa polypeptides than the yellow njavara (V_2) in all the treatments. The present study showed absence of bands between 55-48 kDa and 25-17 kDa in both varieties under under UV-B radiation treatment from panicle initiation stage (T_3).

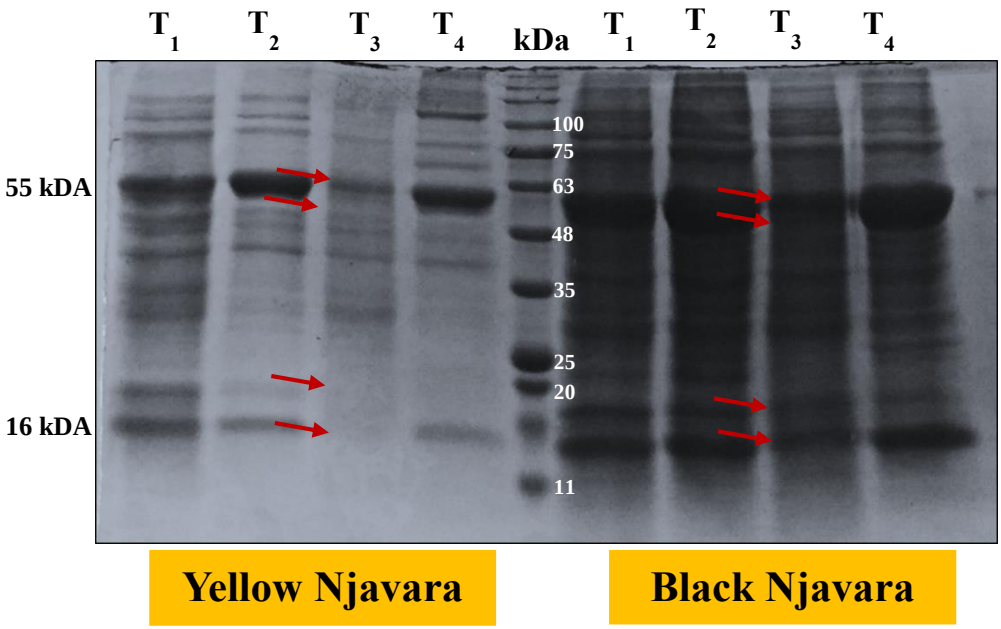


Plate 7: SDS-PAGE protein profile of the two njavara rice varieties under different treatments (T_1 -20% Shade; T_2 -75% field capacity; T_3 - UV-B treatment from panicle initiation stage; T_4 - Control)

4.3.2 Gene expression study using RT-PCR

Gene expression analysis using quantitative real-time PCR (qRT-PCR) was carried out in grains collected during grain filling stage of the crop. Two genes from flavonoid biosynthetic pathway were selected for the present study *viz.*, chalcone synthase (*CHS*) and *CYP75B4* (flavanone 3-hydroxylase family) along with Ubiquitin (*UBQ5*) gene for internal reference.

4.3.2.1 Isolation of RNA

Total RNA was isolated from grains of both the varieties during grain filling stage of the crop raised under four different conditions *viz.*, T_1 (20% shade), T_2 (75% field capacity), T_3 (UV-B treatment from panicle initiation stage) and T_4 (control). Two distinct intact bands at 28S and 18S of rRNA with no apparent degradation were observed on agarose gel (1.5%) (Plate 8).

4.3.2.2 Quality and quantity of isolated RNA

The quantification of RNA was determined by spectrophotometric method. The good quality of RNA are referred by A_{260}/A_{280} value between 1.8 and 2. In the present study good quality of RNA was obtained (Table 25).

4.3.2.3 Preparation and Quality check of cDNA

In the present study cDNA was synthesized using "Thermo Scientific Verso cDNA Synthesis Kit" following the protocol provided by manufacturers. The cDNA synthesis was confirmed by standard PCR technique using housekeeping gene *UBQ5* with gene specific primer. The amplicon of expected size (100 bp) was obtained indicating good quality of cDNA synthesized (Plate 9).

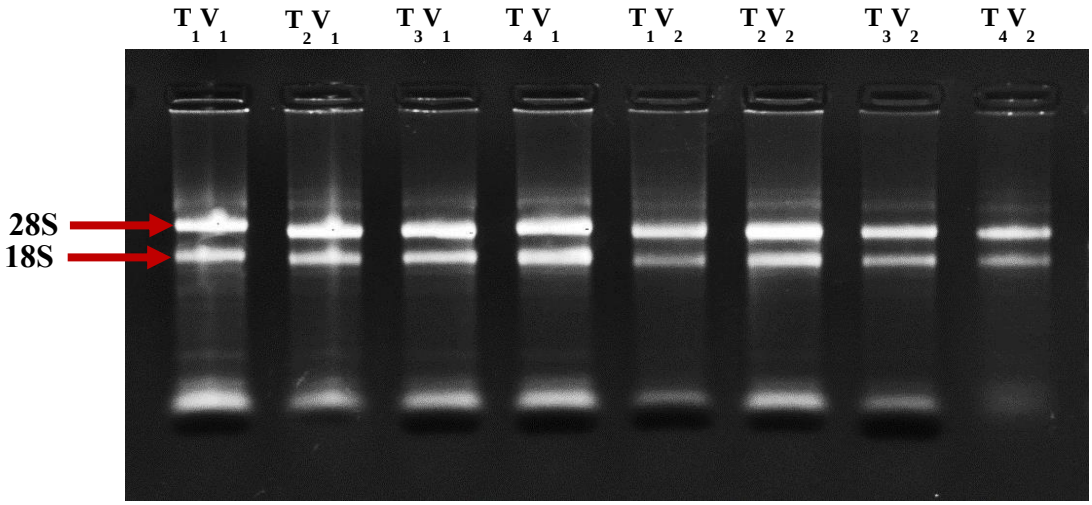


Plate 8: Electrophoresis of total RNA isolated from Black and Yellow njavara varieties (T_1-20% Shade; T_2-75% field capacity; T_3-UV-B treatment from panicle initiation stage; T_4-Control; V_1-Black njavara; V_2-Yellow njavara)

		A_{260}	A_{280}	A_{260}/A_{280} Ratio	Concentration (µg/ml)
Black Njavara	T_1V_1	0.025	0.012	2.1	0.60
	T_2V_1	0.031	0.016	1.9	0.74
	T_3V_1	0.029	0.014	2.1	0.70
	T_4V_1	0.041	0.021	2.0	0.98
Yellow Njavara	T_1V_2	0.027	0.014	1.9	0.65
	T_2V_2	0.035	0.019	1.8	0.84
	T_3V_2	0.019	0.010	1.9	0.46
	T_4V_2	0.034	0.018	1.9	0.82

Table 25: Quality and quantity of total RNA extracted from two njavara medicinal rice grains under different treatments (T_1- 20% Shade; T_2- 75% field capacity; T_3- UV-B treatment from panicle initiation stage; T_4- Control; V_1- Black njavara; V_2- Yellow njavara)

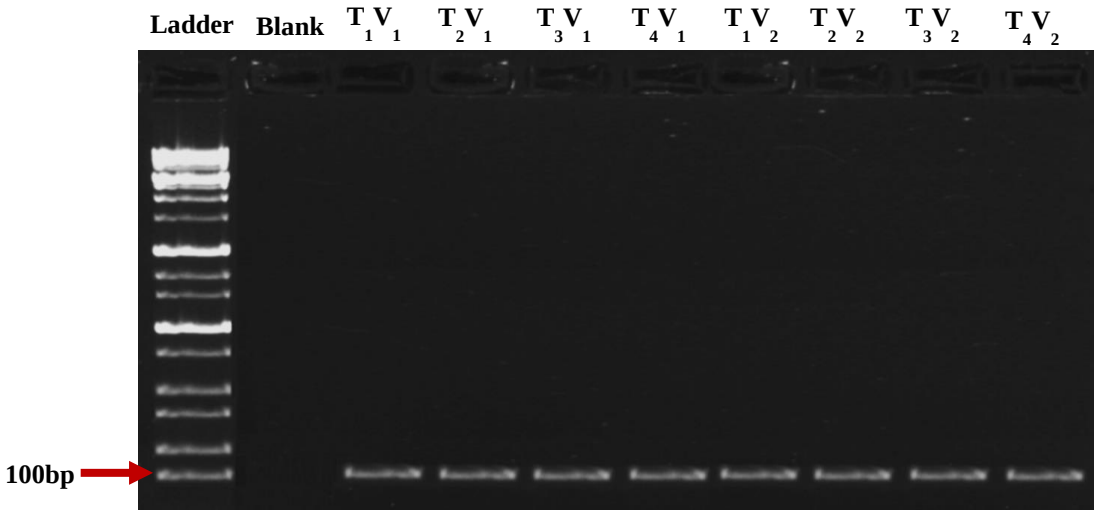


Plate 9: Gel profile of the amplicon from cDNA using *UBQ5* specific primers (Ladder-100 bp; T_1-20% Shade; T_2-75% field capacity; T_3-UV-B treatment from panicle initiation stage; T_4-Control; V_1-Black njavara; V_2-Yellow njavara)

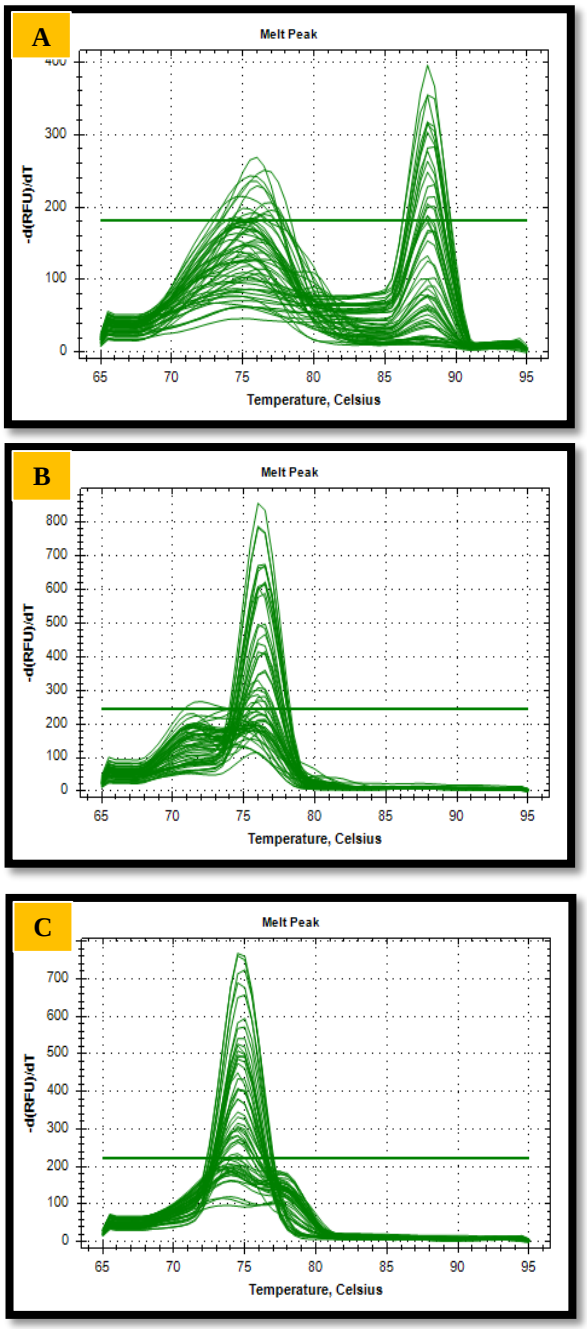


Plate 10: Melt curve analysis under different conditions by qRT-PCR (A-*CHS*; B-*CYP75B4*; C- *UBQ5*)

4.3.2.4 Analysis of quantitative real-time PCR (qRT-PCR)

The cDNA synthesized (from different treatments of both varieties) were subjected to qRT-PCR using primers with *CHS* and *CYP75B4* genes along with *UBQ5* as reference gene. The melting curve of all three genes have shown single peak obtained from the three technical replications in different treatments and varieties (Plate 10).

Differential relative expression of both query genes in grains were seen under different treatments and in both varieties. In the black njavara (V_1), relative expression of *CHS* gene was found higher under UV-B treatment from panicle initiation stage (T_3) by 2.93 fold, followed by 75% field capacity (T_2) by 1.82 fold compared to control (T_4) (Figure 2). Whereas, treatment 20% shade (T_1) recorded low relative expression of *CHS* by 0.15 fold compared to control. Relative expression of *CYP75B4* was seen up-regulated in T_2 by 3.60 fold followed by T_3 by 2.18 fold compared to control in black njavara. Treatment T_1 recorded down-regulation of *CYP75B4* by 0.18 fold compared to control (Figure 2).

In the yellow njavara (V_2) relative expression of *CHS* and *CYP75B4* were found higher in treatment T_3 by 2.46 and 2.01 fold respectively, followed by treatment T_2 in which, relative expression was 0.58 and 0.90 fold for *CHA* and *CYP75B4* respectively compared to control (Figure 3). Under treatment T_1, relative expression was lower for both query genes, *CHS* (0.32 fold) and *CYP75B4* (0.76 fold) compared to control (Figure 3).

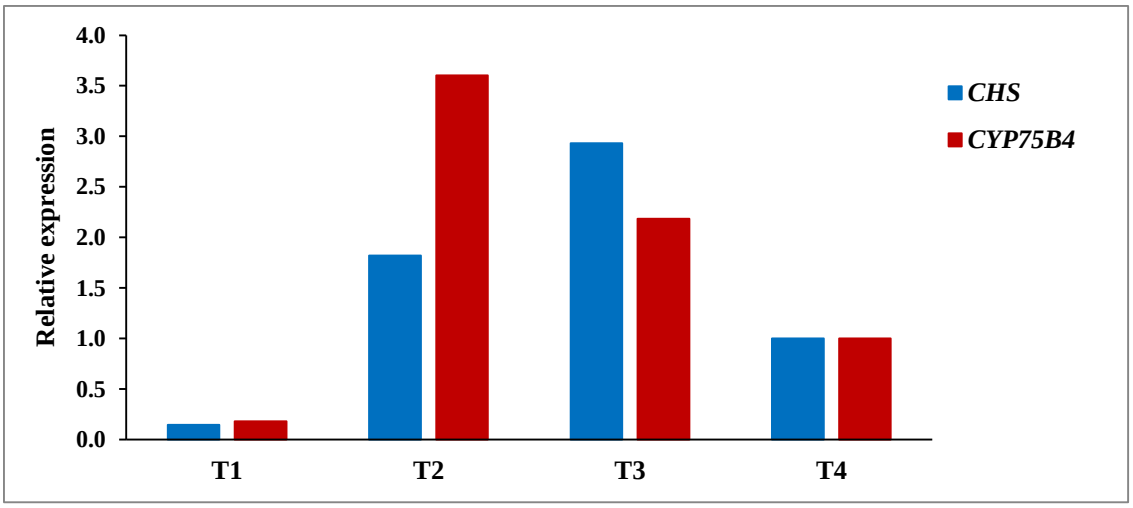


Figure 2: Relative expression of *CHS* and *CYP75B4* under different conditions in black njavara variety (T_1- 20% Shade; T_2-75% field capacity; T_3-UV-B treatment from panicle initiation stage; T_4-Control)

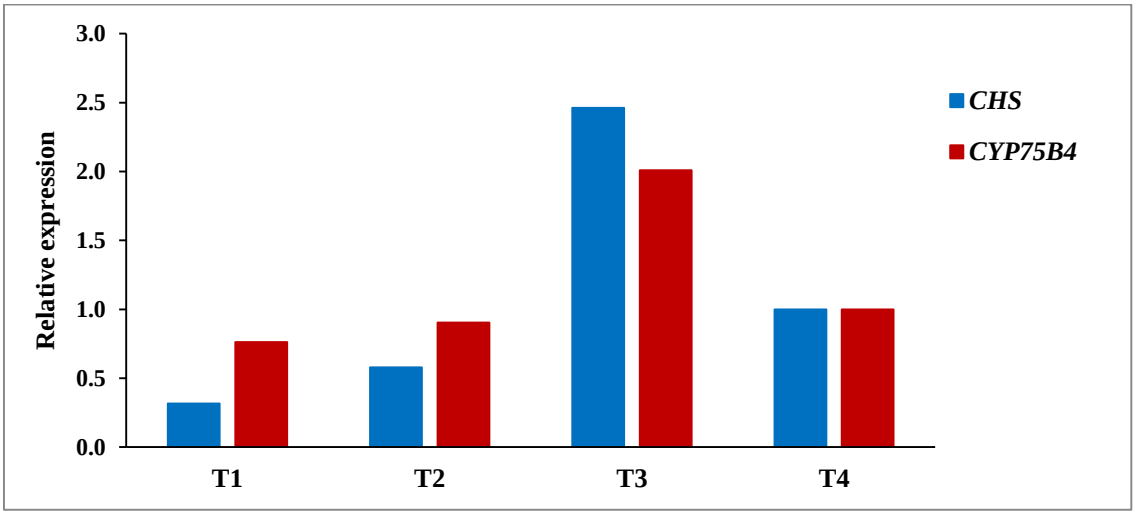


Figure 3: Relative expression of *CHS* and *CYP75B4* under different conditions in yellow njavara variety (T_1- 20% Shade; T_2-75% field capacity; T_3-UV-B treatment from panicle initiation stage; T_4-Control)

Discussion

5. DISCUSSION

Rice is the staple food for more than a billion people all over the world and over thousand varieties are grown around the world. Njavara is a unique rice landrace described in ancient Sanskrit treatises of Ayurveda for its nutritive and medicinal properties. Njavara rice is native to Kerala, India, traditionally used as an efficient health food as well as for external application under Panchakarma treatment. It is used in wide range of treatments such as diseases related to circulatory, respiratory, digestive ailments, skin inflammation and other skin related infections. Flavones, a chemotherapeutic agent belonging to a particular group of flavonoids, occur at higher concentration in njavara rice and have also shown anti-inflammatory effect in carrageenan-induced rat paw edema (Mohanlal *et al.*, 2011). The antioxidant properties of njavara rice also help to maintain the sugar level of diabetic patients (Reshmi and Nandini, 2013).

Abiotic stresses are the major factors responsible for the production of various antioxidants and secondary metabolites in plants. Abiotic stresses *viz.*, temperature, humidity, light intensity, water, CO_2, ultraviolet light and minerals are found to enhance the accumulation of almost all classes of secondary metabolites such as simple and complex phenols, flavonoids as well as different kinds of terpenes and alkaloids (Akula and Gokare, 2011; Selmar and Maik, 2013). These abiotic factors are widely used as elicitors to increase the production or to induce de novo synthesis of secondary metabolites under *in vitro* systems (Dicosmo and Misawa, 1985). A number of studies have shown that different kinds of elicitors could increase the secondary metabolite production in cell, tissue and organ cultures (Sudha and Ravishankar, 2003; Karuppusamy, 2009). However, depending on the physiological and developmental stages of plants, the production of secondary metabolites varies greatly and usually they are produced at very low concentrations (Rao and Ravishankar, 2002).

In the present study 'Physiological, biochemical and molecular studies in medicinal rice (*Oryza sativa* L.), Njavara, as influenced by abiotic stresses' was conducted to elicit information on the physiological, biochemical and molecular attributes associated to secondary metabolites accumulation due to abiotic stresses *viz.,* shade, drought, and UV-B stress in medicinal rice njavara. The results obtained from the study are discussed in this chapter.

5.1 EFFECT OF SHADE AND DROUGHT ON PHYSIOLOGICAL CHARACTERS

Two types of njavara *viz.,* black and yellow njavara were grown under four different abiotic stress condition in pot culture (20% shade, 40% shade, 50% field capacity and 75% field capacity) along with control. Influence of low light and water deficit conditions on physiological parameters of both the varieties are discussed below.

5.1.1 Plant height

Plant height is a very important agronomic trait of crops that directly affect crop architecture, apical dominance, biomass, resistance to lodging and crowding and ultimately the yield. In the present study, low light and water deficit stresses were found to differentially affect the plant height in both the varieties. Under shade condition increase in plant height was recorded and in which maximum increment in plant height was seen in treatment T_2 (40% shade) in the both varieties compared to open condition (T_5) (Figure 4). Black njavara (V_1) recorded increase in plant height by 20.37% and yellow njavara (V_2) by 1.66% under T_2 treatment compared to control (Table 1). Similar results were reported in rice by Sunilkumar and Geethakumari (2002) and Alridiwirsah *et al.* (2018). According to Schoch (1972) low light stress stimulate rapid cell division and cellular expansion which in turn lead to an increase in plant height. Many researchers have reported that the increase in the plant height with increase in shade levels mainly indicate a phototropic response taking place to modify the

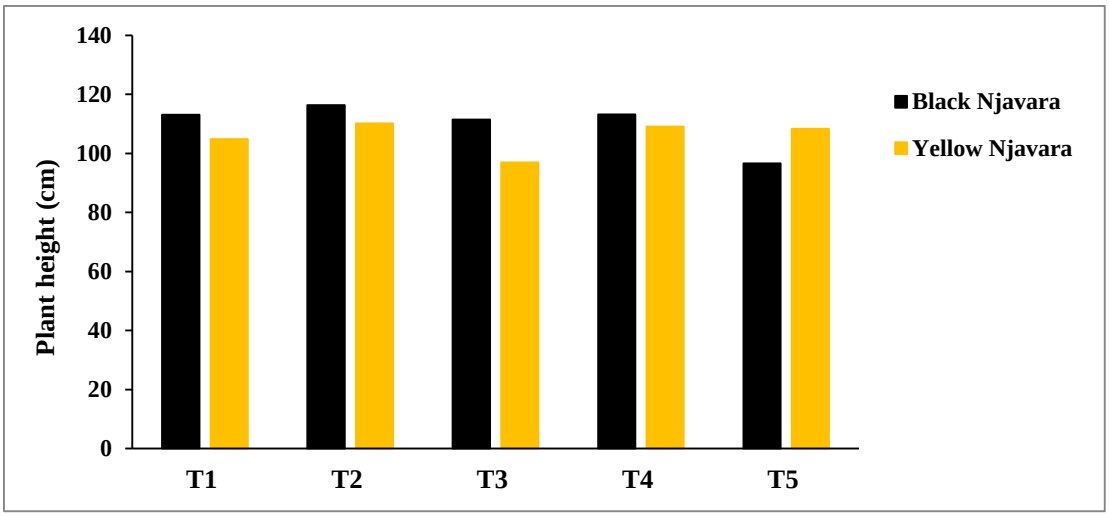


Figure 4: Effect of different shade levels and field capacity levels on plant height (cm) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4- 75% field capacity; T_5- Control)

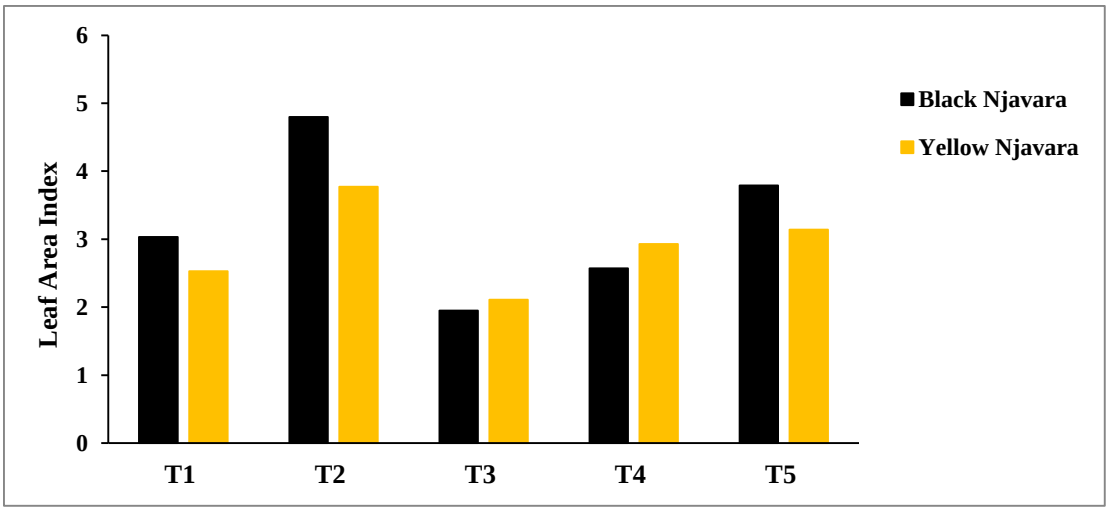


Figure 5: Effect of different shade levels and field capacity levels on leaf area index (LAI) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

distribution of leaves so as to help the plants to get enough light (Yang *et al.,* 2007; Wang *et al.,* 2009; Mapes and Xu, 2014).

Drought stress had no negative effect on black njavara (V_1) and plant height was found to increase by 15.34% and 17.19% under T_3 (50% field capacity) and T_4 (75% field capacity) respectively compared to control (Table 1). But in the case of yellow njavara (V_2), plant height was found to reduce under T_3 treatment by 10.51% compared to control. Whereas, under T_4 treatment, plant height was on par with control. There are many reports in rice indicating that plant height reduced significantly under water stress conditions (Alghabari and Ihsan, 2018; Kamarudin *et al.,* 2018; Singh *et al.,* 2018). But in the present study, black njavara (V_1) recorded increased plant height under water stress condition, and this result obtained indicate the tolerance capacity of black njavara to drought condition (Rani, 2010; Mohanlal, 2011). Reduction in plant height under drought stress has been reported primarily due to the reduction in cell turgor, leading to inhibition in cell division, cell elongation and expansion. The limitation of water was also found to impair the process of mitosis leading to more senescence (Bhatt and Rao, 2005, Kaya *et al.,* 2006; Hussain *et al.,* 2008; Henry *et al.,* 2016).

5.1.2 Leaf area index (LAI), Specific leaf area (SLA) and Number of tillers

Leaf surface carry out many important processes which play vital role in a biogeochemical cycle in the environment and any change in leaf character leads to changes in plant growth and development (Wright *et al.,* 2004; He *et al.,* 2006). In the present study leaf characters like LAI and SLA recorded higher values under treatment T_2 (40% shade) in both varieties but between varieties black njavara (V_1) recorded higher values than yellow njavara (V_2) (Figure 5 and 6). LAI increased by 26.65% in variety V_1 and 20.02% in variety V_2 under treatment T_2 whereas, SLA increased by 15.18% and 13.67% in both varieties respectively under T_2 (Table 2 and 3). Similar results were reported in other crops like rice (Aumonde *et al.,* 2013), wheat (La *et al.,*

2010) and barley (Gunn *et al.,* 1999) also. Many researchers have suggested that increase in parameters like LAI and SLA help the plants to capture sufficient light and to have better utilization of available light for the production of photoassimilates (Evans and Poorter, 2001; Lima *et al.,* 2008). Number of tillers per plant showed a negative effect under shade treatments (Figure 7). In black njavara, number of tillers per plant reduced by 32.28% under T_1 (20% shade) and 30.80% under T_2 (40% shade) compared to T_5 (control). Similarly in yellow njavara, tillers per plant reduced by 38.03% and 34.68% under T_1 and T_2 respectively (Table 4). Emmanuel and Mary (2014) and Ginting *et al*. (2015) reported that in rice, the number of tillers significantly decreased with increase in shade levels. Reduction in tiller number under shade condition may be due to the reason that the tiller buds not growing into productive tillers due to insufficient photoassimilates (Ginting *et al*. 2015; Sridevi and Chellamuthu, 2015).

LAI, SLA and number of tillers per plant reduced under drought treatments and maximum reduction was observed under treatment T_3 (50% field capacity) compared to T_5 (control) in the present study (Figure 5, 6 and 7). In black njavara (V_1), LAI, SLA and tiller number reduced by 48.55%, 17.91% and 46.11% respectively under treatment T_3 (Table 2, 3 and 4). In yellow njavara (V_2), LAI (32.80%), SLA (25.82%) and tillers per plant (47.62%) were also found to be reduced under 50% field capacity (T_3) condition compared to control. These results are in agreement with Singh *et al.* (2018), who reported that when water stress was given to rice varieties there was significant decrease in leaf parameters as well as tiller numbers compared to control. Sorkhi and Fateh (2019) also reported that LAI was found to decrease by 55.96 to 30.57 percent under drought condition in pinto bean. Changes in leaf morphology are the initial signs of water deficit condition seen in plants. Under low water situation leaf characters such as leaf turgor, accumulation of assimilates, emergence rates and leaf temperature get modified which are important for leaf expansion and leaf elongation (Reddy *et al.,* 2003; Anjum *et al.,* 2016). Drought condition also disturb processes of cell division

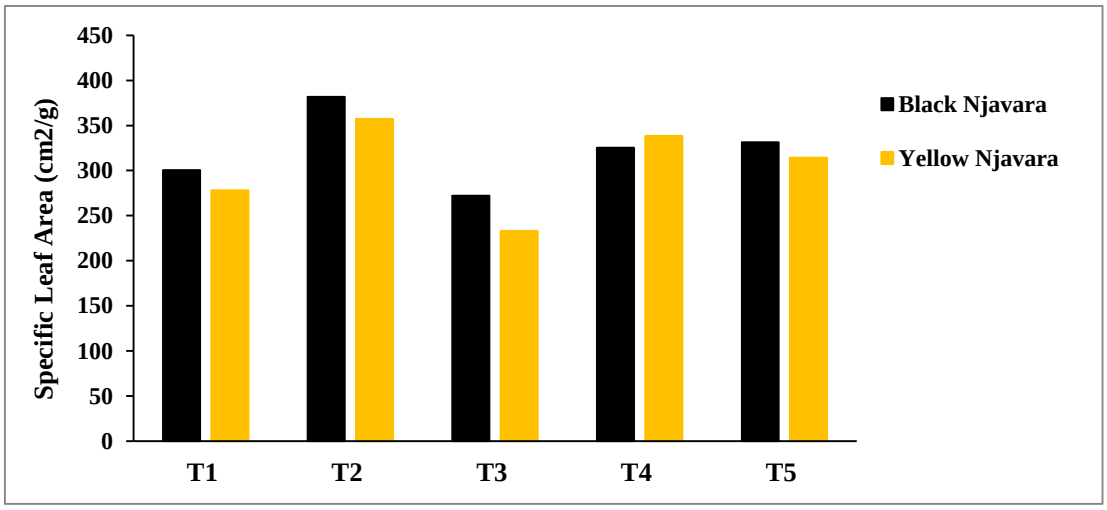


Figure 6: Effect of different shade levels and field capacity levels on specific leaf area (SLA) (cm^2/g) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

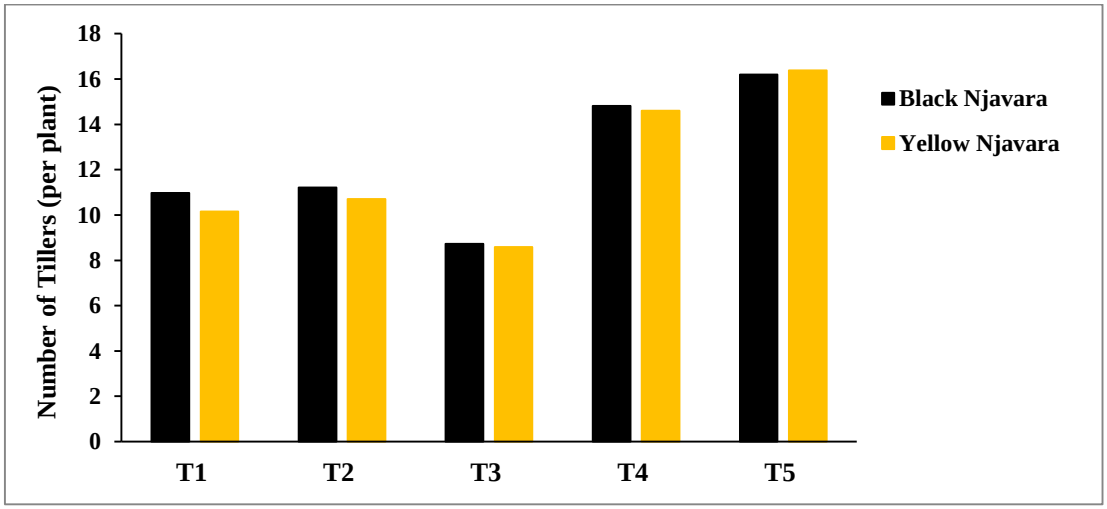


Figure 7: Effect of different shade levels and field capacity levels on number of tillers per plant of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

and cell development which further affect cells and results in reduction in leaf area (Henry *et al.,* 2016). Anjum *et al.* (2017) reported that under drought, mortality of apical part of leaf and increased leaf rolling cause significant reduction in leaf area.

5.1.3 Leaf gas exchange parameters

Leaf gas exchange parameters like photosynthesis, stomatal conductance and transpiration are highly sensitive to any changes in the environment and even slight change in light intensity can lead to changes in leaf gas parameters in plants (Yamori *et al.,* 2016). In the present study, photosynthetic rate, stomatal conductance and transpiration rate decreased under shade condition compared to control in both the varieties. But between the varieties, yellow njavara (V_2) recoded lowest photosynthetic rate, stomatal conductance and transpiration rate than black njavara (V_1) (Figure 8, 9 and 10). Treatment T_2 (40% shade) showed maximum reduction in photosynthetic rate (40.65%), stomatal conductance (22.98%) and transpiration rate (57.22%) in variety V_1 compared to T_5 (control) (Table 5, 6 and 7). In yellow njavara the reduction was 40.31%, 21.39% and 53.45% respectively for the above mentioned parameters under T_2 treatment. These findings are in agreement with many reports in different crops such as rice (Panda *et al.,* 2019), wheat (Acreche *et al.,* 2009), mustard (Zhu *et al.,* 2017), sorghum (Li *et al.,* 2014) and soybean (Feng *et al.,* 2019). There are several reasons behind decrease in leaf gas exchange parameters but the primary reason thought is that, under low light condition closure of stomata take place and the number of stomata per millimeter decrease which lead to reduction in stomatal conductance and transpiration (Farquhar and Sharkey, 1982; Liu *et al.,* 2014). Ribulose bisphosphate carboxylase (Rubisco) activity (which is important enzyme to carry photosynthetic process) decrease significantly under shade condition (Shi *et al.,* 2006). Shade also changes the rate of non-photochemical quenching, electron transfer and quantum yield of PS II which ultimately affects the photosynthetic efficiency of plants (Jiao and Li, 2001).

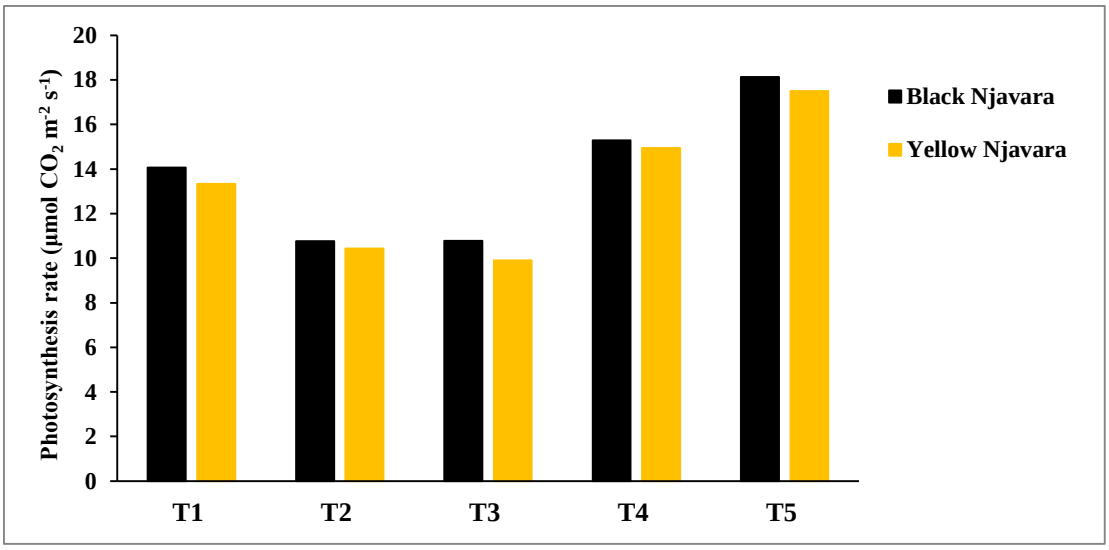


Figure 8: Effect of different shade levels and field capacity levels on photosynthesis rate (µmol CO_2 m^{-2} s^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

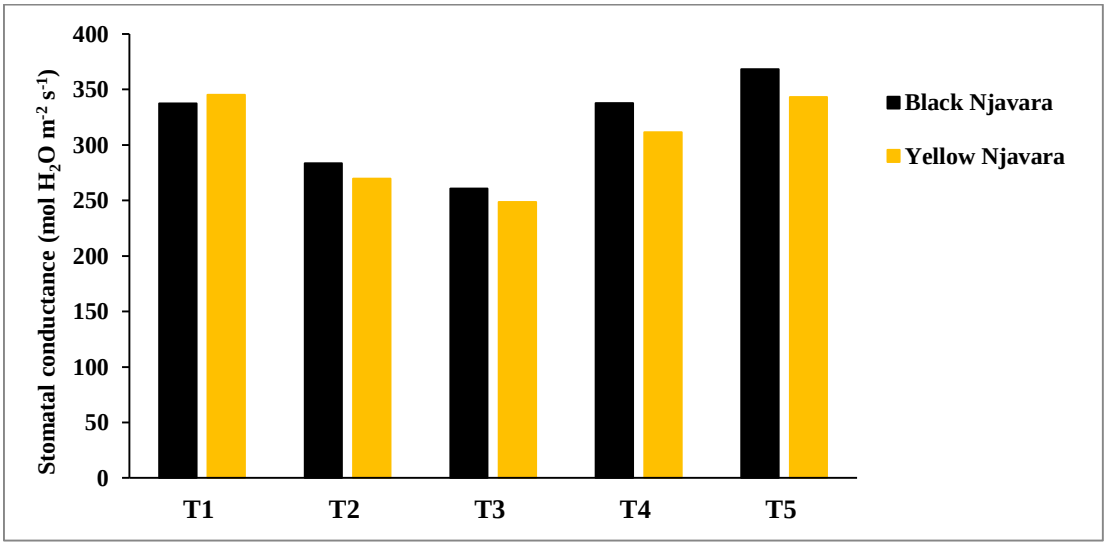


Figure 9: Effect of different shade levels and field capacity levels on stomatal conductance (mol H_2O m^{-2} s^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

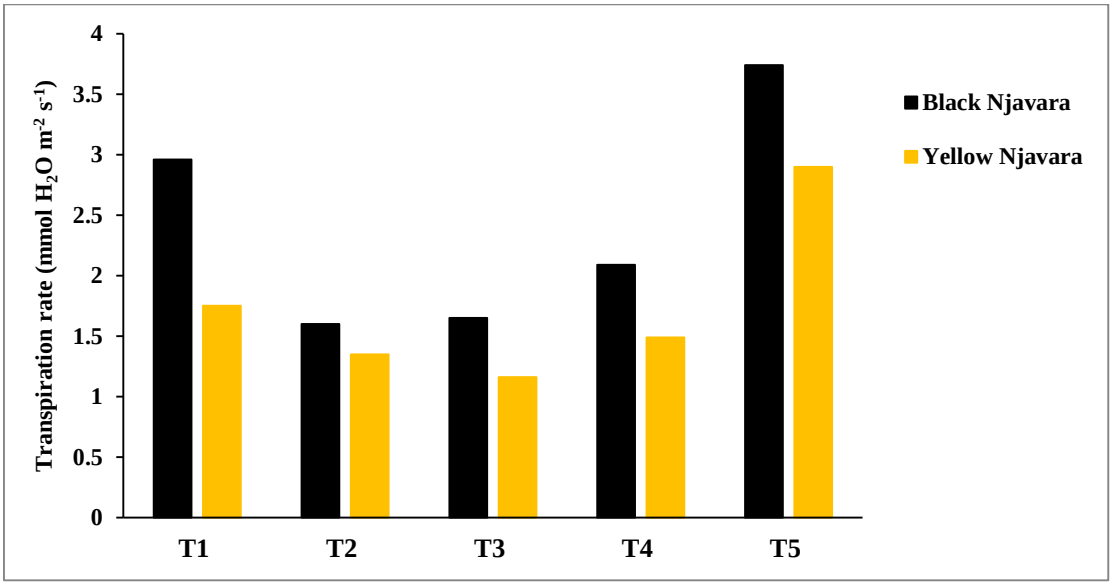


Figure 10: Effect of different shade levels and field capacity levels on transpiration rate (mmol H_2O m^{-2} s^{-1}) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

In the present study drought adversely affected the leaf gas exchange parameters in both varieties. Yellow njavara (V_2) was observed more sensitive than black njavara (V_1) towards water deficit condition (Figure 8, 9 and 10). In black njavara (V_1), treatment T_3 (50% field capacity) showed maximum reduction in photosynthetic rate (40.54%), stomatal conductance (29.19%) and transpiration rate (55.88%) compared to T_5 (control) (Table 5, 6 and 7). In yellow njavara (V_2), the reduction was 43.40%, 27.52% and 60.00% respectively for the above mentioned parameters under T_3 treatment. There are several other studies also which reported similar results in rice (Gu *et al*., 2012; Lauteri *et al.,* 2014; Dhivyapriya *et al.,* 2016; Kumar *et al.,* 2020). Under water deficit condition, excessive production of abscisic acid takes place which then translocate to guard cells and promote the closure of stomata and leads to decrease in stomatal conductance and transpiration rate (Yokota *et al.,* 2002; Fathi and Tari, 2016). Low water status has been reported to decrease the activity of important photosynthetic enzymes such as Rubisco, PEPCase and NADP-malic enzyme which also alter the rate of leaf gas exchange parameters (Reddy *et al.,* 2004; Zhou *et al.,* 2007). Bota *et al.* (2004) reported that under drought condition, the activity of Rubisco binding inhibitors increase in the leaves.

5.2 EFFECT OF SHADE AND DROUGHT ON BIOCHEMICAL CHARACTERS

5.2.1 Total flavonoid in leaves and total phenol content

Total flavonoid and total phenol content were found to reduce in both varieties under shade condition. However, yellow njavara (V_1) recorded lowest content of flavonoid and phenol content than black njavara (V_2) (Figure 11 and 12). Flavonoid content in leaves decreased under T_2 (40% shade) by 12.75% and 11.44% in V_1 and V_2 respectively compared to T_5 (control) (Table 8). Similarly phenol content was also found to decrease under T_2 by 20.25% and 23.97% in both the varieties (V_1 & V_2) respectively (Table 9). Similar results has been reported by Panigrahy *et al.* (2019) in rice. Kumari *et al.* (2009) reported that under shaded condition the activity of PAL

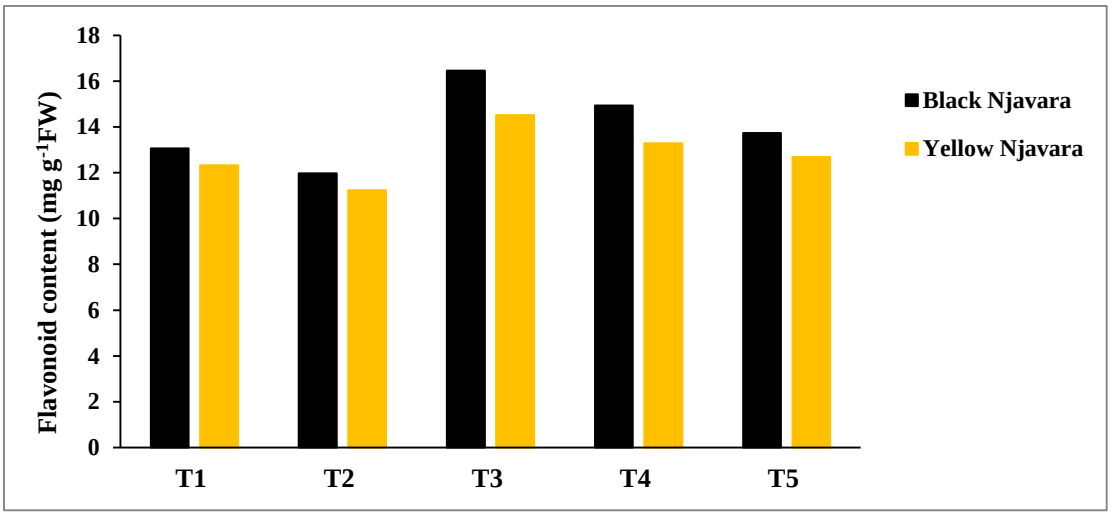


Figure 11: Effect of different shade levels and field capacity levels on flavonoid content in leaves (mg g^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

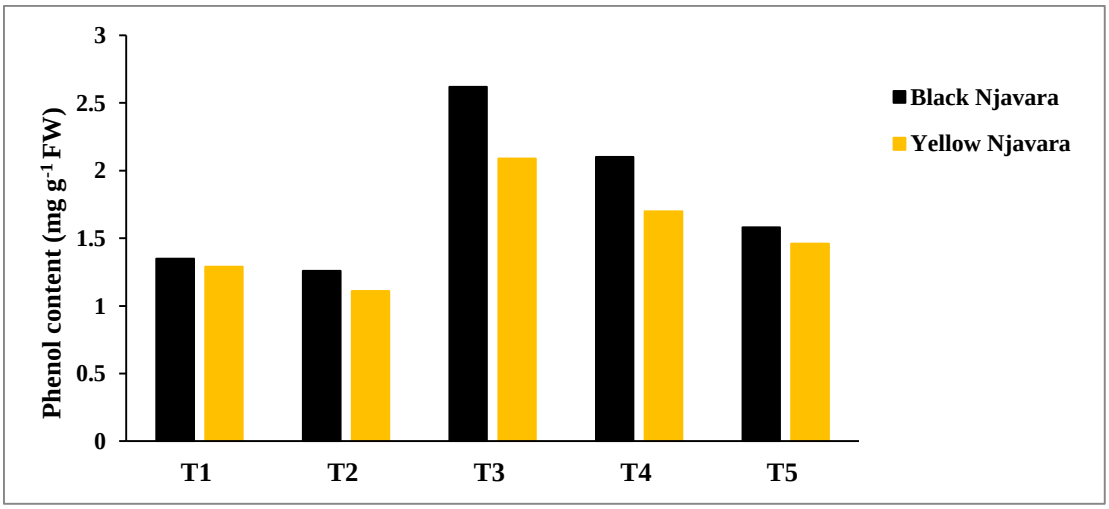


Figure 12: Effect of different shade levels and field capacity levels on phenol content (mg g^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control)

enzyme decreases and the activity (PAL enzyme) increases by high light intensity conditions. Warren *et al.* (2003) also demonstrated higher accumulation of phenolic compounds under high light intensity.

Drought conditions had positive effects on flavonoid content as well as phenol content in the present study (Figure 11 and 12). Black njavara produced higher content of flavonoid content and phenol content than the yellow njavara under water deficit condition. Treatment T_3 (50% field capacity) recorded higher flavonoid content in V_1 (19.88%) and V_2 (14.51%) over control (Table 8). Phenol content also was found to increase under treatment T_3 by 65.82% and 43.15% in variety V_1 and V_2 respectively (Table 9). These results confirm with the findings in rice (Quan *et al.,* 2016), tomato (Klunklin and Savage, 2017), *Amaranthus* (Sarker and Oba, 2018) and *Aloe vera* (Habibi, 2018). Flavonoid and phenol act as antioxidants in plant and they can maintain cell redox status under stressed conditions (Maggi-Capeyron *et al.,* 2001; Yun *et al.,* 2008). Nakabayashi *et al.* (2014) demonstrated that in Arabidopsis, under low water stress, flavonoid content increased and played important role to provide resistance under stressed conditions.

5.2.2 Chlorophyll content

In the present study, low light and drought stresses were found to differentially affected chlorophyll content in both the varieties (Table 10). Under shade stress, chlorophyll content increased in both the varieties (Table 26). In black njavara (V_1) chlorophyll 'a' increased under treatment T_2 (40% shade) by 2.88% compared to control (T_5). However, increased chlorophyll 'b' (62.64%) and total chlorophyll (25.22%) content were recorded under treatment T_1 (20% shade). On contrary to that, in yellow njavara (V_2) chlorophyll 'a' (15.91%) content was found to increase under treatment T_1 (20% shade) compared to control. And increased chlorophyll 'b' and total chlorophyll content were recorded under treatment T_2 (40% shade) (58.88% and 31.51% respectively). Many other researchers have also reported similar results in rice

crop (Viji *et al.,* 1997; Restrepo and Garcés, 2013; Muhidin *et al.,* 2018). Taiz and Zeiger (2002) reported that under shade, plants have the ability to enhance the chlorophyll content by three-four folds with large and thin leaves and this responses of plants allow them to capture sufficient light required for photosynthesis by maintaining enough number of photosynthetic antennae in the leaves (Dai *et al.,* 2009). The present study also revealed that the shade conditions influenced higher chlorophyll 'b' content and lower chlorophyll a/b ratio (Figure 13). However the lower chlorophyll a/b ratio indicates higher amount of light harvesting chlorophyll binding proteins (Burke *et al.,* 1979). Previous studies also revealed that the chlorophyll a/b ratio lower in photosystem-II than photosystem-I (Thayer and Bjorkman, 1992), suggesting chlorophyll 'b' enriched outer antennae pigments relative to the core complexes of photosystem-I and photosystem-II (Baig *et al.,* 2005). Reducing the chlorophyll a/b ratio might be a way to minimize the stress damage by adjusting their pigment composition in the leaves.

Under water deficit condition, decrease in chlorophyll pigment (chlorophyll 'a', chlorophyll 'b' and total chlorophyll) was evident compared to control (Table 10). Under water deficit condition, both the varieties recorded reduced chlorophyll content and among the varieties black njavara (V_1) recorded maximum reduction than yellow njavara (V_2) (Table 26). Chlorophyll 'a', chlorophyll 'b' and total chlorophyll reduced by 12.95%, 45.05% and 18.70% respectively under T_3 (50% field capacity) in black njavara (V_1) compared to the control. Similarly in yellow njavara (V_2) chlorophyll 'a' (0.76%), chlorophyll 'b' (41.12%) and total chlorophyll (16.39%) reduced under treatment T_3 (50% field capacity) .There are many studies which reported similar results in rice crop (Pirdashti *et al.,* 2009; Cha-um *et al.,* 2010; Sikuku *et al.,* 2012). The primary reasons attributed to the decrease of chlorophyll content in plants under low water stress are impaired chlorophyll biosynthetic pathway, chlorophyll degradation, loss of chloroplast membrane and increase in lipid peroxidation (Fu and Huang, 2001; Maisura *et al.,* 2014; Pandey and Shukla, 2015).

Chlorophyll content (mg g^{-1} FW)							
	Mean Chl 'a'	**Mea Chl 'b'**	**Mean Total chl**	**Chloro -phyll a/b ratio**	**Chl 'a' % change over control**	**Chl 'b' % change over control**	**Total chl % change over control**
V_1T_1	1.40	1.48	2.88	0.95	0.72	62.64	25.22
V_1T_2	1.43	1.44	2.87	0.99	2.88	58.24	24.78
V_1T_3	1.21	0.50	1.87	2.42	-12.95	-45.05	-18.70
V_1T_4	1.33	0.70	2.03	1.90	-4.32	-23.08	-11.74
V_1T_5	1.39	0.91	2.30	1.53	0.00	0.00	0.00
V_2T_1	1.53	1.44	2.90	1.06	15.91	34.58	21.85
V_2T_2	1.50	1.70	3.13	0.88	13.64	58.88	31.51
V_2T_3	1.31	0.63	1.99	2.08	-0.76	-41.12	-16.39
V_2T_4	1.40	0.75	2.22	1.87	6.06	-29.91	-6.72
V_2T_5	1.32	1.07	2.38	1.23	0.00	0.00	0.00
V_1	1.35	1.01	2.36	1.34			
V_2	1.41	1.12	2.52	1.26			
T_1	1.46	1.40	2.86	1.04			
T_2	1.47	1.63	3.09	0.90			
T_3	1.26	0.54	1.80	2.33			
T_4	1.37	0.72	2.12	1.90			
T_5	1.34	1.02	2.39	1.31			

Table 26: Mean of chlorophyll content (mg g^{-1} FW) and a/b ratio of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4-75% field capacity; T_5- Control; V_1- Black Njavara; V_2- Yellow Njavara).

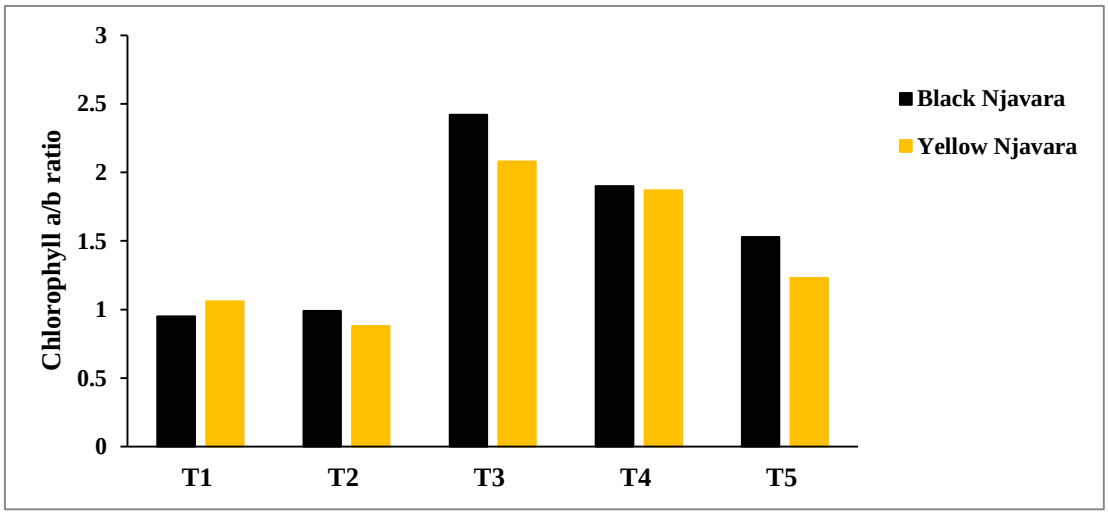


Figure 13: Chlorophyll a/b ratio under different shade levels and field capacity levels of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4- 75% field capacity; T_5- Control)

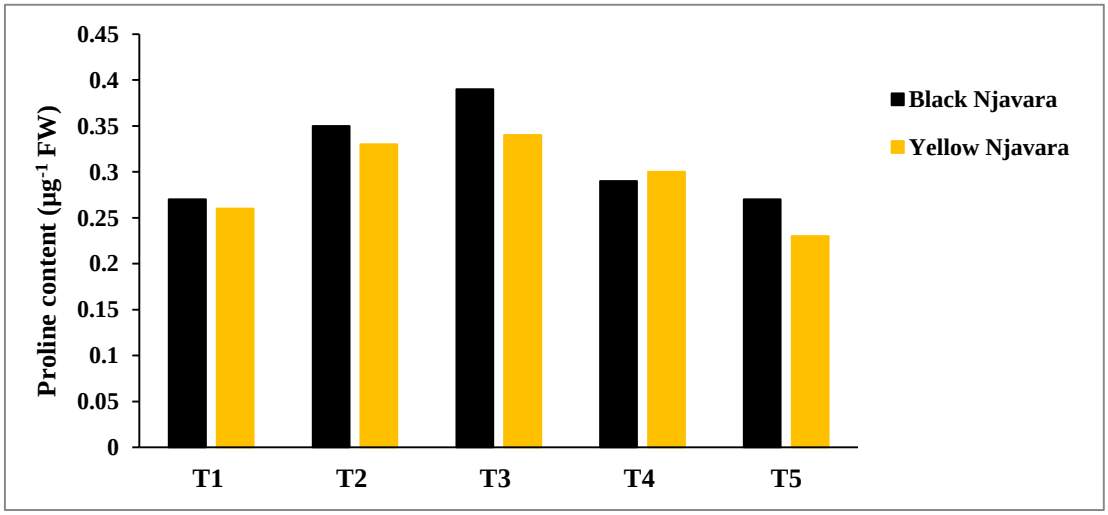


Figure 14: Effect of different shade levels and field capacity levels on proline content (μg^{-1} FW) of njavara rice varieties (T_1- 20% shade; T_2- 40% shade; T_3- 50% field capacity; T_4- 75% field capacity; T_5- Control)

5.2.3 Proline content

In the present study, proline content was found to significantly increase under shade stress as well as under drought stress treatments in both the varieties compared to the non-stressed treatments (Figure 14). Under shade stress, the treatment T_2 (40% shade) recorded higher proline content in both the varieties (V_1-29.63%; V_2-43.48%) compared to control (T_5). However, under T_1 (20% shade) proline content was found on par between black njavara (V_1) and yellow njavara (V_2) and the increase accounted to 13.04% compared to the plants grown under 100% light (T_5). Mo *et al*. (2015) also reported that in two aromatic rice varieties grown under 67% reduced light, the proline content was found higher than those grown under the open condition.

Black njavara (V_1) recorded higher proline content (44.44%) under T_3 (50% field capacity) than the control (T_5) but under T_4 (75% field capacity) the increase was only 7.41% (Table 11). Similarly, in yellow njavara (V_2) higher proline content was recorded under T_3 (47.83%) followed by T_4 (30.43) than the control (T_5). Increase in proline content under drought stress in rice plants has been reported by various researchers (Chutipaijit *et al.,* 2012; Lum *et al.,* 2014; Kamarudin *et al.,* 2018). Also there are reports indicating the increase of proline content in plants under vast environment stresses such as drought, temperature, shade, salinity, UV etc (Rhodes *et al.,* 2002; Munns, 2005; Sharma and Dietz, 2006). In such conditions proline play an important role *viz.,* acting as an osmolyte, stabilizing the membranes, maintaining protein structure, scavenging free radicles and maintaining redox potential of cells (Hayat *et al.,* 2012; Liang *et al.,* 2013; Ashraf and Foolad, 2007; Chun *et al.,* 2018).

5.2.4 Flavonoid content in grain

Treatment T_4 (75% field capacity) recorded significantly increased flavonoid content in grains of black njavara (16.23%) and yellow njavara (39.76%) followed by

treatment T_1 (20% shade) by 3.82% and 26.36% in both the varieties respectively compared to control (Table 12).

The present study revealed that, mild stress *viz.*, T_1 (20% shade) and T_4 (75% field capacity) could increase the accumulation of flavonoids in grains. But under severe stress *i.e.* 40% shade (T_2) and 50% field capacity (T_3) there was no increase in the flavonoid content of the grains. This can be attributed to the effect of low light conditions on restricting the activity of important enzymes like phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) since they are light induced enzymes (Kumari *et al.,* 2009). However under severe stress, 40% shade (T_2) and 50% field capacity (T_3), the flavonoid content was found higher in leaves (Table 11) and may have been involved in other functions such as acting as anti-oxidant, maintaining cell redox status and detoxifying harmful effect of H_2O_2 (Nagah and Seal, 2005; Yun *et al.,* 2008; Hernandez *et al.,* 2009).

5.2 EFFECT OF UV-B RADIATION ON VARIOUS CHARACTERS

Black njavara and yellow njavara were grown under four different treatments in ventilated polyhouse. Plants were subjected to UV-B (280-320 nm) radiation for 4 hours per day according to the treatments. The four different treatments included subjecting the plants to UV-B (280-320 nm) radiation during different critical stages of plants *viz.,* from vegetative stage, from panicle initiation stage and from flowering stage till maturity and a control (without UV-B radiation). UV-B radiation was provided using UV-B fluorescent tubes and the average radiation measured at plant canopy level was 4 Wm^{-2}. The effect of UV-B radiation on physiological and biochemical parameters are discussed below.

5.2.1 Physiological characters

Both black njavara and yellow njavara recorded lower plant height under UV-B radiation treatments compared to control condition. In black njavara (V_1), there was

up to 10.85, 7.40 and 4.40 percent reduction in plant height under T_1 (UV-B radiation treatment from vegetative stage), T_2 (UV-B radiation from panicle initiation stage and T_3 (UV-B radiation from flowering stage) respectively compared to plants grown without UV-B radiation (T_4) (Figure 15). Similar results were observed in yellow njavara (V_2) also wherein the reduction in plant height was 9.52, 4.58 and 3.38 percent under above mentioned treatments respectively compared to T_4. There are reports indicating that elevated UV-B radiation has negative influence on plant height in rice (Mohammed *et al.*, 2007; Wagh and Nandini, 2019), cotton (Kakani *et al.*, 2003), wheat (Kataria and Guruprasad, 2012), soybean (Liu *et al.*, 2013) and in barley (Jun *et al.*, 2010).

Leaf area index (LAI) and specific leaf area (SLA) were found to decrease under elevated UV-B radiation treatments in both varieties. However between the varieties, black njavara (V_1) showed higher LAI and SLA than the yellow njavara (V_2) under elevated UV-B radiation condition (Figure 16 and 17). In black njavara, maximum reduction in LAI and SLA was found under UV-B radiation from vegetative stage (T_1) (57.98% and 30.58% respectively) followed by UV-B radiation from panicle initiation stage (T_2) (50.42% and 30.19% respectively) and the least reduction was recorded under treatment UV-B radiation from flowering stage (T_3) (30.81% and 22.03% respectively) than control (T_5). Similarly in yellow njavara, maximum reduction in LAI and SLA was recorded under treatment T_1 (66.78% and 26.47% respectively) followed by T_2 (50.66% and 24.50% respectively) and the least reduction was recorded in T_3 (29.93 and 20.27% respectively) than control (T_4). The current findings are in agreement with those reported in green gram (Rajendiran and Ramanujam, 2003) and *A. thaliana* (Boeger and Poulson, 2006).

The results on number of tillers per plant indicated significant variation between varieties and treatments. Among the different treatments, UV-B radiation from vegetative stage (T_1) recorded the least number of tillers per plant followed by

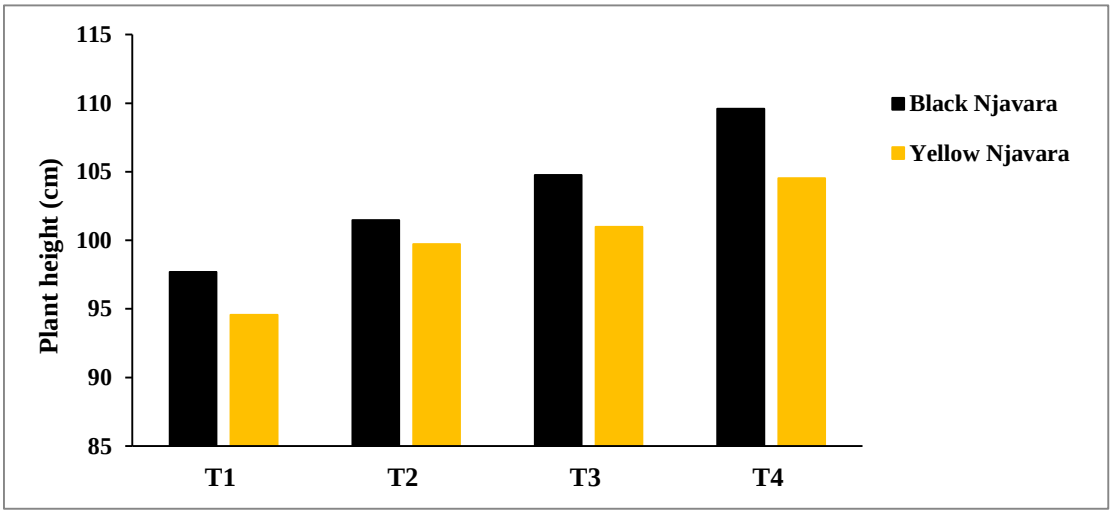


Figure 15: Effect of UV-B radiation on plant height (cm) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

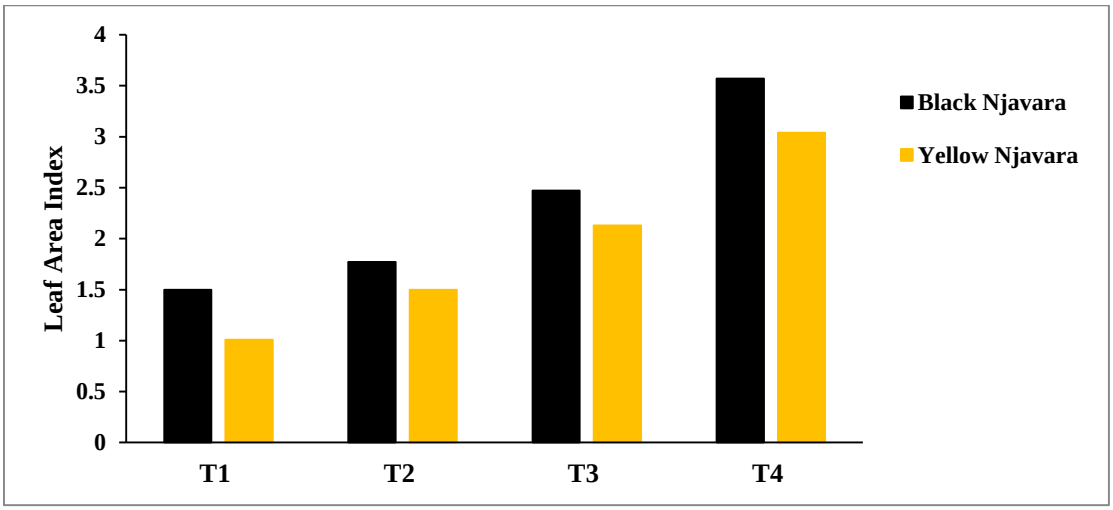


Figure 16: Effect of UV-B radiation on leaf area index (LAI) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

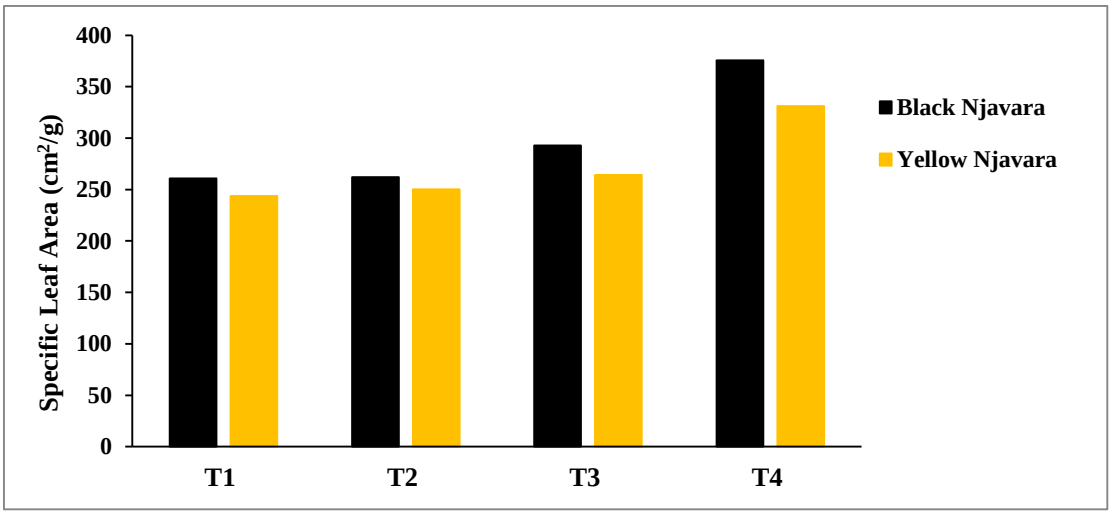


Figure 17: Effect of UV-B radiation on specific leaf area (cm^2/g) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

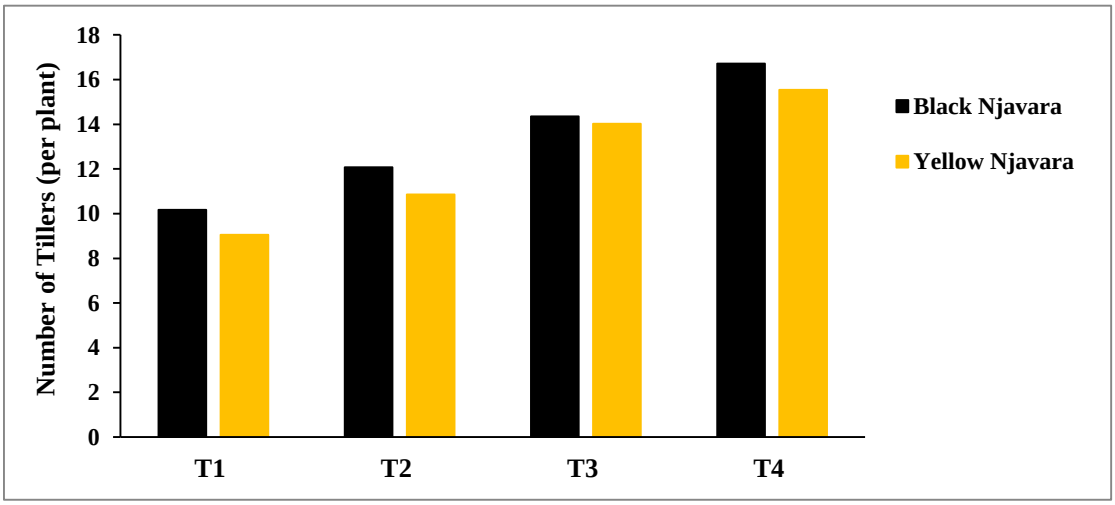


Figure 18: Effect of UV-B radiation on number of tillers per plant at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

treatment UV-B radiation from panicle initiation stage (T_2) and UV-B radiation from flowering stage (T_3) and the maximum number of tillers was recorded under control condition (T_4) in both the varieties (Figure 18). Between both the varieties, black njavara produced higher number of tillers per plant under the above mentioned treatments than yellow njavara (Table 16). In black njavara, tillers per plant reduced by 39.17%, 27.75% and 14.11% under T_1, T_2 and T_3 respectively compared to control (T_4). Similarly in yellow njavara, number of tillers per plant under T_1, T_2 and T_3 reduced by 41.74%, 30.16% and 9.77% respectively compared to control. Similar findings of decrease in tillers per plant in rice under UV-B radiation have been reported by other researchers also (Kumagai *et al.,* 2001; Mohammed and Tarpley, 2009, Wagh, 2015).

Reduction in morphological characters like plant height, LAI, SLA and tillers under UV-B radiation are mainly due to reduction cell division and cell elongation (Milchunas *et al.,* 2004; De Lima-Bessa *et al.,* 2008; Hectors *et al.,* 2010). It is well documented that UV radiation is strongly absorbed by proteins leading to changes in protein structure and loss of functions. Similarly, Zaremba *et al.* (1984) has reported that the tubulin protein strongly absorb UV radiation which leads to disruption of microtubule formation during cell division that might delay the cell division. Logemann *et al.* (1995) demonstrated repression of transcription of cell cycle genes such as Cyclin-dependent kinase 2 (cdk2) and cyclin in UV irradiated suspensions of *Petroselinium crispum* cells. There are also reports indicating that the reduction in morphological traits under UV-B radiation might be attributed to the reduction of IAA activity and imbalance of hormones *via,* photo oxidation of plant hormones (Rozema *et al.,* 2001; Jansen *et al.,* 2002; Hopkins *et al.,* 2002).

5.2.2 Leaf gas exchange parameters

Sunlight is the primary source of energy for plants and any change in quality and quantity of sunlight can lead to changes in gas exchange parameters like photosynthesis rate, stomatal conductance and transpiration rate. Under UV-B

radiation, reduction in rate of photosynthesis was accompanied by decrease in stomatal conductance and transpiration rate (Figure 19, 20 and 21). This finding is in agreement with the results obtained by Surabhi *et al.* (2009) in cowpea, Yu *et al.* (2013) in rice, Reddy *et al.* (2013) in corn and Januskaitiene (2013) in rapeseed. In black njavara, the treatment UV-B radiation from vegetative stage (T_1) recoded lowest photosynthetic rate, stomatal conductance and transpiration rate (38.75%, 31.97% and 41.95% respectively) compared to the control (T_4). Similarly, a reduction in photosynthetic rate, stomatal conductance and transpiration rate was recorded under treatment T_1 (30.72%, 27.73% 38.57% and respectively) in yellow njavara.

Reduction in leaf gas exchange parameters under UV-B radiation is mainly due to deterioration of processes like photophosphorylation reactions of the thylakoid membrane, CO_2-fixation reactions of the Calvin cycle and stomatal control of CO_2 supply (Kataria *et al.,* 2014). In detail, UV-B radiation mainly impairs photosystem-II (PS-II) through damaging water-oxidizing manganese (Mn) cluster, the reaction centers of the D1 and D2 protein, quinine electron acceptors and tyrosine electron donors (Jansen *et al.,* 1998; Vass *et al.,* 2005; Dobrikova *et al.,* 2013). Reduction in photosynthesis under UV-B radiation is mainly due to the degradation of Rubisco enzyme under UV-B exposure (Wilson *et al.,* 1995; Yu *et al.,* 2013). UV-B radiation is strongly absorbed by aromatic amino acid, tryptophan which is present in Rubisco protein and which leads to degradation of proteins (Hartman and Harpel, 1994; Kataria *et al.,* 2014). It is also reported that UV-B radiation induce reduction in stomatal conductance which leads to reduction in CO_2 assimilation (Jansen and Van-Den-Noort, 2000; Lu, 2009; Reddy, 2013). Nogues *et al.* (1999) demonstrated that the stomatal closing effect of UV-B radiation was larger on adaxial stomata than abaxial stomata when equally exposed because adaxial guard cell receive higher UV-B radiation than abaxial guard cell due to UV-B-adsorbing pigments such as flavonoids, particularly in the epidermis (Bilger *et al.,* 1997; Allen *et al.,* 1998).

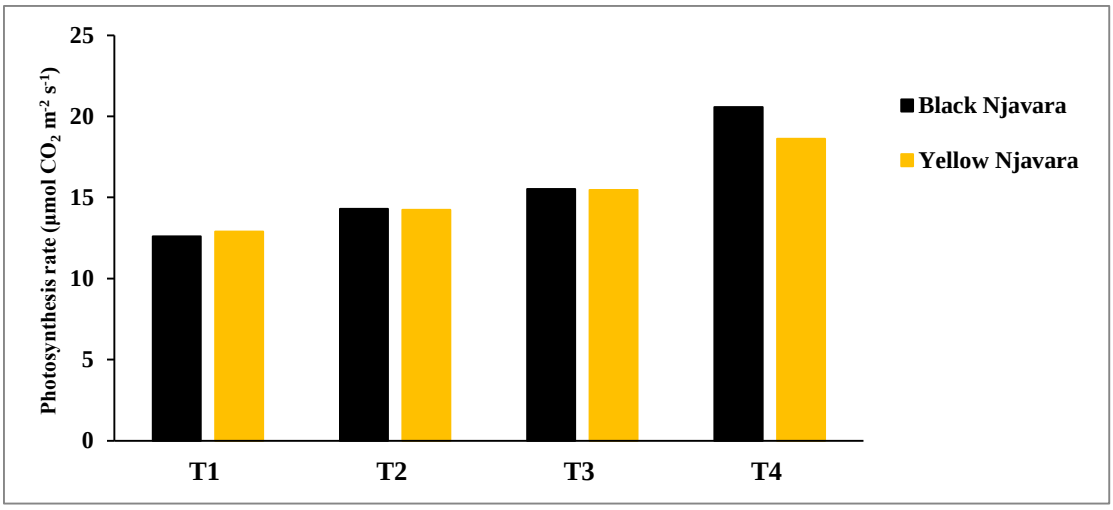


Figure 19: Effect of UV-B radiation on photosynthesis rate (µmol CO_2 m^{-2} s^{-1}) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

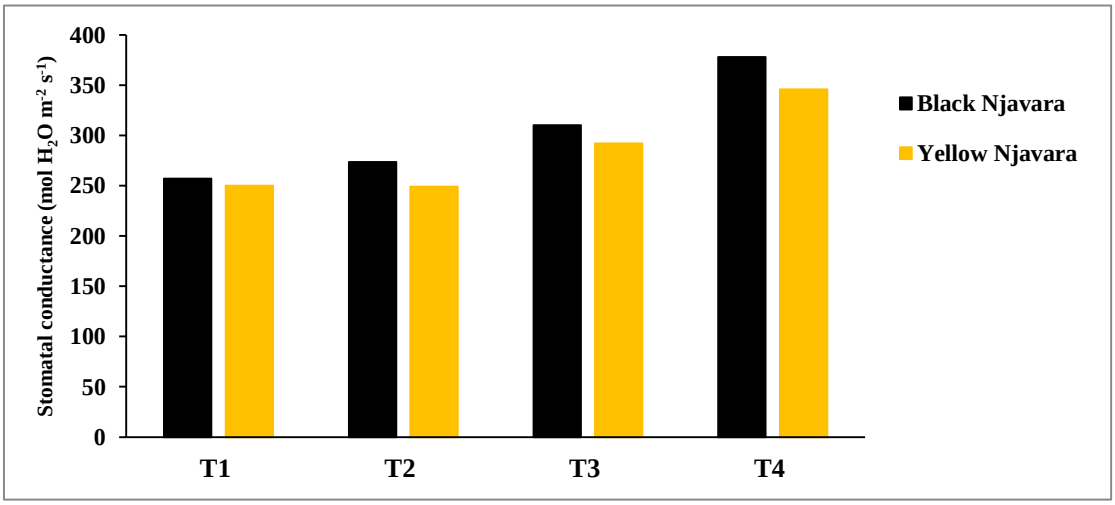


Figure 20: Effect of UV-B radiation on stomatal conductance (mol H_2O m^{-2} s^{-1}) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

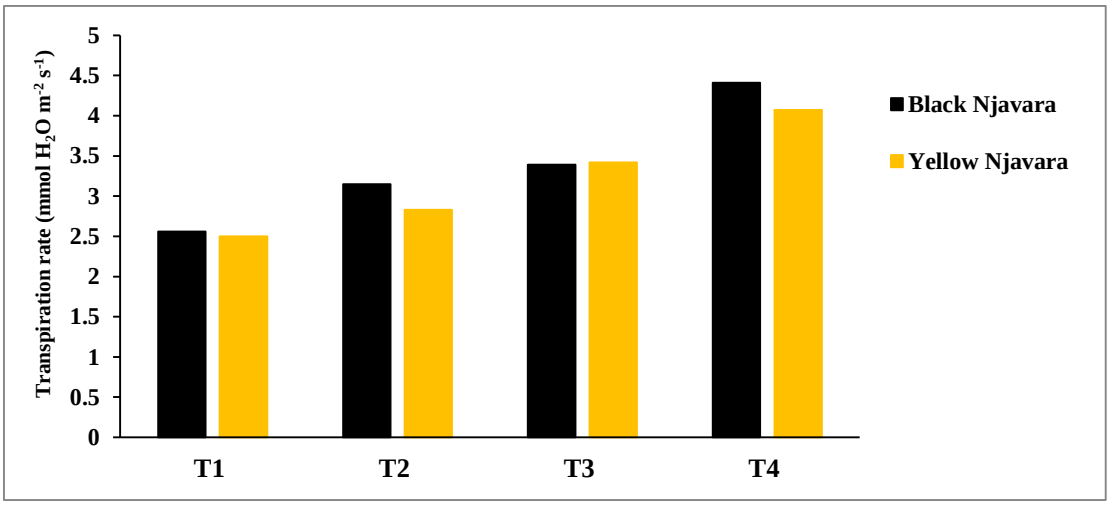


Figure 21: Effect of UV-B radiation on transpiration rate (mmol H_2O m^{-2} s^{-1}) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

5.2.3 Effect of UV-B radiation biochemical characters

Total flavonoid and phenol content in leaves, which are involved in photo protection and as antioxidants recorded an increasing trend under UV-B radiation in both varieties. Flavonoid and phenol content in leaves were found higher in black njavara (V_1) than yellow njavara (V_2) under UV-B radiation (Figure 22 and 23). In black njavara, maximum increment in flavonoid content (31.68%) and phenol content (146.24%) were found in the treatment with UV-B radiation from vegetative stage (T_1) compared to the control (T_4). In yellow njavara, the increment was 36.75% and 125.84% (in flavonoid and phenol content respectively) in the above mentioned treatment. The present result is in agreement with the finding of Ambasht and Agrawal (1998); Yuan *et al.* (2010); Wagh, 2015) in rice. Accumulation of flavonoids and phenols are key components of acclimation response under UV-B radiation to increase the capabilities of photo repair and antioxidants in plants. Flavonoid accumulates in epidermal layer of shoots and leaves on exposure to UV-B radiation and protects cell components by attenuating the impinging at the epidermis (Braun *et al.,* 1993; Olsson *et al.,* 1998). Over exposure to UV-B radiation leads to production of reactive oxygen species (ROS) and associated oxidative damage in plants. Phenols inhibit oxidative damage to macromolecules in the cells and regulates oxidative status of the cell *viz.,* suppressing hydrogen donor and singlet oxygen (Nagah and Seal, 2005; Yun *et al.,* 2008).

Decrease in chlorophyll pigment content (chlorophyll 'a', chlorophyll 'b' and total chlorophyll) was observed during exposure of plants under UV-B radiation in both the varieties (Table 27). However, black njavara (V_1) retained significantly higher chlorophyll content than yellow njavara (V_2) under UV-B radiation. In black njavara, the highest degradation of chlorophyll 'a', chlorophyll 'b' and total chlorophyll (37.02%, 45.71% and 39.64% respectively) were observed under treatment with UV-B radiation from vegetative stage (T_1) than control (T_4). Yellow njavara also recorded

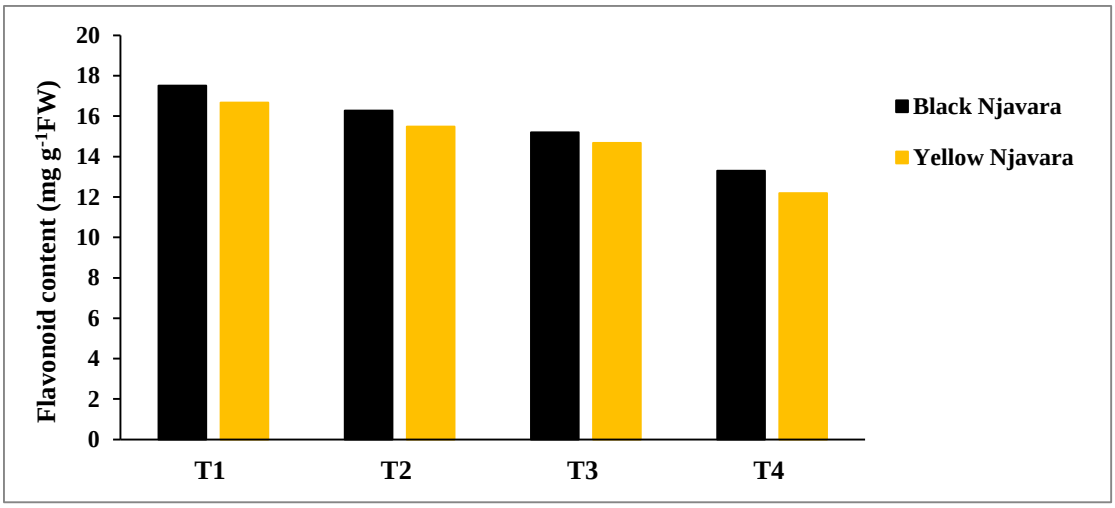


Figure 22: Effect of UV-B radiation on flavonoid content (mg g^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

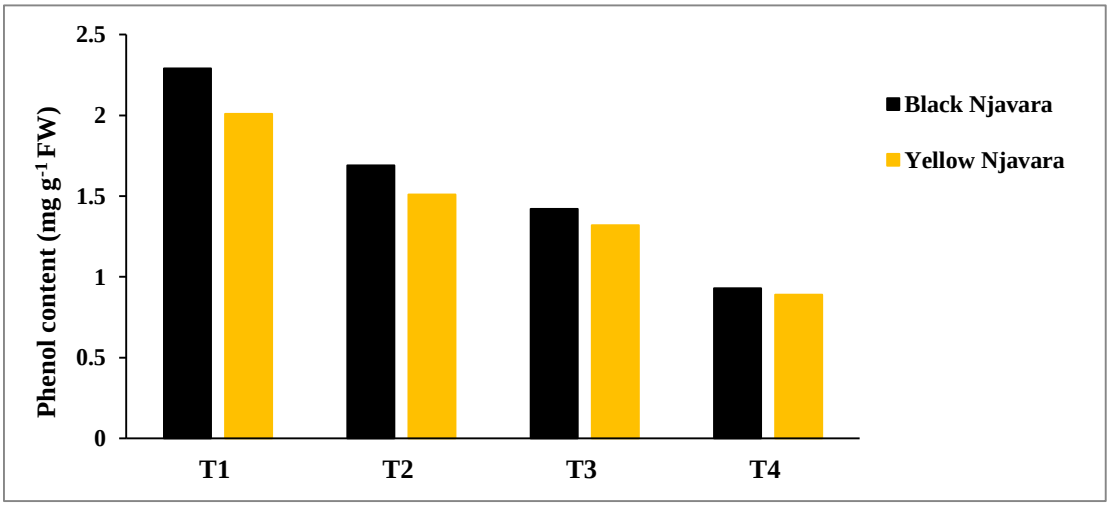


Figure 23: Effect of UV-B radiation on phenol content (mg g^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

Chlorophyll content (mg g^{-1} FW)							
	Mean Chl 'a'	**Mea Chl 'b'**	**Mean Total chl**	**Chloro -phyll a/b ratio**	**Chl 'a' % change over control**	**Chl 'b' % change over control**	**Total chl % change over control**
V_1T_1	1.41	0.54	1.95	2.62	-37.02	-45.71	-39.64
V_1T_2	1.54	0.70	2.24	2.19	-31.10	-29.04	-30.49
V_1T_3	1.69	0.65	2.34	2.59	-24.38	-34.09	-27.31
V_1T_4	2.24	0.99	3.22	2.26	0.00	0.00	0.00
V_2T_1	1.27	0.67	1.94	1.89	-32.80	-22.99	-29.57
V_2T_2	1.56	0.70	2.25	2.24	-17.13	-20.11	-18.02
V_2T_3	1.38	0.61	1.99	2.28	-26.69	-30.46	-27.75
V_2T_4	1.88	0.87	2.75	2.16	0.00	0.00	0.00
V_1	1.72	0.72	2.44	2.39			
V_2	1.52	0.71	2.23	2.14			
T_1	1.34	0.61	1.94	2.21			
T_2	1.55	0.70	2.25	2.21			
T_3	1.54	0.63	2.17	2.44			
T_4	2.06	0.93	2.99	2.23			

Table 27: Mean of chlorophyll content (mg g^{-1} FW) and a/b ratio of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control; V_1- Black Njavara; V_2- Yellow Njavara)

similar results wherein chlorophyll ‘a’ (32.80%), chlorophyll ‘b’ (22.99%) and total chlorophyll (29.57%) reduced under UV-B radiation treatment from vegetative stage (T_1) than control (T_4). There are studies indicating similar results in different plant species such as rice (Du *et al.,* 2011), *Amaranthus tricolor* (Kataria and Guruprasad, 2014), jack bean (Choi and Roh, 2003) and medicinal plant ‘*Tropaeolum majus’* (Germ *et al.,* 2015). However some studies have suggested that increase or decrease in chlorophyll content in response to UV-B radiation depend on the plant species (Sun and Payn, 1999; Barsig and Malz, 2000). UV-B radiation mainly damage to important enzymes ‘aminolevulinic acid’ and precursor ‘protochlorophyllide’ involved in chlorophyll biosynthesis pathway and inhibits the chlorophyll biosynthesis (Boddi *et al.,* 1995; Marwood and Greenberg, 1996; Ranjbarfordoei *et al.,* 2011). Some reports indicate that UV-B radiation significantly reduce carotenoid content, which play an important role in protecting chlorophyll from photo oxidation (Agrawal and Rathore, 2007; Mishra *et al.,* 2008; Cicek *et al.,* 2012). Hence in the present study, reduction in chlorophyll content under UV-B radiation treatment may be attributed due to the degradation of carotenoids.

In the present study proline content was observed to significantly vary between treatments and varieties (Table 23). Among the varieties, yellow njavara (V_2) recorded higher proline content than black njavara (V_1) under UV-B radiation treatments (Figure 24). Higher accumulation of proline content was observed under treatment UV-B radiation from vegetative stage (T_1) in black njavara (115.71%) as well as in yellow njavara (117.71%) than the control (T_4). Further, it was followed by treatment UV-B radiation from panicle initiation stage (T_2) and treatment UV-B radiation from flowering stage (T_3). Fedina *et al.* (2010) reported that under UV-B radiation, proline content increased by 78%, 54% and 28% in three rice varieties *viz.,* Norin 1, Surjamkhi and Sasanishiki respectively compared to control. Saradhi *et al.* (1995) found that the accumulation of proline under UV radiation is mainly to protect the cells from peroxidative damage which serves as an important factor for providing tolerance to UV

radiation in plants. Salama *et al.* (2011) reported that there are three possible reasons which cause accumulation of proline: first, proline synthesis from glutamic acids, which is dependent on the abscisic acid concentration, second, inhibition of proline oxidation to other soluble compounds and third, inhibition of protein synthesis.

The data on total flavonoid content in grains clearly showed that UV-B radiation significantly increased the flavonoid content in grains of both varieties (Table 24). In black njavara (V_1), the treatment T_2 (UV-B treatment from panicle initiation stage) recorded higher flavonoid content in grains followed by T_3 (UV-B treatment from flowering stage) by 27% and 10.87% respectively compared to control (T_4). Whereas least accumulation of flavonoid content in grains (1.81%) was recorded in T_1 (UV-B treatment from vegetative stage). Similarly in yellow njavara (V_2), UV-B treatment from panicle initiation stage (T_2) recorded higher flavonoid content in grains (22.54%) compared to control (T_4). However, under UV-B treatment from vegetative stage (T_1) and UV-B treatment from flowering stage (T_3) the increment in flavonoid in grains was same (7.92) in yellow njavara. The biosynthesis of flavonoids in plants due to increased expression of genes, such as *chalcone synthase* and *phenylalanine ammonialyase* of the phenylpropanoid pathway has been reported and there are many studies indicating that UV radiation can act as strong elicitor to increase the expression of these genes (Brosche *et al.*, 2002; Casati and Walbot, 2003; Hideg *et al.*, 2013). In the present study, treatment T_2 (UV-B treatment from panicle initiation stage) recorded highest flavonoid content in grains which revealed that UV-B radiation treatment from panicle initiation stage has acted as optimum elicitor to accumulate flavonoid in grains. However, treatment T_1 (UV-B radiation from vegetative stage) received higher dose of UV-B radiation throughout the growing period but could not produce high flavonoid content in the grains. Which may have been utilized in other functions where it screened harmful radiation and protected cells and cell organelles and helped in regulating stress responses by controlling auxin transport (Braun *et al.*, 1993; Olsson *et al.*, 1998; Winkel-Shirley, 2002).

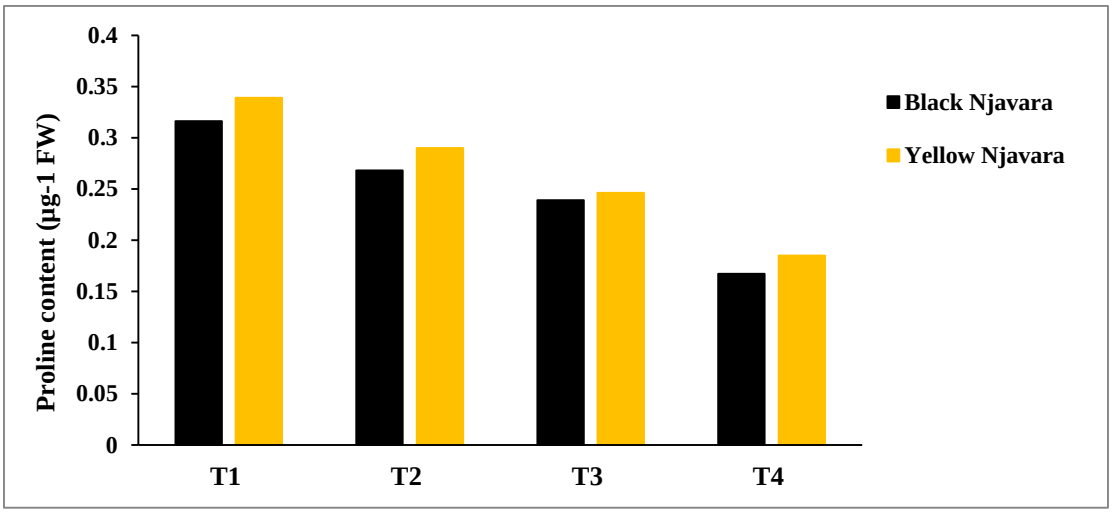


Figure 24: Effect of UV-B radiation on proline content (μg^{-1} FW) at different growth stages of njavara rice varieties (T_1- UV-B treatment from vegetative stage; T_2- UV-B treatment from panicle initiation stage; T_3- UV-B treatment from flowering stage; T_4- Control)

5.4 MOLECULAR ANALYSIS

The treatments which resulted in the highest accumulation of flavonoid content in grains were selected from experiment 1 (20% shade and 75% field capacity) and experiment 2 (UV-B radiation treatment from panicle initiation stage) for molecular analysis of both the varieties of njavara rice (black and yellow) in experiment 3 (Table 12 and 24). The plants were grown in pots and subjected to respective treatments and the results on protein profiling and gene expression studies are discussed below.

5.4.1 Protein profiling

In the present study, SDS-PAGE mediated protein profiling showed differential expression of leaf proteins in different treatments and varieties at grain filling stage (Plate 7). There are reports indicating that, UV-B radiation significantly reduced the expression of polypeptides ranging from 18-24 and 43-55 kDa in rice (Britto *et al.*, 2011), legumes (Shanthi and Janetta, 2015) and *Alaria esculenta* (Bischof *et al.*, 2000). In the present study also, under UV-B radiation treatment from panicle initiation stage (T_3), proteins of molecular weight between 55-48 kDa and 25-17 kDa were found to either exhibit lesser band intensity or absent in both the varieties. However, treatments 20% shade (T_1), 75% field capacity (T_2) and control (T_4) showed higher intensity of protein bands at molecular weight of 55 kDa and 16 kDa in both the varieties. UV-B radiation has been reported to denature proteins and damage nucleic acid which lead to reduction in the amount of many proteins. The damage to nucleic acids affect synthesis of proteins such as RuBisCO which constitute about 50% of the total soluble proteins in plants (Strid *et al.*, 1990). Caldwell (1993) and Kulandaivelu and Noorudeen, (1993) have reported the loss of 47 kDa polypeptide, due to damage to photosystem-II complex under UV radiation and similar results were observed in the present study also in both the varieties under UV-B radiation. Seidler (1994) reported that polypeptides of molecular weight 23 kDa and 17 kDa are associated with oxygen evolving machinery (water oxidizing complex). Also there are many investigations which

indicate that primarily under UV-B radiation, there will be damage to water oxidizing complex which result in the inactivation of the electron transport chain (Renger *et al.,* 1989; Vass *et al.,* 1996; Lidon *et al.,* 2012; Kataria *et al.,* 2014).

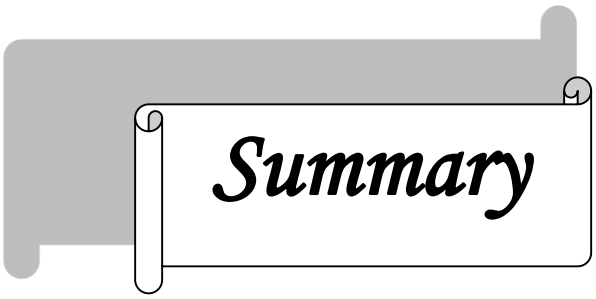
5.4.2 Gene expression study using qRT-PCR

Among the many secondary metabolite pathways in plants, flavonoid biosynthetic pathway is one of the best studied pathways and many important genes involved have been investigated in response to different stresses (Zhou *et al.,* 2018; Gharibi *et al.,* 2019; Sharma *et al.,* 2019). In the present study, comparison of expression patterns of rice flavonoid pathway genes *viz., CHS* and *CYP75B4*, in the seeds (at grain filling stage) of two njavara rice varieties (black and yellow) subjected to different treatments was carried out. Both the genes (*CHS* and *CYP75B4*) were expressed predominantly under T_3 (UV-B radiation treatment from panicle initiation stage) and T_2 (75% field capacity) and the least expression was found under T_1 (20% shade) condition in both the varieties (Figure 2 and 3).

Chalcone synthase (*CHS*) is the first enzyme in flavonoid biosynthetic pathway and later CHI (Chalcone isomerase) catalyze chalcone to naringenin, which is then converted into other flavonoid compounds under series of actions of different enzymes. Similarly, *CYP75B4* (*F3'H* gene family) play an important role in the production of different types of flavonoids specifically 'tricin' also. Park *et al.* (2016) reported that *F3'H* (Flavonoid 3'-hydroxylase) helps to add different types of flavonoids. Lam *et al.* (2015) reported that the rice *CYP75B4* mutants were unable to produce tricin whereas, transgenic Arabidopsis with *CYP75B4* accumulate flavones 'tricin' and concluded that *CYP75B4* also catalyzes 5'-hydroxylation of 3'-methoxylated flavone chrysoeriol to form selgin and later selgin gets converted into tricin with the help of O-methyltransferase. Vasquez-Robinet *et al.* (2008) in potato and Ma *et al.* (2014) in wheat reported that *CHS* and *F3'H (*Flavonoid 3'-hydroxylase) had higher expression during the period of drought stress. Also, Ryan *et al.* (2002) in Petunia and Casati and

Walbot, (2003) in maize showed higher expression of flavonoid biosynthesis genes under UV-B radiation treatment.

The present study also revealed that the expression of *CHS* and *CYP75B4* (*F3'H* gene family) were very low under treatment 20% shade (T_1) compared to control. Similar findings were reported in rice (Chen *et al.,* 2013), tea plant (Liu *et al.,* 2018) and *Syringa oblata* (Liu *et al.,* 2019). This results may be due to the different regulatory networks of individual gene activities in rice grain such as *PAL* and *CHS* genes in requirement for light exposure (Wu *et al.*, 2016; Liu *et al.,* 2017). Also in the present experiment, expression of both the (*CHS* and *CYP75B4*) were found higher in black njavara (V_1) than the yellow njavara (V_2). There are several studies indicating that the expression of important genes involved in flavonoid biosynthesis is higher in pigmented rice (like black, red and brown rice) than normal white rice (Chen *et al.,* 2013; Park *et al.,* 2016; Poulev *et al.,* 2019). This may be due to the reason that in pigmented rice grains, most of the flavonoids are derived from 3'4'-dihydroxylated leucocyanidin, (which is derived from 4′-hydroxylated leucopelargonidin) and is absent in pigmented rice, which implies that the activity of F3'H (Flavonoid 3'-hydroxylase) is prominent in pigmented rice grains (Park *et al.,* 2016).


Summary

6. SUMMARY

Rice is the basic food for more than a billion people all over the world. There are several different medicinal varieties of rice being cultivated and used in various parts of the world. Njavara rice (a medicinal rice) indigenous to Kerala, India, is a unique grain plant in the *Oryza* genus and has been cultivated over 2500 years. The ancient Ayurvedic texts mentioned detail information about nutritional, medicinal qualities and use of Njavara especially in Panchakarma treatment in "Njavara Kizhi" and "Njavara Theppu". It is used in the treatment of various diseases related to circulatory, respiratory and digestive ailments in traditional medicine. The isolated flavonoid compound from njavara rice have shown good antioxidant activity and anti-inflammatory effect in carrageenan-induced rat paw edema. The antioxidant properties of njavara rice also help to maintain the sugar level of diabetic patients. Recently, it is reported that njavara rice has got anti-cancer properties too. Also an anti-cancer gene associated with 'Bowman-Brisk trypsin inhibitor protein' has been identified in njavara rice.

Abiotic stresses *viz.,* temperature, humidity, light intensity, water, CO_2, ultraviolet light and minerals are found to enhance the accumulation of almost all classes of secondary metabolites such as simple and complex phenols, flavonoids as well as different kinds of terpenes and alkaloids. These abiotic factors are widely used as elicitors to increase the production or to induce de novo synthesis of secondary metabolites under *in vitro* system. A number of studies have shown that different kinds of elicitors could increase the secondary metabolite production in cell, tissue and organ culture. However, depending on physiological and developmental stages of the plant, the production of secondary metabolites varies greatly. Thus, the present study was proposed to understand physiological, biochemical and molecular attributes associated to secondary metabolites accumulation due to abiotic stresses *viz.,* shade, drought and

UV-B stress in medicinal rice njavara. The study consisted of three separate experiments.

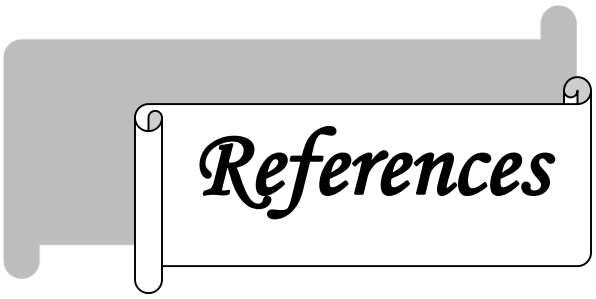
Experiment one was a pot culture study laid out in CRD with two varieties of njavara rice and five treatments. The study included a combination of two levels of shade stress (20% shade and 40% shade), two levels of water deficit stress (50% field capacity and 75% field capacity) and a control. The observations were taken at different critical stages of the crop *viz.,* vegetative stage, panicle initiation stage, flowering stage and harvesting stage. The results revealed that the morphological characters such as plant height, leaf area index and specific leaf area were higher under 40% shade (T_2) whereas, under 50% field capacity (T_3), they were found to be highly reduced in both varieties. However, number of tillers per plant was reduced under shaded and water deficit conditions compared to control. Photosynthetic rate, stomatal conductance and transpiration rate were found to significantly reduce at all growth stages studied under shade and water deficit condition compared to the control and the maximum reduction was recorded under 50% field capacity (T_3) followed by 40% shaded condition (T_2) compared to the control (T_5). Among the varieties, yellow njavara recoded lowest photosynthetic rate, stomatal conductance and transpiration rate than black njavara. The biochemical characters, like chlorophyll content (chlorophyll 'a', 'b' and total chlorophyll) was found higher under 40% shaded condition (T_2) at all growth stages studied in both the varieties. However, shade conditions exhibited lower chlorophyll a/b ratio and higher chlorophyll 'b' content. Flavonoids and phenol content of leaves were found higher under 50% field capacity (T_3) and lower under 40% shade (T_2) condition at all the growth stages studied. The proline content was found to significantly increase under both shade and water deficit treatments in both the varieties. The total flavonoid content in the grains was found higher under treatment 75% field capacity (T_4) in black njavara (16.23%) and yellow njavara (39.76%) followed by treatment 20% shade (T_1) by 3.82 percent and 26.36 percent in both the varieties respectively compared to control.

The second experiment was carried out in pot culture in CRD with four treatments. The crop was subjected to UV-B (280-320 nm) radiation with the help of UV-B fluorescent tubes during the different critical stages of the crop i.e. from vegetative stage, from panicle initiation stage and from flowering stage till harvesting in ventilated polyhouse. The UV-B tubes were switched on for 4 hours daily from 10 am to 2 pm and the average intensity of UV-B radiation at the canopy level of plants was maintained at 4 Wm^{-2}. The control was maintained in another compartment of polyhouse without UV-B tubes. The results showed that the morphological characters *viz.,* plant height, leaf area index, specific leaf area and tiller number reduced significantly under UV-B radiation treatment and the maximum reduction was observed in treatment T_1 (UV-B treatment from vegetative stage) in both the varieties. Leaf gas exchange parameters *viz.,* photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll content decreased significantly under UV-B radiation treatments compared to the treatment without UV-B radiation. However flavonoid, phenol and proline contents which are having protective function to UV-B radiation were found to increase under all UV-B treatments. The accumulation of total flavonoid in grains was found significantly higher in UV-B radiation treatments in both the varieties. In black njavara (V_1), the treatment T_2 (UV-B treatment from panicle initiation stage) recorded highest increase in flavonoid content (27%) compared to control (T_4). Similarly, in yellow njavara, flavonoid content in grains increased by 22.54 percent compared to control (T_4).

The experiment three was conducted with three treatments selected based on the accumulation of flavonoids in grains from experiment 1 (20% shade and 75% field capacity) and experiment 2 (UV-B radiation treatment from panicle initiation stage). The protein profiling and gene expression studies were carried out during grain filling stage of the crop. SDS-PAGE mediated protein profiling showed differential expression of leaf proteins in different treatments and in the treatment T_3 (UV-B radiation from panicle initiation stage), proteins of molecular weight between 55-48

kDa and 25-17 kDa were found to either exhibit lesser band intensity or absent in both the varieties. However, treatments 20% shade (T_1), 75% field capacity (T_2) and control (T_4) showed higher intensity of protein bands of molecular weight 55 kDa and 16 kDa in both the varieties. In the present study, comparison of expression patterns using qRT-PCR of rice flavonoid pathway genes (*CHS* and *CYP75B4*) in the seeds (at grain filling stage) of both the njavara rice varieties was carried out. The results revealed that the expression of both CHS and *CYP75B4* genes were higher in black glumed njavara than the yellow glumed njavara. Among the different treatments, in T_3 (UV-B radiation treatment from panicle initiation stage) and T_2 (75% field capacity) both these genes were over expressed. However in T_1 (20% shade) both the genes (*CHS* and *CYP75B4)* were down regulated in both njavara rice varieties.

The present results revealed that, based on growth, physiological and biochemical characters, black njavara performed better under different combination of stresses than the yellow njavara. This result also reflected on the accumulation of flavonoids in grains in which black njavara grains recorded higher flavonoid content compared to yellow njavara grains. However chlorophyll content and proline content were found to have negative influence on the flavonoid content of grains, which were higher in yellow njavara as compared to black njavara. Further, this results were also confirmed by molecular analysis. SDS-PAGE mediated protein profiling showed higher intensity of protein bands in black njavara compared to yellow njavara. Expression patterns using qRT-PCR of rice flavonoid pathway genes were also found higher in black njavara. Based on the current results it is concluded that, the black glumed njavara performed better than yellow glumed njavara under all the stress conditions studied and can be exploited better for its therapeutic value. Application of mild stress levels *viz.*, water deficit (75% field capacity) or UV-B radiation treatment from panicle initiation stage may be utilized to enhance the medicinal quality of this crop.


References

7. REFERENCES

Acreche, M.M., Briceno-Félix, G., Sánchez, J., and Slafer, G. 2009. Grain number determination in an old and a modern Mediterranean wheat as affected by pre-anthesis shading. *Crop Pasture Sci.* 60: 271-279.

Agrawal, S.B. and Rathore, D. 2007. Changes in oxidative stress defense system in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. *Environ. Exp. Bot.* 59: 21-33.

Ahmad, F., Din, S., Perveen, A., and Afzal, M. N. 2013. Investigating critical growth stage of cotton subject to water deficit stress. *Iranian J. Plant Physiol.* 4(1): 873-880.

Ahmadikhah, A. and Marufinia, A. 2016. Effect of reduced plant height on drought tolerance in rice. *3 Biotech*. 6: 221.

Ajum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., and Lei, W. 2011. Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.* 6(9): 2026-2032.

Akula, R. and Gokare, A. R. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling Behav.* 6(11): 1720-1731.

Akula, R. and Ravishankar, G.A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* 6: 1720-1731.

Alagupalamuthirsolai, M., Ankegowda, S.J., and Krishnamurthy, K.S. 2018. Effect of different shade levels on growth, physiology and biochemical characteristics of

small cardamom (*Elettaria cardamomum* Maton). *Curr. J. App. Sci. Technol.* 28(3): 1-9.

Alghabari, F. and Ihsan, M.Z. 2018. Effects of drought stress on growth, grain filling duration, yield and quality attributes of barley (*Hordeum vulgare* L.). *Bangladesh J. Bot.* 47(3): 421-428.

Allen, D.J., Nogues, S., and Baker, N.R. 1998. Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *J. Exp. Bot.* 49: 1775-1788.

Allen, D.J., Nogues, S., and Baker, N.R. 1998. Ozone depletion and increased UV-B radiation: Is there a real threat to photosynthesis? *J. Exp. Bot.* 49(328): 1775-1788.

Alridiwirsah, E.M., Harahap, Akoeb, E.N., and Hanum, H. 2018. Growth and production of new superior rice varieties in the shade intensity. Earth Environ. Sci. 122: 01202.

Ambasht, N.K. and Agrawal, M. 1998. Influence of supplemental UV-B radiation on photosynthetic characteristics of rice plants. *Photosynthetica* 34: 401-408.

Ancillotti, C., Bogani, P., Biricolti, S., Calistri, E., Checchini, L., Ciofi, L., Gonnelli, C., Bubba, M.D. 2015. Changes in polyphenol and sugar concentrations in wild type and genetically modified Nicotiana langsdorffii Weinmann in response to water and heat stress. *Plant Physiol. Biochem.* 97: 52-61.

Anjum, S.A., Ashraf, U., Zohaib, A., Tanveer, M., Naeem, M., Ali, I., Tabassum, T., and Nazir, U. 2017. Growth and developmental responses of crop plants under drought stress: a review. *Zemdirbyste Agric.* 104(3): 267-276.

Anjum, S.A., Jian-hang, N., Ran, W., Jin-huan, L., Mei-ru, L., Ji-xuan, S., Jun, L., Zohaib, A., San-gen, W., and Xue-feng, Z. 2016. Regulation mechanism of

exogenous 5-aminolevulinic acid on growth and physiological characters of *Leymus chinensis* (Trin.) under high temperature stress. *Philippine Agric. Sci.* 99(3): 253-259.

Arite, T., Iwata, H., Ohshima, K., Maekawa, M., Nakajima, M., Arite, T., Iwata, H., Ohshima, K., Maekawa, M., Nakajima, M., Kojima, M., Sakakibara, H., and Kyozuka, J. 2007. $DWARF_{10}$, an $RMS_1/MAX_4/DAD_1$ ortholog, controls lateral bud outgrowth in rice. *Plant J.* 51(6): 1019-1029.

Ashraf, A.M. and Lokanadan, S. 2017. A review of rice landraces in India and its inherent medicinal values -the nutritive food values for future. *Int. J. Curr. Microbiol. App. Sci.* 6(12): 348-354.

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

Ashraf, M. and Harris, P.J.C. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 166: 3-16.

Aswatha, R.H.N., Shreedhara, C.S., Falguni, G.P., and Sachin, Z.B. 2008. Antioxidant studies of aqueous extract of Phyllanthus reticulates poir. *Pharmacologyonline* 1: 351-364.

Aumonde, T.Z., Pedó, T., Borella, J., Amarante, L., and Villela, F.A. 2013. Seed vigor, antioxidant metabolism and initial growth characteristics of red rice seedlings under different light intensities. *Acta Botanica Brasilica* 27(2): 311-317.

Babatunde, O. and Oseni, K. 2016. Optimization and response surface modelling of antioxidant activities of *Amaranthus Virides* seed flour extract. *Ann. J. Food Sci. Techn.* 17(1): 114-123.

Baig, M.J., Anand, A., Mandal, P.K., and Bhatt, R.K. 2005. Irradiance influences contents of photosynthetic pigments and proteins in tropical grasses and legumes. *Photosynthetica* 43: 47-53.

Bakiyalakshmi, S.V. and Boominathan, M. 2014. In-Vitro pharmacological activity of flavonoid isolated from Njavara (*Oryza sativa*). *BioMed. Res. J.* 1(1): 1-10.

Bandurska, H., Pietrowska-Borek, M. and Cieslak, M. 2012. Response of barley seedlings to water deficit and enhanced UV-B irradiation acting alone and in combination. *Acta Physiol. Plant* 34: 161-171.

Barmudoi, B. and Bharali, B. 2016. Effects of light intensity and quality on physiological changes in winter rice (*Oryza sativa* L.). *Int. J. Environ. Agric. Res.* 2(3): 65-76.

Barsig, M. and Malz, R. 2000. Fine structure, carbohydrates and photosynthetic pigments of sugar maize leaves under UV-B radiation. *Environ. Exp. Bot.* 43(2): 121-130.

Basahi, J.M., Ismail, I.M., and Hassan, I.A. 2014. Effects of enhanced UV-B radiation and drought stress on photosynthetic performance of lettuce (*Lactuca sativa* L. Romaine) plants. *Ann. Res. Rev. Biol.* 4(11): 1739-1756.

Bennett, R.N. and Wallsgrove, R.M. 1994. Secondary metabolites in plant defense mechanisms. *New Phytol.* 127: 617-633.

Bergquist, S.A.M., Gertsson, U.E., Nordmark, L.Y.G., and Olsson, M.E. 2007. Effects of shade nettings, sowing time and storage on baby spinach flavonoids. *J. Sci. Food Agric.* 87: 2464-2471.

Bhatt, R.M. and Rao, N.K.S. 2005. Influence of pod load response of okra to water stress. *Ind. J. Plant Physiol.* 10(1): 54-59.

Bhattacharya, R., Pal, S., Bhoumick, A., and Barman, P. 2012. Annual arability and distribution of ultraviolet index over India using TEMIS data. *Int. J. Engng. Sci. Technol.* 4(11): 4577-4583.

Bilger, W., Veit, M., Schreiber, L., and Schreiber, U. 1997. Measurement of leaf epidermal transmittance of UV radiation by chlorophyll fluorescence. *Physiol. Plant* 101: 754-763.

Bischof, K., Hanelt, D., and Wiencke, C. 2000. Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* 211: 555-562.

Blaustein, A.R. and Searle, C. 2013. *Ultraviolet radiation, Encyclopedia of Biodiversity* (2nd Ed.). Academic Press, Kidlington, Oxford, UK, 296-303p.

Blokhina, O., Virolainen, E., and Fagerstedt, KV. 2003. Antioxidants, oxidative damage and oxygen deprivation stress. *Ann. Bot.* 91: 179-194.

Boddi, B., Oravecz, A. R., and Lehoczki, E. 1995. Effect of cadmium on organization and photoreduction of protochlorophyllide in dark-grown leaves and etioplast inner membrane preparations of wheat. Photosynthetica 31(3): 411-420.

Boeger, M.R.T. and Poulson, M. 2016. Effects of ultraviolet-B radiation on leaf morphology of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *Acta Bot. Bras.* 20(2): 329-338.

Bolat, I., Dikilitas, M., Ercisli, S., Ikinci, A., and Tonkaz, T. 2014. The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and quince rootstocks. *The Scientific World J.* 2014: 769732.

Bota, J., Flexas, J., and Medrano, H. 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol.* 162: 671-681.

Botwright-Acuna, T.L., Lafitte, H.R., and Wade, L.J. 2008. Genotype and environment interactions for grain yield of upland rice backcross lines in diverse hydrological environments. *Field Crops Res.* 108(2): 117-125.

Bouman, B.A.M., Peng, S., Castaòeda, A.R., and Visperas, R.M. 2005. Yield and water use of irrigated tropical aerobic rice systems. *Agric. Water Manag.* 74(2): 87-105.

Braca, A., Sortino, C., Politi, M., Morelli, I., and Mendez, J. 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethanopharmacol.* 70: 379-381.

Braun, J. and Tevini, M. 1993. Regulation of UV-B protective pigment synthesis in the epidermal layer of Rye seedlings (*Secale cereale* L. cv. Kustro). *Photochem. Photobiol.* 57: 318-323.

Brazier-Hicks, M., Evans, K.M., Gershater, M.C., Puschmann, H., Steel, P.G., and Edwards, R. 2009. The C-glycosylation of flavonoids in cereals. *J. Biol. Chem.* 284: 17926-17934.

Britto, A.J.D., Sujin R.M., and Sebastian, S.R. 2011. Morphological and molecular variation of five rice varieties to ultra violet-B radiation stress. *J. Stress Physiol. Biochem.* 7(3): 80-86.

Brosche, M., Schuler, M.A., Kalbina, I., Connor, L., and Strid, A. 2002. Gene regulation by low level UV-B radiation: identification by DNA array analysis. *Photochem. Photobiol. Sci.* 1: 656-664.

Burke, J.J., Steinback, K.E., and Arntzen, C.J. 1979. Analysis of light harvesting pigment-protein complexes of wild type and a chlorophyll-b-less mutant of barley. *Plant Physiol.* 63: 237-243.

Caldwell, C.R. 1993. Ultraviolet-induced photodegradation of cucumber (*Cucumis sativus* L.) microsomal and soluble protein tryptophanyl residues in vitro. *Plant Physiol.* 101: 947-953.

Caldwell, M.M., Bornman, J.F., Ballare, C.L., Flint, S.D., and Kulandaivelu, G. 2007. Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. *Photochem. Photobiol. Sci.* 6: 252-266.

Carlo D.G., Mascolo, N., Izzo, A.A., Capasso, F. 1999. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. *Life Sci.* 65: 337-353.

Casati, P. and Walbot, V. 2003. Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. *Plant Physiol.* 132: 1739-1754.

Cen, Y.P. and Bornman, J.F. 1990. The response of bean plants to UV-B radiation under different irradiances of background visible light. *J. Exp. Bot.* 41: 1489-1495.

Chauhan, B.S. 2015. Effect of shade on growth and yield of weedy rice (*Oryza sativa* L.) biotypes and a rice (*Oryza sativa* L.) cultivar from Asia. *J. Crop Improv.* 27(3): 272-280.

Cha-um, S., Yooyongwech, S., and Supaibulwatana, K. 2010. Water deficit stress in the reproductive stage of four Indica rice (*Oryza sativa* L.) genotypes. *Pak. J. Bot.* 42(5): 3387-3398.

Chen, J.M., and Cihlar, J. 1996. Retrieving leaf area index for boreal conifer forests using Landsat TM images. *Remote Sensing Environ.* 55: 153-162.

Chen, X., Itani, T., Wu, X., Chikawa, Y., and Irifune, K. 2013. Physiological factors affecting transcription of genes involved in the flavonoid biosynthetic pathway in different rice varieties. *Plant Signaling Behav.* 8: e27555.

Chen, Z.J., Wu, J.Q., Ma, Y., Wang, P., Gu, Z.X., and Yang, R.Q. 2018. Advances in biosynthesis, regulation and bioactivity of phenolic acids in plant food raw materials. *Food Sci.* 39: 321-328.

Cheng, J., Chu, P., Chen, D., Bai, Y., and Niu, S. 2016. Functional correlations between specific leaf area and specific root length along a regional environmental gradient in Inner Mongolia grasslands. *Funct. Ecol.* 30: 985-997.

Cheynier, V., Comte, G., Davies, K.M., Lattanzio, V., and Martens, S. 2013. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem.* 72: 1-20.

Choi, B.Y. and Roh, K.S. 2003. UV-B Radiation Affects Chlorophyll and Activation of Rubisco by Rubisco Activase in *Canavalia ensiformis* L. Leaves. *J. Plant Biol.* 46(2): 117-121.

Chun, S.C., Paramasivan, M., and Chandrasekaran, M. 2018. Proline accumulation influenced by osmotic stress in arbuscular *Mycorrhizal* symbiotic plants. *Front. Microbiol.* 9: 2525.

Chung, I.M., Park, M.R., Chun, J.C., and Yun, S.J. 2003. Resveratrol accumulation and resveratrol synthase gene expression in response to abiotic stresses and hormones in peanut plants. *Plant Sci.* 164: 103-109.

Chutipaijit, S., Cha-Um, S., and Sompornpailin, K. 2012. An evaluation of water deficit tolerance screening in pigmented Indica rice genotypes. *Pak. J. Bot.* 44(1): 65-72.

Cicek, N., Fedina, I., Cakirlar, H., Velitchkova, M., and Georgieva, K. 2012. The role of short term high temperature pretreatment on the UV-B tolerance of barley cultivars. *Turk. J. Agric. For.* 36: 153-165.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B. Steege, H.T., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G., and Poorter, H. 2003. Handbook of protocols for standardized and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51: 335-380.

Cornic, G. and Massacci, A. 1996. Leaf photosynthesis under drought stress. In: Baker, N.R. (ed.), *Photosynthesis and the Environment.* Kluwer Academic Publishers, The Netherlands.

Czeczuga, B. 1987. Carotenoid contents in leaves grown under various light intensities. *Biochem. Sys. Ecol.* 15: 523-527.

Dai, Y., Shen, Z., Liu, Y., Wang, L., Hannaway, D., and Lu, H. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environ. Exp. Bot.* 65: 177-182.

Dalirie, M.S., Sharifi, R.S., and Farzaneh, S. 2010. Evaluation of yield, dry matter accumulation and leaf area index in wheat genotypes as affected by terminal drought stress. *Notulae Botanicae Horti. Agrobotanici Cluj-Napoca.* 38(1): 182-186.

Dar, R.A., Shahnawaz, M., and Qazi, P.H. 2017. General overview of medicinal plants: A review. *J. Phytopharmacol.* 6(6): 349-351.

Davidson-Hunt, I. 2000. Ecological ethno botany: stumbling toward new practices and paradigms. *MASA J.* 16: 1-13.

Deepa, G., Singh, V., and Naidu, K.A. 2008. Nutrient composition and physicochemical properties of Indian medicinal rice-Njavara. *Food Chem.* 106: 165-171.

Deepa, G., Venkatachalam, L., Bhagyalakshmi, N., Shashidhar, H.E., Singh, V., and Naidu, K.A. 2009. Physicochemical and genetic analysis of an endemic rice variety, Njavara (*Oryza sativa* L.), in comparison to two popular south Indian cultivars, Jyothi (PTB 39) and IR 64. *J. Agric. Food Chem.* 57: 11476-11483.

Delouche, J.C., Burgos, N.R., Gealy, D.R., de San Martin, G.Z., Labrada, R., Larinde, M., and Rosell, C. 2007. Weedy rice-origin, biology, ecology, and control. FAO Plant Production and Protection Paper 188. Rome: Food and Agriculture Organization.

Deng, F., Wang, L., Yao, X., Wang, J.J., Ren, W.J., and Yang, W.Y. 2009. Effects of different-growing-stage shading on rice grain-filling and yield. *J. Sichuan Agric. Univ.* 27(3): 265-269.

Deng, Y.M., Li, C.C., Shao, Q.S., Ye, X.Q., and She, J.M. 2012. Differential responses of double petal and multi petal jasmine to shading: I. Photosynthetic characteristics and chloroplast ultrastructure. *Plant Physiol. Biochem.* 55: 93-102.

De Souza, P. I., Egli, D. B., and Bruening, W. P. 1997. Water stress during seed filling and leaf senescence in soybean. *Agron. J.* 89(5): 807-812.

Devi, R.R. and Arumughan, C. 2007. Antiradical efficacy of phytochemical extracts from defatted rice bran. *Food Chem. Toxicol.* 45: 2014-2021.

Dhivyapriya, D., Kalamani, A., Ramchander, S., Raveendran, M., and Robin, S. 2016. Estimation of gas exchange parameters in backcross introgressed lines of rice (*Oryza sativa* L.) with different combinations of drought QTLs. *App. Biol. Res.* 18(2): 106-114.

Diaz-Napal, G.N., Defago, M., Valladares, G., and Palacios, S. 2010. Response of Epilachna paenulata to two flavonoids, Pinocembrin and quercetin, in a comparative study. *J. Chem. Ecol.* 36: 898-904.

Dicosmo, F. and Misawa, M. 1985. Eliciting secondary metabolism in plant cell cultures. *Trends Biotechnol.* 3: 318-322.

Dobrikova, A.G., Vassilena, K., and Emilia, L.A. 2013. Damage and protection of the photosynthetic apparatus from UV-B radiation. I. Effect of ascorbate. *J. Plant Physiol.* 170: 251-257.

Du, H., Liang, Y., Pei, K., and Ma, K. 2011. UV Radiation-responsive proteins in rice leaves: A proteomic analysis. *Plant Cell Physiol.* 52(2): 306-316.

Elsy, C.R., Rosamma, C.A., and Potty, N.N. 1992. Njavara-A rice variety with special characters. *Oryza* 29(1): 55-56.

Emmanuel, G.A. and Mary, D.M. 2014. Effect of light intensity on growth and yield of a Nigerian local rice variety-Ofada. *Int. J. Plant Res.* 4(4): 89-94.

Ennajeh, M., Vadel, A.M., Cochard, H., and Khemira, H. 2010. Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *J. Hortic. Sci. Biotechnol.* 85(4): 289-294.

Evans, J.R. and Poorter, H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ.* 24: 755-767.

Fabre, D., Adriani, D.E., Dingkuhn, M., Ishimaru, T., Punzalan, B., Lafarge, T., Clément-Vidal, A., and Luquet, D. 2016. The *qTSN4* effect on flag leaf size, photosynthesis and panicle size, benefits to plant grain production in rice, depending on light availability. *Front. Plant Sci.* 7: 623.

Fan, Y., Chen, J., Cheng, Y., Raza, M.A., Wu, X., Wang, Z., Liu, Q., Wang, R., Wang, R., Yong, T., Liu, W., Liu, J., Du, J., Shu, K., Yang, W., and Yang, F. 2018. Effect of shading and light recovery on the growth, leaf structure, and photosynthetic performance of soybean in a maize-soybean relay-strip intercropping system. *PLoS ONE* 13(5): 1-15.

Farahani, H., Valadabadi, A., Daneshian, J., and Khalvati, M. 2009. Medicinal and aromatic plants farming under drought conditions. *J. Hort. Forestry.* 1(6): 86-92.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. 2009. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.* 29. 185-212.

Farquhar, G.D. and Sharkey, T.D. 1982. Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.* 33: 317-345.

Fathi, A. and Tari, D.B. 2016. Effect of drought stress and its mechanism in plants. *Int. J. Life Sci.* 10(1): 1-6.

Favaretto, V.F., Martinez, C.A., Soriani, H.H., Furriel, R.P.M. 2011. Differential responses of antioxidant enzymes in pioneer and late-successional tropical tree species grown under sun and shade conditions. *Environ. Exp. Bot.* 70: 20-28.

Fedina, I., Hidema, J., Velitchkova, M., Georgieva, K., and Nedeva, D. 2010. UV-B induced stress responses in three rice cultivars. *Biologia Plantarum* 54(3): 571-574.

Feng, L., Raza, M.A., Li, Z., Chen, Y., Khalid, M.H.B., Du, J., Liu, W., Wu, X., Song, C., Yu, L., Zhang, Z., Yuan, S., Yang, W., and Yang, F. 2019. The Influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. *Front. Plant Sci.* 9: 1952.

Fening, J.O., Quansah, C., and Sarfo-Kantanka, A. 2009. Response of three forage legumes to soil moisture stress. *J. Sci. Technol.* 29(3): 24-30.

Foyer, C.H. and Fletcher, J.M. 2001. Plant antioxidants: colour me healthy. *Biologist.* 48: 115-120.

Fu, J. and Huang, B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.* 45: 105-114.

Fu, J. and Huang, B. 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.* 45: 105-114.

Furness, N., Upadhyaya, M.K., and Ormrod, D.P. 1999. Seedling growth and leaf surface morphological responses of three rangeland weeds to ultraviolet-B radiation. *Weed Sci.* 47: 427-434.

Galland, M., Boutet-Mercey, S., Lounifi, I., Godin, B., Balzergue, S., Grandjean, O., Morin, H., Perreau, F., Debeaujon, I., and Rajjou, L. 2014. Compartmentation and dynamics of flavone metabolism in dry and germinated rice seeds. *Plant Cell Physiol.* 55(9): 1646-1659.

Germ, M., Spahic, I., and Gaberščik, A. 2015. Morphological, biochemical and physiological responses of Indian cress (*Tropaeolum majus*) to elevated UV-B radiation. *Periodicum Biologorum* 117(3): 357-364.

Gharibi, S., Tabatabaei, B.E.S., Saeidi, G., Talebi, M., and Matkowski, A. 2019. The effect of drought stress on polyphenolic compounds and expression of flavonoid biosynthesis related genes in Achillea pachycephala Rech.f. *Phytochem.* 162: 90-98.

Gibson, K.D., Fischer, A.J., and Foin, T.C. 2004. Compensatory responses of late water grass (*Echinochloa phyllopogon*) and rice to resource limitations. *Weed Sci*. 52(2): 271-280.

Ginting, J., Damanik, B.S.J., Sitanggang, J.M., and Muluk, C. 2015. Effect of shade, organic materials and varieties on growth and production of upland rice. *Int. J. Sci. Technol. Res.* 4(1): 68-74.

Gonçalves, J.F.C.G., Barreto, D.C.D.S., Junior, U.M.D.S., Fernandes, A.V., Sampaio, P.T.B., and Buckeridge, M.S. 2005. Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaeodora* Ducke) under different light intensities. *Braz. J. Plant Physiol.* 17(3): 325-334.

Green, A.E.S., Sawada, T., and Shettle, E.P. 1974. The middle ultraviolet reaching the ground. *Photochem. Photobiol.* 19: 251-559.

Gunn, S., Farrar, J. F., Collis, B. E., and Nason, M. 1999. Specific leaf area in barley: Individual leaves versus whole plants. *New Phytol.* 143: 45-51.

Gunn, S., Farrar, J.F., Collis, B.E., and Nason, M. 1999. Specific leaf area in barley: Individual leaves versus whole plants. *New Phytol.* 143: 45-51.

Habibi, G. 2018. Effects of mild and severe drought stress on the biomass, phenolic compounds production and photochemical activity of *Aloe vera* (L.) Burm.f. *Acta Agric. Slovenica*. 111(2): 463-476.

Hakala, K., Jauhiainen, L., Koskela, T., Kayhko, P., and Vorne, V. 2002. Sensitivity of crops to increased ultraviolet radiation in northern growing conditions. *J. Agron. Crop Sci.* 188: 8-18.

Hakala, K., Jauhiainen, L., Koskela, T., Kayhko, P., and Vorne, V. 2002. Sensitivity of crops to increased ultraviolet radiation in northern growing conditions. *J. Agron. Crop Sci.* 188: 8-18.

Handa, N., Kohli, S.K., Sharma, A., Thukral, A.K., Bhardwaj, R., Abd_Allah, E.F., Alqarawi, A.A., and Ahmad, P. 2019. Selenium modulates dynamics of antioxidative defence expression, photosynthetic attributes and secondary metabolites to mitigate chromium toxicity in *Brassica juncea* L. plants. *Environ. Exp. Bot.* 161: 180-192.

Hare, P.D. and Cress, W.A. 1997. Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul.* 21: 79-102.

Hartman, F.C. and Harpel, M.R. 1994. Structure, function, regulation, and assembly of DRibulose 1, 5-bisphosphate carboxylase oxygenase. *Annu. Rev. Biochem.* 63: 197-234.

Hassan, I.A., Basahi, J.M., Haiba, N.S., and Kadi, M.W. 2013. Investigation of climate changes on metabolic response of plants; Interactive effect of drought stress and excess UV-B. *J. Earth Sci. Clim. Change* 4: 129.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., and Ahmad, A. 2012. Role of proline under changing environments. *Plant Signaling Behav*. 7(11): 1456-1466.

Hazra, B., Biswas, S., and Mandal, N. 2008. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *Complem. Altern. Med.* 8: 63-72.

He, J.S., Fang, J., Wang, Z., Guo, D., Flynn, D.F.B., and Geng, Z. 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia* 149: 115-122.

Henry, A., Wehler, R., Grondin, A., Franke, R., and Quintana, M. 2016. Environmental and physiological effects on grouping of drought-tolerant and susceptible rice varieties related to rice (*Oryza sativa*) root hydraulics under drought. *Ann. Bot.* 118: 711-724.

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

Hetherington, A.M., Woodward, F.I. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424: 901-908.

Hideg, E., Barta, C., Kalai, T., Vass, I., Hideg, K., and Asada, K. 2002. Detection of singlet oxygen and superoxide with fluorescent sensors in leaves under stress by photoinhibition or UV radiation. *Plant Cell Physiol.* 43: 1154-1164.

Hideg, E., Jansen, M.A.K., and Strid, A. 2013. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? *Trends Plant Sci.* 18(2): 107-115.

Hidema, J., Zhang, W.-H., Yamamoto, M., Sato, T., and Kumagai, T. 2005. Changes in grain size and grain storage protein of rice (*Oryza sativa* L.) in response to elevated UV-B radiation under outdoor conditions. *J. Radiat. Res.* 46: 143-149.

Hiscox, J.D. and Israelstam, G.F. 1979. A method for extraction of chlorophylls from leaf tissue without maceration. *Can. J. Bot.* 57: 1332-1334.

Hoekstra, F.A., Golovina E.A., and Buitink, J. 2001. Mechanisms of plant desiccation tolerance. *Trends Plant Sci.* 6: 431-438.

Hoekstra, F.A., Golovina, E.A., and Buitink, J. 2001. Mechanisms of plant desiccation tolerance. *Trends Plant Sci.* 6: 431-438.

Holley, J. and Cherla, K. 1998. The Medicinal Plants Sector in India. International Development Research Centre. South Asia Regional Office, IDRC, Canada Medicinal and Aromatic Program in Asia (MAPPA), New Delhi, India.

Hopkins, L., Bond, M., and Tobin, A. 2002. Ultraviolet-B radiation reduces the rates of cell division and elongation in the primary leaf of wheat (*Triticum aestivum* L. cv Maris Huntsman). *Plant Cell Environ.* 25: 617-624.

Hura, T., Hura, K., Grzesiak, M., Rzepka, A. 2007. Effect of long-term drought stress on leaf gas exchange and fluorescence parameters in C_3 and C_4 plants. *Acta Physiol. Plant* 29:103-113.

Hussain, M., Malik, M.A., Farooq, M., Ashraf, M.Y., and Cheema, M.A. 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* 194: 193-199.

Hussain, M., Malik, M.A., Farooq, M., Ashraf, M.Y., and Cheema, M.A. 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* 194(3): 193-199.

Ihle, C. 1997. Degradation and release from the thylakoid membrane of Photosystem II subunits after UV-B irradiation of the liverwort Conocephalum conicum. *Photosynth. Res.* 54: 73-78.

Jaleel, C.A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R., and Panneerselvam, R. 2009. Drought stress in plants: A review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.* 11: 100-105.

Jaleel, C.A., Manivannan. P., Sankar, B., Kishorekumar, A., Gopi, R., Somasundaram, R., and Panneerselvam, R. 2007. Water deficit stress mitigation by calcium chloride in Catharanthus roseus: effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf. B Biointerfaces* 60(1): 110-116.

Jansen, M.A.K. 2002. Ultraviolet-B radiation effects on plants: induction of morphogenic responses. *Physiol. Plant.* 116: 423-429.

Jansen, M.A.K. and Van-Den-Noort, R.E. 2000. Ultraviolet-B radiation induces complex alteration in stomatal behavior. *Physiol. Plant.* 110: 189-194.

Jansen, M.A.K., Gaba, V., and Greenberg, B.M. 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. *Trends Plant Sci.* 3(4): 131-135.

Jansen, M.A.K., Hideg, E.V., and Fernando, J.C.L. 2012. UV-B radiation: "When does the stressor cause stress?" *Emir. J. Food Agric.* 24(6): 1.

Januskaitiene, I. 2013. Low UV-B radiation and substrate acidity impact on photosynthetic parameters of *Brassica napus. Biologija.* 59(2): 219-230.

Jiao, D.M. and Li, X. 2001. Cultivar differences in photosynthetic tolerance to photooxidation and shading in rice (*Oryza sativa* L.). *Photosynthetica* 39(2): 167-175.

Jordan, B.R., James, P.E., Strid, A., and Anthony, R.G. 1994. The effect of ultraviolet-B radiation on gene expression and pigment composition in etiolated and green pea leaf tissue UV-B induced changes are gene-specific and dependent upon the developmental stage. *Plant Cell Environ.* 17: 45-54.

Jose, M., Raj, R., Varghese, G., Thomas, G. 2010. Is Njavara (*Oryza sativa*), the ancient medicinal rice endemic to Kerala, India, a distinct gene pool? In 28th International Rice Research Conference. Hanoi, Vietnam: IRRI.

Joseph, J., Francies, R.M., Zachariah, G., and Kumar S.A.V. 2007. Characterization of navara (*Oryza sativa* L.) a traditional medicinal rice of Kerala for qualitative traits. *Indian J. Agric. Res.* 41(4): 267-271.

Jun, W., YunSheng, L., YongXiu, L., HuanYou, C. 2010. Effect of enhanced ultraviolet-B radiation on physiological and ecological parameters in barley. *J. Agro-Environ. Sci.* 29(6): 1033-1038.

Kakani, V.G., Reddy, K.R., Zhao, D., and Mohammed, A.R. 2003. Ultraviolet-B radiation effects on cotton (*Gossypium hirsutum* L.) morphology and anatomy. *Ann. Bot.* 91: 817-826.

Kakani, V.G., Reddy, K.R., Zhao, D., Sailaja, K. 2003. Field crop responses to ultraviolet-B radiation: a review. *Agric. Forest Meteorol.* 120: 191-218.

Kalaivani, R., Arulmozhi, P., and Bakiyalakshmi, S.V. 2016. A Study on medicinal properties of traditional rice karung kavuni and neutraceutical formulation. *Int. J. Food Nutr. Sci.* 5(1): 86- 90.

Kallscheuer, N., Vogt, M., and Marienhagen, J. 2017. A novel synthetic pathway enables microbial production of polyphenols independent from the endogenous aromatic amino acid metabolism. *ACS Synth. Biol.* 6: 410-415.

Kamara, A.Y., Menkir, A., Badu-Apraku, B., and Ibikunle, O. 2003. The influence of drought stress on growth, yield and yield components of selected maize genotypes. *J. Agric. Sci.* 141(1): 43-50.

Kamarudin, Z.S., Yusop, M.R., Mohamed, M.T.M., Ismail, M.R., and Harun, A.R. 2018. Growth performance and antioxidant enzyme activities of advanced mutant rice genotypes under drought stress condition. *Agron.* 8: 279.

Kamoshita, A., Rodriguez, R., Yamauchi, A., and Wade, L. 2004. Genotypic variation in response of rainfed lowland to prolonged drought and re-watering. *Plant Prod. Sci.* 7(4): 406-420.

Karimi, E., Jaafar, H.Z.E., Ghasemzadeh, A., and Ibrahim, M.H. 2013. Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. *Aus. J. Crop Sci.* 7(7): 1016-1023.

Karuppusamy, S. 2009. A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *J. Med. Plants Res.* 3: 1222-1239.

Kataria, S. and Guruprasad, K.N. 2012. Solar UV-B and UV-A/B exclusion effects on intraspecific variations in crop growth and yield of wheat varieties. *Field Crops Res.* 125: 8-13.

Kataria, S. and Guruprasad, K.N. 2014. Exclusion of solar UV components improves growth and performance of *Amaranthus tricolor* varieties. *Scientia Hortic.* 174: 36-45.

Kataria, S., Jajoo, A., and Guruprasad, K.N. 2014. Impact of increasing Ultraviolet-B (UV-B) radiation on photosynthetic processes. *J. Photochem. Photobiol. B: Biol.* 137: 55-66.

Kaya, M.D., Okçub, G., Ataka, M., Çıkılıc, Y., and Kolsarıcıa, O. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.* 24: 291-295.

Keller, P. and Luettge, U. 2005. Photosynthetic light-use by three bromeliads originating from shaded sites (*Ananas ananassoides*, *Ananas comosus* cv. Panare) and exposed sites (*Pitcairnia pruinosa*) in the medium Orinoco basin, Venezuela. *Biol. Plant.* 49: 73-79.

Kennedy, D.O. and Wightman, E.L. 2011. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. *Adv. Nutr.* 2: 32-50.

Khan, A.A. and Kabir, M.R. 2014. Evaluation of spring wheat genotypes (*Triticum aestivum* L.) for heat stress tolerance using different stress tolerance indices. *Cercetări Agron. Moldova* 4(160): 49-63.

Khayatnezhad, M. and Gholamin, R. 2012. The effect of drought stress on leaf chlorophyll content and stress resistance in maize cultivars (*Zea mays*). *Afr. J. Microbiol.* 6(12): 2844-2848.

Khokhar, S. and Owusu-Apenten, R.K. 2003. Iron binding characteristics of phenolic compounds: Some tentative structure-activity relations. *Food Chem.* 81(1): 133-140.

Klunklin, W. and Savage, G. 2017. Effect on Quality characteristics of tomatoes grown under well-watered and drought stress conditions. *Foods* 6(8): 56.

Knekt, P., Jarvinen, R., Reunanen, A., and Maatela, J. 1996. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* 312(7029): 478-481.

Krishnaswamy, K. and Raghuramulu, N. 1998. Bioactive phytochemicals with emphasis on dietary practice. *Ind. J. Medica. Res.* 108: 167-181.

Krizek, D.T., Mirecki, R.M., and Britz, S.J. 2006. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cucumber. *Physiologia Plantarum* 100: 886-893.

Kubasek, W.I., Shirley, B.W., McKilop, A., Goodman, H.M., Briggs, W., and Ausubel, F.M. 1992. Regulation of flavonoid biosynthetic genes in germinating Arabidopsis seedlings. *Plant Cell* 4: 1229-1236.

Kulandaivelu, G. and Noorudeen, A.M. 1993. Comparative study of the action of ultraviolet-C and ultraviolet- B radiation on photosynthetic electron transport. *Physiol. Plant.* 58(3): 389-394.

Kumagai, T., Hidema, J., Kang, H.S., Sato, T. 2001. Effects of supplemental UV-B radiation on the growth and yield of two cultivars of Japanese lowland rice

(*Oryza sativa* L.) under the field in a cool rice-growing region of Japan. *Agric. Ecosyst. Environ.* 83: 201-208.

Kumar, P.S., Elsy, C.R., Nazeem, P.A., and Augustin, A. 2010. Use of different marker systems to estimate genetic diversity in the traditional medicinal rice cultivar of Kerala. *Int. J. Plant Breed. Genet.* 4: 89-103.

Kumar, R., Sharma, S., and Singh, B. 2011. Influence of transplanting time on growth, essential oil yield and composition in clary sage (*Salvia sclarea* L.) plants grown under mid hills of north-western Himalayas. *J. Essen. Oil Bearing Plants* 14: 260-265.

Kumar, S., Dwivedi, S.K., Basu, S., Kumar, G., Mishra, J.S., Koley, T.K., Rao, K.K., Choudhary, A.K., Mondal, S., Kumar, S., Bhakta, N., Bhatt, B.P., Paul, R.K., and Kumar, A. 2020. Anatomical, agro-morphological and physiological changes in rice under cumulative and stage specific drought conditions prevailed in eastern region of India. *Field Crops Res.* 245: 107658.

Kumari, R., Singh, S., and Agrawal, S.B. 2009. Effects of supplemental ultraviolet-B radiation on growth and physiology of *Acorus calamus* L. (sweet flag). *Acta. Biol. Cracov. Ser. Bot.* 51: 19-27.

Lai, P., Li, K.Y., Lu, S., and Chen, H.H. 2009. Phtochemicals and antioxidant properties of solvent extracts from Japonica rice bran. *Food Chem.* 117: 538-544.

Lake, J.A., Field, K.J., Davey, M.P., Beerling, D.J., and Lomax, B.H. 2009. Metabolomic and physiological responses reveal multi-phasic acclimation of Arabidopsis thaliana to chronic UV radiation. *Plant Cell Environ.* 32(10): 1377-1389.

Lam, P.Y., Liu, H., and Lo, C. 2015. Completion of tricin biosynthesis pathway in rice: Cytochrome P450 75B4 is a unique chrysoeriol 5'-hydroxylase. *Plant Physiol.* 168: 1527-1536.

Lambers, H., Chapin, F.S., and Pons, T.L. 2008. Plant Physiological Ecology. Springer, New York, NY.

Lauteri, M., Haworth, M., Serraj, R., Monteverdi, M.C., and Centritto, M. 2014. Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. *PLoS ONE* 9(10): 109054.

Li, H., Jiang, D., Wollenweber, B., Dai, T., and Cao, W. 2010. Effects of shading on morphology, physiology and grain yield of winter wheat. *Europ. J. Agron.* 33(4): 267-275.

Li, J., Yang, L., Jin, D., Nezames, C.D., Terzaghi, W., and Deng, X.W. 2013. UV-B-induced photomorphogenesis in Arabidopsis. *Protein Cell* 4: 485-492.

Li, M., Li, Y., Zhang, W., Li, S., Gao, Y., Ai, X., Zhang, D., Liu, B., and Li, Q. 2018. Metabolomics analysis reveals that elevated atmospheric CO_2 alleviates drought stress in cucumber seedling leaves. *Anal. Biochem.* 559: 71-85.

Li, T., Liua, L.N., Jianga, C.D., Liub, Y.J., and Shi, L. 2014. Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum. *J. Photochem. Photobiol. B: Biol.* 137: 31-38.

Liang, X., Zhang, L., Natarajan, S.K., and Becker, D.F. 2013. Proline Mechanisms of Stress Survival. *Antioxid. Redox. Signal.* 19(9): 998-1011.

Lichtenthaler, H.K., Buschmann, C., Doll, M., Fietz, H.J. Bach, T., Kozel, U., Meier, D., and Rahmsdorf, U. 1981. Photosynthetic activity, chloroplast ultrastructure,

and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynth. Res.* 2: 115-141.

Lidon, F.C. and Ramalho, J.C. 2011. Impact of UV-B irradiation on photosynthetic performance and chloroplast membrane components in *Oryza sativa* L. *J. Photochem. Photobiol. B: Biol.* 104: 457-466.

Lidon, F.J.C., Reboredo, F.H., Leitao, A.E., Silva, M.M.A., Duarte, M.P., and Ramalho, J.C. 2012. Impact of UV-B radiation on photosynthesis-an overview. *Emir. J. Food Agric.* 24: 546-556.

Lima, J.D., Silva, B.M.S.E., Moraes, W.S., Dantas, V.A.V., and Almeida, C.C. 2008. Efeitos da luminosidade no crescimento de mudas de Caesalpinia ferrea Mart. ex Tul. (Leguminosae, Caesalpinoideae). *Acta Amazônica* 38(1): 5-10.

Liu, B., Liua, X., Lia, Y., and Herbertc S.J. 2013. Effects of enhanced UV-B radiation on seed growth characteristics and yield components in soybean. *Field Crops Res.* 154: 158-163.

Liu, G.F., Han, Z.X., Feng, L., Gao, L.P., Gao, M.J., Gruber, M.Y., Zhang, Z.L., Xia, T., Wan, X.C., and Wei, S. 2017. Metabolic flux redirection and transcriptomic reprogramming in the albino tea cultivar 'Yu-Jin-Xiang' with an emphasis on catechin production. *Sci. Rep-UK.* 7: 45062.

Liu, H.L., Yin, Z.J., Xiao, L., Xu, Y.N., and Qu, L.Q. 2012. Identification and evaluation of omega-3 fatty acid desaturase genes for hyperfortifying alpha-linolenic acid in transgenic rice seed. *J. Exp. Bot.* 63(8): 3279-3287.

Liu, L., Gitz, D.C., and McClure, J.W. 1995. Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Physiologia Plantarum* 93: 725-733.

Liu, L., Li, Y., She, G., Zhang, X., Jordan, B., Chen, Q., Zhao, J., and Wan, X. 2018. Metabolite profiling and transcriptomic analyses reveal an essential role of UVR8-mediated signal transduction pathway in regulating flavonoid biosynthesis in tea plants (*Camellia sinensis*) in response to shading. *BMC Plant Biol.* 18: 233.

Liu, Q., Wu, X., Chen, B., Ma, J., and Gao, J. 2014. Effects of low light on agronomic and physiological characteristics of rice including grain yield and quality. *Rice Sci.* 21(5): 243-251.

Liu, Q.H., Zhou, X.B., Yang, L.Q., Li, T., and Zhang, J.J. 2009. Effects of early growth stage shading on rice flag leaf physiological characters and grain growth at grain-filling stage. *Chin. J. Appl. Ecol.* 20(9): 2135-2141.

Liu, Q.H., Zhou, X.B., Yang, L.Q., Li, T., and Zhang, J.J. 2009. Effects of early growth stage shading on rice flag leaf physiological characters and grain growth at grain-filling stage. *Chin. J. Appl. Ecol.* 20(9): 2135-2141.

Liu, Y., Chen, X., Wang, J., Cui, W., Xing, X., Chen, X., Ding, W., God'spower, B., Eliphaz, N., Sun, M., and Li, Y. 2019. Transcriptomic analysis reveals flavonoid biosynthesis of *Syringa oblata* Lindl. in response to different light intensity. *BMC Plant Biol.* 19: 487.

Logemann, E., Tavernaro, A., Schlz, W., Somssich, I. E., and Hanlbrok, K. 1999. UV light selected co-induces supply pathway from primary metabolism and flavonoid secondary product formation in parsley. *Plant Biol.* 7: 1903-1907.

Logemann, E., Wu, S.-C., Schröder, J., Schmelzer, E., Somssich, E., and Hahlbrock, K. 1995. Gene activation by UV light, fungal elicitor or fungal infection in Petroselinum crispum is correlated with repression of cell cycle-related genes. *Plant J.* 8: 865-876.

Lu, Y., Duan, B., Zhang, X., Korpelainen, H., Berninger, F., and Li, C. 2009. Intraspecific variation in drought response of Populus cathayana growth under ambient and enhanced UV-B radiation. *Ann. For. Sci.* 66: 613-624.

Lum, M.S., Hanafi, M.M., Rafii, Y.M., and Akmar, A.S.N. 2014. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *J. Anim. Plant Sci.* 24(5): 1487-1493.

Ma, D., Sun, D., Wang, C., Li, Y., and Guo, T. 2014. Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress. *Plant Physiol. Biochem.* 80: 60-66.

Mackerness, S.A.H. 2000. Plant responses to ultraviolet-B (UV-B: 280-320 nm) stress: What are the key regulators? *Plant Growth Reg.* 32: 27-39.

Maggi-Capeyron, M.F., Ceballos, P., Cristol, J.P., Delbosc, S., Le Doucen, C., Pons, M., Leger, C.L., and Descomps, B. 2001. Wine phenolic antioxidants inhibit AP-1 transcriptional activity. *J. Agric. Food Chem.* 49: 5646-5652.

Maisura, Chozin, M.A., Lubis, I., Junaedi, A., and Ehara, H. 2014. Some physiological character responses of rice under drought conditions in a paddy system. *J. ISSAAS* 20(1): 104-114.

Malick, C.P. and Singh, M.B. 1980. *Plant enzymology and histoenzymology.* Kalyani Publications, New Delhi, 286p.

Manetas, Y. 2003. The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of Verbascum speciosum are due to solar UV-B radiation. *New Phytol.* 158(3): 503-508.

Mapes, C. and Xu, Y. 2014. Photosynthesis, vegetative habit and culinary properties of sage (*Salvia officinalis*) in response to low-light conditions. *Can. J. Plant Sci.* 94: 881-889.

Marwood, C.A. and Greenberg, B.M. 1996. Effect of supplementary UVB radiation on chlorophyll systems during chloroplast development in *Sspirodela oligarrhiza. J. Photochem. Photobiol.* 64: 664-670.

Mathobo, R., Diana M., and Martin, S.J. 2017. The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.). *Agric. Water Manag.* 180: 118-125.

Matysik, J., Alia, Bhalu, B., and Mohanty, P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* 82: 525-532.

Mazid, M., Khan, T.A., and Mohammada, F. 2012. Medicinal plants of rural India: A review of use by Indian folks. *Indo Global J. Pharma. Sci.* 2(3): 286-304.

Mendes, M.M., Gazarine, L.C., and Rodrigues, M.L. 2001. Acclimation of *Myrtus communis* to contrasting Mediterranean light environments-on structure and chemical composition of foliage and plant water relations. *Environ. Exp. Bot.* 45: 165-178.

Menon, M.V. and Potty, N.N. 1999. Nutritional specificity and quality properties of medicinal rice "Njavara". *Oryza* 36(4): 315-317.

Milchunas, D.G., King, J.Y., Mosier, A.R., Moore, J.C., Morgan, J.A., Quirk, M.H., and Slusser, J.R. 2004. UV Radiation Effects on Plant Growth and Forage Quality in a Shortgrass Steppe Ecosystem. *Photochem. Photobiol.* 79(5): 404-410.

Mishra, V., Srivastava, G., Prasad, S.M., and Abraham, G. 2008. Growth, photosynthetic pigments and photosynthetic activity during seedling stage of cowpea (*Vigna unguiculata*) in response to UV-B and dimethoate. *Pestic. Biochem. Phys.* 92: 30-37.

Mo, Z., Li, W., Pan, S., Fitzgerald, T.L., Xiao, F., Tang, Y., Wang, Y., Duan, M., Tian, H., and Tang, X. 2015. Shading during the grain filling period increases 2-acetyl-1-pyrroline content in fragrant rice. *Rice* 8: 9.

Mohammed, A.R. and Tarpley, L. 2009. Effects of elevated ultraviolet-B radiation on productive tillers, spikelet sterility and grain characteristics of southern US rice (*Oryza sativa* L.) cultivars. *J. Agron. Crop Sci.* 195: 292-300.

Mohammed, A.R., Rounds, E.W., and Tarpley, L. 2007. Response of Rice (*Oryza sativa* L.) tillering to sub-ambient levels of ultraviolet-B radiation. *J. Agron. Crop Sci.* 193: 324-335.

Mohammed, H.A., Alshalmani, S.K., and Abdellatif A.G. 2013. Antioxidant and quantitative estimation of phenolic and flavonoids of three halophytic plants growing in Libya. *J. Pharmaco. Phytochem.* 2(3): 89-94.

Mohanlal, S., Parvathy, R., Shalini, V., Helen, A., and Jayalekshmy, A. 2011. Isolation, characterization and quantification of tricin and flavonolignans in the medicinal rice Njavara (*Oryza sativa* L.), as compared to staple varieties. *Plant Foods Hum. Nutr.* 66: 91-96.

Monakhova, O.F. and Chernyadèv, I.I. 2002. Protective role of kartolin-4 in wheat plants exposed to soil drought. *Appl. Biochem. Micro.* 38: 373-380.

Moniruzzaman, M., Islam, M.S., Hossain, M.M., Hossain, T., and Miah, M.G. 2009. Effects of shade and nitrogen levels on quality Bangladhonia production. *Bangladesh J. Agric. Res.* 34: 205-213.

Moonmoon, S. and Islam, M.T. 2017. Effect of drought stress at different growth stages on yield and yield components of six rice (*Oryza sativa* L.) genotypes. *Fundam. Appl. Agric.* 2(3): 285-289.

Morales, L.O., Tegelberg, R., Brosché, M., Keinänen, M., Lindfors, A., and Aphalo, P.J. 2010. Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in Betula pendula leaves. *Tree Physiol.* 30(7): 923-934.

Mu, H., Jiang, D., Wollenweber, B., Dai, T., Jing, Q., and Cao, W. 2010. Long-term low radiation decreases leaf photosynthesis, photochemical efficiency and grain yield in winter wheat. *J. Agron. Crop Sci.* 196: 38-47.

Muhidin, Syamun, E., Kaimuddin., Musa, Y., Sadimantara, G.R., Usman., Leomo, S., and Rakian, T.C. 2018. Shading effect on generative characters of upland red rice of Southeast Sulawesi, Indonesia. *Earth Environ. Sci.* 157: 012017.

Muller, J.E. and Whitsitt, M.S. 1996. Plant cellular response to water deficit. *Plant Growth Reg.* 20: 41-46.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytol.* 167: 645-63.

Murthy, K.R.S. 2001. Vagbatta'sAshtangaHrdayam (Text, English translation, notes, appendix, indices). Varanasi: Krishna Das Academy.

Nagah, A.M. and Seal, C.J. 2005. *In vitro* procedure to predict apparent antioxidant release from whole grain foods measured using three different analytical methods. *J. Sci. Food Agric.* 85: 1177-1185.

Naikoo, M.I., Dar, M.I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F.A., and Naushin, F. 2019. Role and regulation of plants phenolics in abiotic stress tolerance: An Overview. In: *Plant Signaling Molecules*, Elsevier, Amsterdam, pp. 157-168.

Nakabayashi, R., Yonekura-Sakakibara, K., Urano, K., Suzuki, M., Yamada, Y., Nishizawa, T., Matsuda, F., Kojima, M., Sakakibara, H., Shinozaki, K., Michael A.J., Tohge T., Yamazaki, M., and Saito, K. 2014. Enhancement of oxidative and drought tolerance in *Arabidopsis* by over accumulation of antioxidant flavonoids. *Plant J.* 77: 367-379.

Nichols, S.N., Hofmann, R.W., and Williams, W.M. 2015. Physiological drought resistance and accumulation of leaf phenolics in white clover interspecific hybrids. *Environ. Exp. Bot.* 119: 40-47.

Nikolaeva, M.K., Maevskaya, S.N., Shugaev, A.G., and Bukhov, N.G. 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. *Russian J. Plant Physiol.* 57(1): 87-95.

Nogues, S., Allen, D.J., Morison, J.I.L., and Baker, N.R. 1999. Characterization of stomatal closure caused by ultraviolet-B radiation. *J. Plant Physiol.* 121: 489-496.

Nonami, H. 1998. Plant water relations and control of cell elongation at low water potentials. *J. Plant Res.* 111: 373-382.

Olsson, L.C., Veit, M., Weissonbock, G., and Bornmann, J.F. 1998. Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. *Phytochem.* 4: 1021-1028.

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

Panda, D., Biswal, M., Behera, L., Baig, M.J., Dey, P., Nayak, L., Sharma, S., Samantaray, S., Ngangkham U., and Kumar, A. 2019. Impact of low light stress on physiological, biochemical and agronomic attributes of rice. *J. Pharmac. Phytochem.* 8(1): 1814-1821.

Pandey, V. and Shukla, A. 2015. Acclimation and tolerance strategies of rice under drought stress. *Rice Sci.* 22(4): 147-161.

Panigrahy, M., Ranga, A., Das, J., and Panigrahi, K.C.S. 2019. Shade tolerance in Swarnaprabha rice is associated with higher rate of panicle emergence and positively regulated by genes of ethylene and cytokinin pathway. *Sci. Rep.* 9(1): 6817.

Parfitt, J.M.B., Concenco, G., Downing, K., Larue, J., and da Silva, J.T. 2017. Rice growth under water stress levels imposed at distinct developmental stages. *Revista de Ciências Agrárias.* 40(3): 587-596.

Patil, P., Biradar, P., Bhagawathi, A.U., and Hejjegar, I.S. 2018. A review on leaf area index of horticulture crops and its importance. *Int. J. Curr. Microbiol. App. Sci.* 7(4): 505-513.

Pierce, L.L., Running, S.W., and Walker, J. 1994. Regional-scale relationships of leaf-area index to specific leaf-area and leaf nitrogen content. *Ecol. Appl.* 4: 313-321.

Poulev, A., Heckman, J.R., Raskin, I., and Belanger, F.C. 2019. Tricin levels and expression of flavonoid biosynthetic genes in developing grains of purple and brown pericarp rice. *Peer J.* 7: e6477.

Pourmohammad, A. 2013. Application of molecular markers in medicinal plant studies. *Acta Univ. Sapientiae Agricul. Environ.* 5: 80-90.

Qiu, T., Wu, Y., Shen, Z., Wu, Y., Lu, D., and He, J. 2018. Effects of shading on leaf physiology and morphology in the 'Yinhong' grape plants. *Rev. Bras. Frutic. Jaboticabal.* 40(5): 037.

Quan, N.T., Anh, L.H., Khang, D.T., Tuyen, P.T., Toan, N.P., Minh, T.N., Bach, D.T., Ha, P.T.T., Elzaawely, A.A., Khanh, T.D., and Trung, K.H. 2016. Involvement of secondary metabolites in response to drought stress of rice (*Oryza sativa* L.). *Agric.* 6(2): 23.

Rad, M.H., Assare, M.H., Banakar M.H., and Soltani, M. 2011. Effects of different soil moisture regimes on leaf area index, specific leaf area and water use efficiency in Eucalyptus (*Eucalyptus camaldulensis* Dehnh) under dry climatic conditions. *Asian J. Plant Sci.* 10(5): 294-300.

Rahdari, P. and Hoseini, S.M. 2012. Drought stress: A review. *Int. J. Agron. Plant Prod.* 3(10): 443-446.

Rajendiran, K. and Ramanujam, M.P. 2003. Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum* 46(4): 621-624.

Ramakrishna, A. and Ravishankar, G.A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behav.* 6(11): 1720-1731.

Rani, S. and Sukumari, P. 2016. Productivity and economics of medicinal rice Njavara (*Oryza sativa* L.) as influenced by different establishment techniques in lowland condition of Kerala, India. *Afr. J. Agric. Res.* 11(22): 1984-1989.

Ranjbarfordoei, A., Samson, R., and Damme, P.V. 2011. Photosynthesis performance in sweet almond [*Prunus dulcis* (Mill) D. Webb] exposed to supplemental UV-B radiation. *Photosynthetica* 49: 107-111.

Rao, S.R. and Ravishankar, G.A. 2002. Plant cell cultures: chemical factories of secondary metabolites. *Biotechnol Adv.* 20: 101-153.

Rao, X., Huang, X., Zhou, Z., and Lin, X. 2013. An improvement of the 2(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat. Bioinforma. Biomath.* 3(3): 71-85.

Rao-Akiri, S.V.C., Reddy, S.G., Babu, P.P., Reddy, A.R. 2010. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. *BMC Complement Altern Med.* 10: 4.

Reddy, A.R., Chaitanya, K.V., and Vivekanandan, M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* 161: 1189-1202.

Reddy, K. R., Singh, S.K., Koti, S., Kakani, V.G., Zhao, D., Gao, W., and Reddy, V.R. 2013. Quantifying the effects of corn growth and physiological responses to ultraviolet-B radiation for modeling. *Agron. J.* 105(5): 1367-1377.

Reddy, K.R., Kakani, V.G., Zhao, D., Mohammed, A.R., and Gao, W. 2003. Cotton response to ultraviolet-B radiation, experimentation and algorithm development. *Agric. Forest Meteorol.* 120: 249-265.

Reddy, K.R., Singh, S.K., Koti, S., Kakani, V.G., Zhao, D., Gao, W., and Reddy, V.R. 2013. Quantifying the effects of corn growth and physiological responses to ultraviolet-B radiation for modeling. *Agro. J.* 105: 1367-1377.

Reddy, T.Y., Reddy, V.R., and Anbumozhi, V. 2003. Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: a critical review. *Plant Growth Reg.* 41(1): 75-88.

Ren, B., Cui, H., Camberato, J.J., Dong, S., Liu, P., Zhao, B., and Zhang, J. 2016. Effects of shading on the photosynthetic characteristics and mesophyll cell ultrastructure of summer maize. *Sci. Nat.* 103: 67.

Ren, W.J., Yang, W.Y., Xu, J.W., Fan, G.Q., Wang, L.Y., and Guan, H. 2002. Impact of low-light stress on leaves characteristics of rice after heading. *J. Sichuan Agric. Univ.* 20(3): 205-208.

Renger, G., Voss, M., Graber, P., and Schulze, A. 1986. Effect of UV irradiation on different partial reactions of the primary processes of photosynthesis. In: Worrest, C. and Caldwell, M.M. (eds.), *Stratospheric ozone reduction, solar ultraviolet radiation and plant life.* Springer, Berlin, Heidelberg, pp. 171-184.

Reshmi, G.R. and Rajalakshmi, R. 2012. Drought and UV stress response in *Spilanthes acmella* Murr. (tooth-ache plant). *J. Stress Physiol. Biochem.* 8(4): 110-129.

Reshmi, R. and Nandini, P.V. 2013. Therapeutic value of Indian medicinal rice (*Oryza sativa* L.) Cv. Njavara. *Int. J. Food Nutr. Sci.* 2(1): 78-83.

Restrepo, H. and Garces, G. 2013. Evaluation of low light intensity at three phenological stages in the agronomic and physiological responses of two rice (*Oryza sativa* L.) cultivars. *Agronomía Colombiana* 31(2): 195-200.

Rezai, S., Etemadi, N., Nikbakht, A., Yousefi, M., and Majid, M.M. 2018. Effect of light intensity on leaf morphology, photosynthetic capacity, and chlorophyll content in Sage (*Salvia officinalis* L.). *Hortic. Sci. Technol.* 36(1): 46-57.

Rezai, S., Etemadi, N., Nikbakht, A., Yousefi, M., and Majidi, M.M. 2017. Effect of light intensity on leaf morphology, photosynthetic capacity, and chlorophyll content in Sage (*Salvia officinalis* L.). *Hortic. Sci. Technol.* 36(1): 46-57.

Rezayian, M., Niknam, V., and Ebrahimzadeh, H. 2018. Differential responses of phenolic compounds of Brassica napus under drought stress. *Iran. J. Plant Physiol.* 8: 2417-2425.

Rhodes, D., Nadolska-Orczyk, A., and Rich, P.J. 2002. Salinity osmolytes and compatible solutes. In: Lauchli, A. and Luttge, U. (eds), *Salinity, Environment, Plant, Molecules.* Netherlands: Al-Kluwer Academic Publishers, pp. 181-204.

Rio, D.D., Rodriguez-Mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G., and Crozier, A. 2013. Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signal.* 18: 1818-1892.

Rodriguez-Calzada, T., Qianb, M., Stridb, A., Neugartc, S., Schreinerc, M., Torres-Pachecoa, I., Guevara-Gonzalez, R.G. 2019. Effect of UV-B radiation on morphology, phenolic compound production, gene expression, and subsequent drought stress responses in chili pepper (*Capsicum annuum* L.). *Plant Physiol. Biochem.* 134: 94-102.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., and Heimler, D. 2002. Polyphenols in greenhouse and open-air-grown lettuce. *Food Chem.* 79: 337-342.

Routray, W. and Orsat, V. 2012. Review: Microwave-assisted extraction of flavonoids. *Food Bioprocess. Technol.* 5: 409-424.

Rozema, J., Broekman, R., Lud, D., Huiskes, A.H.J., Moerdijk, T., de Bakker, N., Meijkamp, B., and van Beem, A. 2001. Consequences of depletion of stratospheric ozone for terrestrial antarctic ecosystems: the response of Deschampsia antarctica to enhanced UV-B radiation. *Plant Ecol.* 154: 101-115.

Sadasivam, S. and Manickam, A. 2008. *Biochemical Methods for Agricultural Sciences* (6th Ed.). New Age International (P) Ltd, New Delhi.

Saito, K., Yonekura-Sakakibara, K., Nakabayashi, R., Higashi, Y., Yamazaki, M., Tohge, T., and Fernie, A.R. 2013. The flavonoid biosynthetic pathway in Arabidopsis: Structural and genetic diversity. *Plant Physiol. Biochem.* 72: 21-34.

Sankar, B., Jaleel, C.A., Manivannan, P., Kishorekumar, A., Somasundaram, R., and Panneerselvam, R. 2008. Relative efficacy of water use in five varieties of *Abelmoschus esculentus* (L.) Moench under water-limited conditions. *Colloids Surf. B: Biointerfaces* 62: 125-129.

Saradhi, P.P., Arora, A., and Prasad, K.V. 1995. Proline accumulates inplants exposed to UV radiation and protects them against UV induced peroxidation. *Biochem. Biophys. Res. Commun.* 209: 1-5.

Sarker, U. and Oba, S. 2018. Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of *Amaranthus* leafy vegetable. *BMC Plant Biol.* 18: 258.

Sato, K. and Kim, J.M. 1980. Relationship between environmental conditions and production and consumption activities of individual leaves in the population of rice plant in a paddy field: I. Changes in photosynthesis and dark respiration of individual leaves under field conditions. *Jpn. J. Crop Sci.* 49(2): 243-250.

Schoch, P.G. 1972. Effects of shading on structural characteristics of the leaf and yield of fruit in Capsicum annum. *J. Amer. Soc. Hort. Sci.* 97(4): 461-464.

Schreiner, M., Martínez-Abaigar, J., Glaab, J., and Jansen, M. 2014. UV-B induced secondary plant metabolites. *Biophotonics.* 9(2): 34-37.

Searles, P.S., Flint, S.D., and Caldwell, M.M. 2001. A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia* 127: 1-10.

Sebastian, A., Kumari, R., Kiran, B.R., and Prasad, M.N.V. 2018. Ultraviolet B induced bioactive changes of enzymatic and non-enzymatic antioxidants and lipids in *Trigonella foenum-graecum* L. (Fenugreek). *The EuroBiotech. J.* 2(1): 64-71.

Seidler, A. 1994. Expression of the 23 kDa protein from the oxygen-evolving complex of higher plants in Escherichia coli. *Biochim. Biophys. Acta* 1187(1): 52-58.

Seitz, C., Eder, C., Deiml, B., Kellner, S., Martens, S., and Forkmann, G. 2006. Cloning, functional identification, and sequence analysis of flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase cDNAs reveals independent evolution of flavonoid 3',5'-hydroxylase in the Asteraceae family. *Plant Mol. Biol.* 61: 365-381.

Selmar, D. and Maik, K. 2013. Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. *Plant Cell Physiol.* 54(6): 817-826.

Semida, W.M., Mohamed, S.A., and El-Sawah, N.A. 2017. Cucumber (*Cucumis sativus*) transplants as affected by shade level: microenvironment, growth, photosynthetic efficiency, osmoprotectants, plant water status, and leaf mineral nutrients. *Agri. Res. Tech.* 12(5): 149-157.

Shahzad, M.A., Jan, S.U., Afzal, F., Khalid, M., Gul, A., Sharma, I., Sofo, A., and Ahmad, P. 2016. Drought stress and morphophysiological responses in plants. In: Ahmad P. 2016 (ed.), *Water Stress and Crop Plants: A Sustainable Approach* (1st Ed.). John Wiley and Sons, Ltd., pp. 452-466.

Shalini, V., Bhaskar, S., Kumar, K.S., Mohanlal, S., Jayalekshmy, A., and Helen, A. 2012. Molecular mechanisms of anti-inflammatory action of the flavonoid, tricin from Njavara rice (*Oryza sativa* L.) in human peripheral blood mononuclear cells: possible role in the inflammatory signaling. *Int. Immunopharm.*14: 32-38.

Shanthi, N. and Janetta, N.S.M. 2015. Effect of enhanced solar UV-B radiation (280-320nm) on chloroplast polypeptide profile of some leguminous plants at different growing seasons. *Int. J. Res. Biosci.* 4(3): 42-48.

Shao, Q., Wang, H., Guo, H., Zhou, A., Huang, Y., Sun, Y., and Li, M. 2014. Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of *Anoectochilus roxburghii*. *PLoS ONE* 9(2): e85996.

Shareesh, N. 2007. Molecular documentation of Njavara types of rice for cultivar identification. M.Sc. (Ag) thesis, Kerala Agriculture University, Thrissur.

Sharif, M.O., Wadud, M.A., Mondol, M.A., Tanni, A.D., and Rahman, G.M.M. 2010. Effect of shade of different trees on growth and yield of Aman rice. *J. Agrofor. Environ.* 4(2): 167-172.

Sharma, A., Yuan, H., Kumar, V., Ramakrishnan, M., Kohli, S.K., Kaur, R., Thukral, A.K., Bhardwaj, R., and Zheng, B. 2019. Castasterone attenuates insecticide induced phytotoxicity in mustard. *Ecotoxicol. Environ. Saf.* 179: 50-61.

Sharma, S.S. and Dietz, K.J. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.* 57: 711-726.

Sheehy, J.E., Dionora, M.J.A., and Mitchell, P.L. 2001. Spikelet numbers, sink size and potential yield in rice. *Field Crops Res.* 71: 77-78.

Shehab, G.G., Ahmed, O.K., and El-Beltagi, H.S. 2010. Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). *Not. Bot. Hort. Agrobot. Cluj-Napoca.* 38(1): 139-148.

Shi, X.D., Wen, Z.Q., Liu, Y.F., and Wang, W.W. 2006. Research on the effect of different light stresses on crop growth. *J Anhui Agric. Sci.* 34(17): 4216-4218.

Shih, C.H., Chu, H., Tang, L.K., Sakamoto, W., Maekawa, M., Chu, I.K., Wang, M., and Lo, C. 2008. Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta* 228: 1043-1054.

Shih, C.H., Chu, H., Tang, L.K., Sakamoto, W., Maekawa, M., Chu, I.K.,·Wang, M., and Lo, C. 2008. Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta* 228: 1043-1054.

Sikuku, P.A., Onyango, J.C., and Netondo, G.W. 2012. Physiological and biochemical responses of five nerica rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage. *Agric. Biol. J. N. Am.* 3(3): 93-104.

Singh, R. 2015. Medicinal plants: A review. *J. Plant Sci.* 3(1-1): 50-55.

Singh, S., Prasad, S., Yadav, V., Kumar A., Jaiswal, B., Kumar, A., Khan, N.A., and Dwivedi, D.K. 2018. Effect of drought stress on yield and yield components of rice (*Oryza sativa* L.) genotypes. *Int. J. Curr. Microbiol. App. Sci.* 7: 2752-2759.

Smirnov, O.E., Kosyan, A.M., Kosyk, O.I., and Taran, N.Y. 2015. Response of phenolic metabolism induced by aluminium toxicity in Fagopyrum esculentum moench. plants. *Ukr. Biochem. J.* 87: 129-135.

Sorkhi, F. and Fateh, M. 2019. Effect of drought stress on leaf area index, photosynthesis, stomatal conductance and proline content in two pinto bean cultivars (*Phaseolus vulgaris* L.). *Environ. Stresses Crop Sci.* 12(2): 389-399.

Specht, J.E., Chase, K., Macrander, M., Graef, G.L., Chung, J., Markwell, J.P., Germann, M., Orf, J.H., and Lark, K.G. 2001. Soybean response to water. *Crop Sci.* 41(2): 493-509.

Sreejayan, Kumar U.S., Varghese, G., Jacob, T.M., and Thomas, G. 2010. Stratification and population structure of the genetic resources of ancient medicinal rice (*Oryza sativa* L.) landrace Njavara. *Genet. Resour. Crop Evol.* 58: 697-711.

Sridevi, V. and Chellamuthu, V. 2015. Impact of weather on rice-A review. *Int. J. Appl. Res.* 1(9): 825-831.

Srivastava, R. 2000. Studying the information needs of medicinal plant stakeholders, *Traffic Dispatches*, 15: 5.

Strid, A., Chow, W.S., and Anderson, J.M. 1990. Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum. Biochim. Biophys. Acta* 1020(3): 260-268.

Strizhov, N., Abrahám, E., Okrész, L., Blickling, S., Zilberstein, A., Schell, J., Koncz, C., and Szabados, L. 1997. Differential expression of two P5CS genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in Arabidopsis. *Plant J.* 12(3): 557-569.

Sudha, G. and Ravishankar, G.A. 2003. Elicitation of anthocyanin production in callus cultures of *Daucus carota* and involvement of calcium channel modulators. *Curr Sci.* 84 (6): 775-779.

Sullivan, J.H., Gitz, D.C., Peek, M.S., and McElrone, J.A. 2003. Response of three eastern species to supplemental UV-B radiation: leaf chemistry and gas exchange. *Agric. Forest Meteorol.* 120: 219-228.

Sun, O.J. and Payn, T.W. 1999. Magnesium nutrition and photosynthesis in *Pinus radiata*-Clonal variation and influence of potassium. *Tree Physio1*. 19: 535-540.

Sunilkumar, B. and Geethakumari, V.L. 2002. Shade response of upland rice cultivars (*Oryza sativa* L.) as influenced by silica application. *J. Trop. Agric.* 40: 67-70.

Surabhi, G.K., Reddy, K.R., and Singh, S.K. 2009. Photosynthesis, fluorescence, shoot biomass and seed weight responses of three cowpea (*Vigna unguiculata* (L.) Walp.) cultivars with contrasting sensitivity to UV-B radiation. *Environ. Exp. Bot.* 66: 160-171.

Taiz, L. and Zeiger, E. 2006. *Plant Physiology* (4th Ed.). Sinauer Associate, Massachusetts, 764p.

Taiz, L. and Zeiger, E. 2010. *Plant Physiology* (6th Ed.). Sinauer Associates, Massachusetts, 690p.

Takemiya, A., Inouea, S., Doi, M., Kinoshita, T., and Shimazaki, K. 2005. Phototropins promote plant growth in response to blue light in low light environments. *Plant Cell* 17: 120-127.

Tanabe, S., Ashikari, M., Fujioka, S., Takatsuto, S., Yoshida, S., Yano, M., Yoshimura, A., Kitano, H., Matsuoka, M., Fujisawa, Y., Kato, H., and Iwasaki, Y. 2005. A

novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, dwarf11, with reduced seed length. *Plant Cell* 17: 776-790.

Tanase, C., Bujor, O.C., and Popa, V.I. 2019. Phenolic natural compounds and their influence on physiological processes in plants. In: Watson, R.R. (ed.), *Polyphenols in Plants*, (2nd Ed). Academic Press, Cambridge, MA, USA, pp. 45-58.

Tao, H., Brueck, H., Dittert, K., Kreye, C., Lin, S., and Sattelmacher, B. 2006. Growth and yield formation for rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). *Field Crops Res.* 95(1): 1-12.

Teramura, A.H., Lewis, H.Z., and Sztein, A.E. 1991. Changes in growth and photosynthetic capacity of rice with increased UV-B radiation. *Physiol. Plant.* 83(3): 3773-3380.

Teramura, A.H., Ziska, L.H., and Sztein, A.E. 1991. Changes in growth and photosynthetic capacity of rice with increased UV-B radiation. *Physiol. Plant.* 83: 372-380.

Tevini, M., Braun, J., and Fieser, G. 1991. The protective function of the epidermal layer of rye seedlings against ultraviolet-B radiation. *Photochem. Photobiol.* 53: 329-333.

Thayer, S.S. and Bjorkman, O. 1992. Leaf xanthophylls content and composition in sun and shade determined by HPLC. *Photosynth. Res.* 23: 331-343.

Torras-Claveria, L., Jáuregui, O., Codina, C., Tiburcio, A.F., Bastida, J., and Viladomat, F. 2012. Analysis of phenolic compounds by high-performance

liquid chromatography coupled to electrospray ionization tandem mass spectrometry in senescent and water-stressed tobacco. *Plant Sci.* 182: 71-78.

Tossi, V.E., Lamattina, L., Jenkins, G., and Cassia, R. 2014. UV-B-induced stomatal closure in *Arabidopsis* is regulated by the resistance locus8 photoreceptor in an NO-dependent mechanism. *Plant Physiol.* 164(4): 2220-2230.

Tsai, Y.Z. and Lai, K.L. 1990. The effect of temperature and light intensity on the tiller development of rice. Memoirs of the college of Agriculture, National Taiwan University. 30(2): 22-30.

Tyystjarvi, E. 2008. Photoinhibition of photosystem II and photodamage of the oxygen evolving manganese cluster. *Coord. Chem. Rev.* 252: 361-376.

Ueguchi-Tanaka, M., Fujisawa, Y., Kobayashi, M., Ashikari, M., Iwasaki, Y., Kitano, H., and Matsuoka, M. 2000. Rice dwarf mutant d1, which is defective in the alpha subunit of the heterotrimeric G protein, affects gibberellin signal transduction. *Proc. Natl. Acad. Sci. USA.* 97(21): 11638-11643.

Umadevi, M., Pushpa, R., Sampathkumar, K.P., and Bhowmik, D. 2012. Rice-Traditional medicinal plant in India. J. Pharma. Phytochem. 1(1): 6-12.

UNEP, (United Nations Environment Programme). 2010. Environmental Effects of Ozone Depletion and its Interactions with Climate Change: 2010 Assessment, pp.328.

Vasquez-Robinet, C., Mane, S.P., Ulanov, A.V., Watkinson, J.I., Stromberg, V.K., de Koeyer, D.D., Schafleitner, R., Willmot, D.B., Bonierbale, M., Bohnert, H.J., and Grene, R. 2008. Physiological and molecular adaptations to drought in Andean potato genotypes. *J. Exp. Bot.* 59: 2109e2123.

Vass, I., Sass, L., Spetea, C., Bakou, A., Ghanotakis, D.F., and Petrouleas, V. 1996. UV-B-induced inhibition of photosystem II electron transport studied by EPR and chlorophyll fluorescence. Impairment of donor and acceptor side components. *Biochem.* 35(27): 8964-8973.

Vass, I., Szilard, A., and Sicora, C. 2005. Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus. In: Pessarakli, M. (ed.), *Handbook of Photosynthesis* (2nd Ed.). CRC Press, Taylor and Francis Group, Boca Raton, FL, pp. 827-843.

Verbruggen, N. and Hermans, C. 2008. Proline accumulation in plants: a review. *Amino Acids* 35: 753-759.

Viji, M.M., Thangaraj, M., and Jayapragasam, M. 1997. Effect of low light on photosynthetic pigments, photochemical efficiency and hill reaction in rice (*Oryza sativa* L.). *J. Agron. Crop Sci.* 178: 193-196.

Wagh, Y.S. 2015. Effect of UV-B radiation on physiological and phenological plasticity in rice (*Oryza sativa* L.). M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 108p.

Wagh, Y.S. and Nandini, K. 2019. Changes in Morpho-physiological and yield parameters of rice (*Oryza sativa* L.) in response to ultraviolet-B (UV-B) radiation. *Int. J. Environ. Clim. Change* 9(12): 862-877.

Wahid, A. and Rasul, E. 2005. Photosynthesis in leaf, stem, flower and fruit. In: Pessarakli, M. (ed.), *Handbook of Photosynthesis* (2nd). CRC Press, Florida, pp. 479-497.

Wang, J., Xu, J., Xue Gong, X., Yang, M., Zhang, C., and Li, M. 2019. Biosynthesis, chemistry, and pharmacology of polyphenols from Chinese *Salvia* species: A Review. *Molecules* 24(1): 155.

Wang, Y., Guo, Q., and Jin, M. 2009. Effects of light intensity on growth and photosynthetic characteristics of *Chrysanthemum morifolium*. *Zhongguo. Zhong. Yao. Za. Zhi.* 34(13): 1633-1635.

Warren, M.J., Bassman, J., Fellman, J.K., Mattinson, D.S., and Eigenbrode, S. 2003. Ultraviolet-B radiation of *Populus trichocarpata* leaves. *Tree Physiol.* 23: 527-535.

Wilson, M.I., Ghosh, S., Gerhardt, K.E., Holland, N., Babu, T.S., Edelman, M., Dumbroff, E.B., Greenburg, B.M. 1995. In vivo photomodification of ribulose 1,5-bisphosphate carboxylase oxygenase holoenzyme by ultraviolet-B radiation formation of a 66-kiloDalton variant of the large subunit. *Plant Physiol.* 109: 221-229.

Winkel-Shirley, B. 2002. Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol.* 5: 218-223.

WMO (World Meteorological Organization). 2014. Scientific assessment of ozone depletion: 2014, World Meteorological Organization, Global ozone research and monitoring project, report no. 56, Geneva, Switzerland.

World Health Organization. 2003. Guidelines for the Assessment of Herbal Medicine Programme on Traditional Medicine. Doc. WHO/TRM/91.4.WHO, Geneva.

Wright, I.J. *et al.* 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.

Wu, Q., Chen, Z., Sun, W., Deng, T., and Chen, M. 2016. De novo sequencing of the leaf transcriptome reveals complex light-responsive regulatory networks in *Camellia sinensis* cv. Baijiguan. *Front Plant Sci.* 7: 332.

Yamasaki, H., Sakihama, Y., and Ikehara, N. 1997. Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_2O_2. *Plant Physiol.* 115: 1405-1412.

Yamori, W., Makino, A., and Shikanai, T. 2016. A physiological role of cyclic electron transport around photosystem I in sustaining photosynthesis under fluctuating light in rice. *Nat. Sci. Rep.* 6: 20147.

Yang, F., Huang, S., Gao, R., Liu, W., Yong, T., Wang, X., Wu, X., and Yang, W. 2014. Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio. *Field Crop Res.* 155: 245-253.

Yang, J.C., Liu, K., Zhang, S.F., Wang, X.M., Wang, Z.Q., and Liu, L.J. 2008. Hormones in rice spikelets in responses to water stress during meiosis. *Acta Agronomica Sinica* 34(1): 111-118.

Yang, X.Y., Ye, X.F., Liu, G.S., Wei, H.Q., and Wang, Y. 2007. Effects of light intensity on morphological and physiological characteristics of tobacco seedlings. *Chin. J. Appl. Ecol.* 18: 2642-2645.

Yokota, A., Kawasaki, S., Iwano, M., Nakamura, C., Miyake, C., and Akashi, K. 2002. Citrulline and DRIP-1 protein (ArgE Homologue) in drought tolerance of wild watermelon. *Ann. Bot.* 89: 825-832.

Yoshida, S., Forno, D.A., Cock, J.H., and Gomez, K.A. 1976. *Laboratory manual for physiological studies of rice*, (3rd Ed.). Manila, Philippines: Int. Rice Res. Inst.71p.

Yu, G.H., Li, W., Yuan, Z.Y., Cui, H.Y., Lv, C.G., Gao, Z.P., Han, B., Gong, Y.Z., and Chen, G.X. 2013. The effects of enhanced UV-B radiation on photosynthetic and biochemical activities in super-high-yield hybrid rice Liangyoupeijiu at the reproductive stage. *Photosynthetica* 51: 33-44.

Yu, G.H., Li, W., Yuan, Z.Y., Cui, H.Y., Lv, C.G., Gao, Z.P., Han, B., Gong, Y.Z., and Chen, G.X. 2013. The effects of enhanced UV-B radiation on photosynthetic and biochemical activities in super-high-yield hybrid rice Liangyoupeijiu at the reproductive stage. *Photosynthetica* 51: 33-44.

Yun, K.J., Koh, D.J., Kim, S.H., Park, S.J., Ryu, J.H., Kim, D.G., Lee, J.Y., and Lee, K.T. 2008. Anti-inflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygase-2, and proinflammatory cytokines expressions via nuclear factor-kappa B inactivation. *J. Agric.Food Chem.* 56: 10265-10272.

Zervoudakis, G., Salahas, G., Kaspiris, G., and Konstantopoulou, E. 2012. Influence of light intensity on growth and physiological characteristics of common sage (*Salvia officinalis* L.). *Braz. Arch. Biol. Technol.* 55(1): 89-95.

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., and Jin, J. 2018. Effect of drought on agronomic traits of rice and wheat: A meta-analysis. *Int. J. Environ. Res. Public Health.* 15(5): 839.

Zhao, D., Reddy, K.R., Kakani, V.G., Mohammed, A.R., Read, J.J., and Gao, W. 2004. Leaf canopy photosynthetic characteristics of cotton (*Gossypium hirsutum*) under elevated CO_2 concentration and UV-B radiation. *J. Plant. Physiol.* 161: 581-590.

Zhou, P., Li, Q., Liu, G., Xu, N., Yang, Y., Zeng, W., Chen, A., and Wang, S. 2018. Integrated analysis of transcriptomic and metabolomic data reveals critical

metabolic pathways involved in polyphenol biosynthesis in *Nicotiana tabacum* under chilling stress. *Funct. Plant Biol.* 46: 30-43.

Zhou, Y., Lam, H.M., and Zhang, J. 2007. Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. *J. Exp. Bot.* 58: 1207-1217.

Zhu, H., Li, X., Zhai, W., Liu, Y, Gao, Q., Liu, J., Ren, L., Chen, H., Zhu, Y. 2017. Effects of low light on photosynthetic properties, antioxidant enzyme activity, and anthocyanin accumulation in purple pak-choi (*Brassica campestris* ssp. *Chinensis* Makino). *PLoS ONE* 12(6): e0179305.

Zhu, P., Yang, S.M., Ma, J., Li, S.X., and Chen, Y. 2008. Effect of shading on the photosynthetic characteristics and yield at later growth stage of hybrid rice combination. *Acta. Agron. Sin.* 34(11): 2003-2009.

Zielinski, H., Kozlowska, H., and Lewczuk, B. 2001. Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innovative Food Sci. Emerging Technol.* 2(3): 159-169.

Zlatev, Z.S., Lidon, F.J.C., and Kaimakanova, M. 2012. Plant physiology responses to UV-B radiation. *Emir. J. Food Agric.* 24(6): 481-501.

Zuk-Golaszewska, K., Upadhyaya, M.K., and Golaszewski, J. 2003. The effect of UV-B radiation on plant growth and development. *Plant Soil Environ.* 49: 135-140.

Appendices

APPENDIX I

Preparation of stock solution for SDS-PAGE

A) Acrylamide/Bis-Acrylamide stock (30%) (100ml):

Acrylamide	29.2 g
Bis-Acrylamide	0.8 g

B) Tris-HCl buffer stock, 1.5 M (pH 8.8) (100ml):

Tris-HCL	3.69 g
Tris base	15.39 g

C) Tris-HCl buffer stock, 0.5 M (pH 6.8) (100ml):

Tris-HCL	7.8 g
Tris base	0.45 g

D) Sample buffer:

Bromophenol blue	5 mg in 1 ml D/W (0.5 ml taken)
0.5 M Tris-HCl pH 6.8	2 ml
SDS	0.5 g
Glycerol	1 ml
2-mercaptoethanol	7.013 ml in 1 ml D/W (0.5 ml taken)

E) Electrode buffer (pH 8.3) (1 L):

Tris base	3.03 g
Glycine	14.4 g
SDS	1 g

APPENDIX II

A) Resolving gel (12%) (10 ml):

30% acrylamide stock	3.3 ml
1.5 M Tris-HCL (pH 8.8)	2.5 ml
10% SDS	100 µl
Distilled water	3.99 ml
10% APS	100 µl
TEMED	10 µl

B) Stacking gel (5%) (3 ml):

30% acrylamide stock	510 µl
0.5 M Tris-HCL (pH 6.8)	750 µl
10% SDS	30 µl
Distilled water	1.66 ml
10% APS	40 µl
TEMED	7 µl

APPENDIX III

A) Staining solution (0.1%) (100 ml):

Coomassie brilliant blue R-250	0.1 g
Glacial acetic acid	10 ml
Methanol	40 ml
Distilled water	50 ml

B) Staining solution (0.1%) (100 ml):

Glacial acetic acid	10 ml
Methanol	40 ml
Distilled water	50 ml

APPENDIX IV

10x TBE (Tris borate ethylenediaminetetraacetic acid) pH 8.0:

Tris base	108 g
Boric acid	54 g
0.5 M EDTA	40 ml
DEPC treated water	Up to 1 L

PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR STUDIES IN MEDICINAL RICE (*Oryza sativa* L.), NJAVARA, AS INFLUENCED BY ABIOTIC STRESSES

By

WAGH YOGESH SAHEBRAO

(2015-21-013)

ABSTRACT

Submitted in partial fulfillment of the

requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT PHYSIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2020

Abstract

The study entitled "Physiological, biochemical and molecular studies in medicinal rice (*Oryza sativa* L.), Njavara, as influenced by abiotic stresses" was conducted during 2015 to 2019 at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram. The objective of the study was to elicit information on the physiological, biochemical and molecular attributes associated to secondary metabolites accumulation due to abiotic stresses *viz.,* shade, drought and UV-B stress in medicinal rice Njavara. The study was conducted as three different experiments with black glumed and yellow glumed njavara.

Experiment one was a pot culture study laid out in Completely Randomized Design (CRD) with two varieties and five treatments. The study included a combination of two levels of shade stress and two levels of water deficit stress and a control with four replications and with three pots in each replication. The observations were taken at different critical stages of the crop *viz.,* vegetative stage, panicle initiation stage, flowering stage and harvesting stage. The results revealed that the morphological characters such as plant height, leaf area index and specific leaf area were higher under 40% shade whereas under 50% field capacity, they were found to be highly reduced. Number of tillers per plant was higher under control (T_5) compared to shaded and water deficit conditions. Leaf gas exchange parameters *viz.,* photosynthetic rate, stomatal conductance and transpiration rate were lesser under both experimental conditions compared to the control. However maximum reduction of leaf gas exchange parameters were recorded at 50% field capacity (T_3) followed by 40% shaded condition (T_2) compared to the control (T_5). Among the biochemical characters, maximum chlorophyll content was found under 40% shaded condition (T_2). The biochemical parameters such as flavonoid, phenol and proline content of leaves were found higher under 50% field capacity (T_3) at all the growth stages studied. The total flavonoid

content in the grains was found higher under 75% field capacity (T_4) followed by 20% shade treatment (T_1) in both the varieties.

The second experiment was carried out in pot culture in CRD with four treatments, three replications and four pots per replication. The crop was subjected to UV-B (280-320 nm) radiation with the help of UV-B fluorescent tubes during the different critical stages of plants i.e. from vegetative stage, from panicle initiation stage and from flowering stage till harvesting in ventilated polyhouse. The UV-B tubes were switched on for 4 hours daily from 10 am to 2 pm and the average intensity of UV-B radiation at the canopy level of plants was maintained at 4 Wm^{-2}. The control was maintained in another compartment of polyhouse without UV-B tubes. The results indicated that the morphological characters *viz.*, plant height, leaf area index, specific leaf area and tiller number reduced significantly under UV-B radiation treatment and the maximum reduction was observed in treatment T_1 (UV-B treatment from vegetative stage). Leaf gas exchange parameters as well as chlorophyll content decreased significantly under UV-B radiation treatments compared to the treatment without UV-B radiation. However flavonoid, phenol and proline contents of leaves were found to increase under UV-B treatments (T_1, T_2 and T_3). The accumulation of total flavonoid in grains was found significantly higher in treatment T_2 (UV-B radiation treatment given from panicle initiation stage) in both the varieties.

The treatments which resulted in the highest accumulation of flavonoid content in grains were selected from experiment 1 (20% shade and 75% field capacity) and experiment 2 (UV-B radiation treatment from panicle initiation stage) for molecular analysis in experiment 3. Protein profiling was done in leaves using SDS-PAGE in which, there was variation in the intensity of large subunit (55 kDa) as well as small subunit (16 kDa) of RuBisCO, between the varieties and the treatments. The intensity of those bands were found higher in 20% shade (T_1), 75% field capacity (T_2) and control (T_4) whereas under UV-B radiation treatment from panicle initiation stage (T_3)

relatively lesser intensity was exhibited. Gene expression study in grains using qRT-PCR revealed relatively higher expression of *chalcone synthase (CHS)* and *CYP75B4* genes in black glumed njavara (V_1) than the yellow glumed njavara (V_2) variety. Also the gene expression study revealed that both the genes were over expressed under T_3 (UV-B radiation treatment from panicle initiation stage) and T_2 (75% field capacity). But both the genes (*CHS* and *CYP75B4*) were found down regulated under T_1 (20% shade) condition.

The present study revealed that the flavonoid content in grains is higher in black njavara (V_1) compared to yellow njavara (V_2). The study also indicated that the various parameters studied *viz.,* growth, physiological and biochemical were found to have positive influence on the flavonoid accumulation of grains. But the chlorophyll content was found to have negative influence on the flavonoid content of grains. Proline content was less in black njavara (V_1) under UV-B radiation compared to yellow njavara (V_2). Based on the present study it is concluded that the black glumed njavara performed better than yellow glumed njavara under all the stress conditions studied and can be exploited better for its therapeutic value. Application of mild stress levels *viz.,* water deficit (75% field capacity) or UV-B radiation treatment from panicle initiation stage may be utilized to enhance the medicinal quality of this crop.