EFFECT OF MELATONIN ON MORPHO-PHYSIOLOGY, GROWTH AND YIELD PERFORMANCE OF BORO RICE UNDER DROUGHT STRESS

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This is to certify that thesis entitled **“EFFECT OF MELATONIN ON MORPHO-PHYSIOLOGY, GROWTH AND YIELD PERFORMANCE OF BORO RICE UNDER DROUGHT STRESS”** *submitted to the Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE*** *in* ***AGRICULTURAL*** **BOTANY,** *embodies the result of a piece of bona fide research work carried out by* ***ASHISH KUMAR ROY****, Registration No.:* ***1910338*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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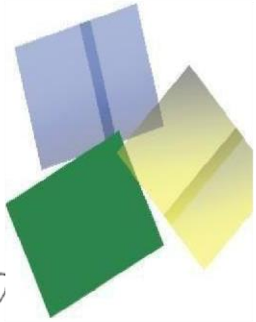
Place: Dhaka, Bangladesh



Dedication

To my parents,
The reason of what I become
today. I am grateful for your
great support
and continuous care.

To my youngest aunt,
The woman with whose blessing,
I started my journey.

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ABSTRACT

Drought stress adversely affect plant growth and development. Melatonin (N-acetyl-5-methoxytryptamine) plays an essential role in abiotic stress but its role in alleviating drought stress in rice is not clear. Therefore, the present study investigated the effect of melatonin application on plant growth, physiological responses, and yield performance in rice under drought stress. *Oryza sativa* L. cv. BRRI dhan29 was the planting material. The experiment was laid out in Randomized Complete Block Design (RCBD). Rice was grown with 0 and 100 µM melatonin alone or in combination with drought. The control plant was watered as per requirement while the others were subjected to drought stress by withholding water. Treatments were applied 15 days after transplanting. The treatments were: Control (C), 100 µM melatonin (M), withholding of water for 10 days (D1), D1+M, withholding of water for 20 days (D2) and D2+M. Drought stress resulted higher proline accumulation in rice plants. This indicates the drought-induced osmotic stress. Relative water content (RWC%) of drought affected rice plants decreased noticeably. Drought caused oxidative stress and disrupts membrane properties which is clear from decreased value of leaf membrane stability index (MSI%). The reduced SPAD value indicates breakdown of chlorophyll pigment or hindering its biosynthesis. Drought decreased the vegetative growth and reproductive development of rice plant. Exposure of rice plants to drought stress decreased plant height, no. of tillers plant^{-1}, leaf area, dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1}, panicle length, no. of spikelet panicle^{-1}, 1000 grain weight and finally decreased the grain yield plant^{-1}. Therefore, drought stress altered the physiological and biochemical processes of rice plants. The visible effect of drought stress was reduction of different growth parameters and alteration of phenological processes. Drought exerted negative effects on the reproductive and yield attributes and finally the yield of rice decreased significantly. Exogenous application of melatonin alleviated the adverse effect of drought stress to some extent, compared to drought affected plants without melatonin. Exogenous melatonin addition improved MSI value under drought stress. Melatonin addition restored the RWC in drought affected rice plants, compared to drought stress alone. Melatonin increased chlorophyll content under drought stress. Melatonin added drought treated rice plants showed improved physiology, growth, reproductive development and the yield. This study suggests that melatonin can be exploited as drought alleviating elicitor.

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LIST OF ABBREVIATIONS

%	Percent
@	At the rate of
$_{0}$C	Degree Celsius
ABA	Abscisic Acid
AEZ	Agro-Ecological Zone
APX	Ascorbate Peroxidase
AsA	Ascorbate
BARI	Bangladesh Agriculture Research Institute
BRRI	Bangladesh Rice Research Institute
Cm	Centimeter
ABA	Abscisic acid
CAT	Catalase
CV%	Percentage of Coefficient of Variation
NCED3	9-cis-epoxycarotenoid dioxygenase 3
DAT	Days After Transplanting
AMT	Ammonium transporter
NRT	Nitrate transporter
NR	Nitrate reductase
NiR	Nitrite reductase
EC	Electrical Conductivity
e.g	As for example
et al.	and others
FAO	Food and Agriculture Orzanization
g/gm	Gram
GS	Glutamine synthetase
GOGAT	Glutamate synthase
SDS	Sodium dodecyl sulfate
GPX	Glutathione Peroxidase
IAA	Indole-3-acetic acid
SNAT	Serotonin N-acetyl transferase
	i.e. that is
GC-MS	Gas chromatography coupled to mass spectrometry
BTH	Benzothiadiazole
Kg	Kilogram
Kg ha^{-1}	Kilogram per hectare
LSD	Least Significant Difference
L	Liter

LIST OF ABBREVIATIONS (Continued)

M	Meter
MG	Methylglyoxal
Mg/L	Milligram per Liter
MoP	Muriate of Potash
N	Nitrogen
NR	Nitrate Reductase
nm	Nano Meter
Ng	Nano Gram
pH	Hydrogen ion concentration(Negative Logarithm)
PI	Panicle Initiation
Pn	Net Photosynthetic Rate
Pro	Proline
POD	Peroxidase
PS I/II	Photo System I/II
qP	Photochemical Quenching
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
RWC	Relative Water Content
ppm	Parts Per Million
M	Melatonin
SAU	Sher-e-Bangla Agricultural University
SOD	Superoxide Dismutase
TSP	Triple Super Phosphate
µM	Micro Molar
µmol/g	Micro Mol per gram
FW	Fresh Weight
Zn	Zink

CHAPTER 1

INTRODUCTION

Drought stress is a major abiotic factor limiting crop production (Hasanuzzaman et al., 2012), more importantly in the rain-fed areas of the world (Nikus et al., 2004). Drought is the most severe stress and the main cause of significant losses in growth, productivity of crop plants and finally their yields (Hasanuzzaman et al., 2012). Drought affects morpho- physiological, bio-chemical and molecular processes in plants resulting in growth inhibition, stomata closure with consecutive reduction of photosynthesis, transpiration, decrease in chlorophyll content and inhibition of photosynthesis and protein changes (Hasanuzzaman et al., 2012).

Rice, the dominant crop in Bangladesh, accounts for more than 90% of total cereal production covering 75% of Country's total cropped area (BBS, 2016; GoB, 2016). In the production year 2014-2015, boro (winter-early summer, also known as rabi) rice accounted for 55%, while aus (summer-early monsoon, also known as kharif 1) rice and aman (monsoon-late autumn, also known as kharif 2) rice respectively contributed 7% and 38% of total rice production, respectively (BBS, 2016). ―Boro‖ is the dry season irrigated rice crop planted from December to early February and harvested between April and June. About 3,000-5,000 liter of irrigation water is required to produce one kilogram of boro rice. Due to climate change the drought prone area of Bangladesh is increasing day by day. The groundwater level is also decreasing. So, irrigated rice production technology may fall under threaten. Therefore, it is vital to know about the drought stress response of rice which is supposed to help managing the drought stress in rice together with other strategies like agronomic approaches or development of drought tolerant rice cultivar.

Alternative cultivation technology is time demanding because various adverse climatic/environmental condition. Melatonin (N-acetyl-5-methoxytryptamine), a pleiotropic and highly conserved molecule, is ubiquitous throughout the animal and plant kingdoms (Hardeland et al., 2011). Melatonin is a naturally occurring compound that is found in humans, animals, plants and microbes. The presence of melatonin in plants has been identified in a range of species including rice. It has been demonstrated that changes occur in the major compounds during processing and one would also expect changes in the minor compounds i.e. melatonin. It performs various functions in plant growth, development, and stress responses (Shi et al., 2016). For example,it increases seed germination, promotes lateral root development, delays leaf senescence, protectes plants from stress, and increases crop yield (Zhang et al., 2013, Ahmad et al., 2020). Moreover, melatonin has been demonstrated as an effective plant biostimulant against biotic and abiotic stresses (Arnao and HernándezRuiz, 2021). Melatonin triggers the accumulation of compatible solutes such as total soluble sugars and proline content (Sharma et al., 2020). Many studies reported that exogenous melatonin could enhance plant tolerance to drought stress (Wei et al., 2015, Zhang et al., 2015). For example, melatonin alleviates oxidative damage during drought stress by directly scavenging ROS resulted in decreases in electrolyte leakage and MDA content and by enhancing antioxidant enzyme activities (Ye et al., 2016, Cherono et al., 2021). Melatonin also has crosstalk with other plant growth regulators such as gibberellin, jasmonic acid, and abscisic acid to regulate various physiological processes in plants under drought stress. In addition, melatonin regulates the transcription of various essential genes involved in antioxidative defense mechanisms (Sharma et al., 2020). Studies have demonstrated its important roles in regulating plant growth, development, and defense against various environmental stresses (Arnao and Hernández-Ruiz, 2014, 2015; Zhang et al., 2015; Nawaz et al., 2016). The beneficial role of melatonin in stress mitigation is broadly attributable to higher photosynthesis, improvement of cellular redox homeostasis and alleviation of oxidative stress, and regulation of the expression of stress responsive genes involved in signal transduction (Li et al., 2012; Bajwa et al., 2014; Zhang and Zhang, 2014). At the end of this research an insight into the effect of different water regimes or drought affected Boro rice will be known which will help to introduce /initiate cultivation of Boro rice under reduced water supply. Moreover the role of melatonin to alleviate the adverse effect of drought stress will be investigated. The result of the present study expected to help both plant physiologists and plant breeder to manipulate the traits of the Boro rice plants to adapt under reduced water or drought condition. Thus, introduction of dry cultivation practices in Boro rice may improve the food security of the country as a whole.

Considering the above mentioned facts, the present study will be undertaken with the following objectives:

I. To investigate the physiological, growth and yield performance of some boro rice cultivars under drought stress.
II. To study the role of melatonin to improve the drought tolerance of boro rice.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Rice, the Major Food Crop

The dominant food crop of Bangladesh is rice, accounting for about 75 percent of agricultural land use (and 28 percent of GDP). Rice production increased every year in the 1980s (through 1987) except FY 1981, but the annual increases have generally been modest, barely keeping pace with the population. Rice production exceeded 15 million tons for the first time in FY 1986. In the mid-1980s, Bangladesh was the fourth largest rice producer in the world, but its productivity was low compared with other Asian countries, such as Malaysia and Indonesia. It is currently the world's forth-largest producer. Highyield varieties of seed, application of fertilizer, and irrigation have increased yields, although these inputs also raise the cost of production and chiefly benefit the richer cultivators.

The cultivation of rice in Bangladesh varies according to seasonal changes in the water supply. The largest harvest is aman, occurring in November and December and accounting for more than half of annual production. Some rice for the aman harvest is sown in the spring through the broadcast method, matures during the summer rains, and is harvested in the fall. The higher yielding method involves starting the seeds in special beds and transplanting during the summer monsoon. The second harvest is aus, involving traditional strains but more often including high-yielding, dwarf varieties. Rice for the aus harvest is sown in March or April, benefits from April and May rains, matures during the summer rain, and is harvested during the summer. With the increasing use of irrigation, there has been a growing focus on another rice-growing season extending during the dry season from October to March. The production of this boro rice, including high-yield varieties, expanded rapidly until the mid-1980s, when production leveled off at just below 4 million tons.[1] Where irrigation is feasible, it is normal for fields throughout

Bangladesh to produce rice for two harvests annually. Between rice-growing seasons, farmers will do everything possible to prevent the land from lying fallow and will grow vegetables, peanuts, pulses, or oilseeds if water and fertilizer are available.

2.2 Drought Stress and its Impacts on Plant Physiology

Plants growing under water deficit conditions face many challenges at the biochemical and molecular level which ultimately causes hindrance to plant's growth and yield . Drought stress causes a decline in photosynthesis by disturbing the mechanism of light harvesting and its utilization, negatively impacting the metabolism of photosynthetic pigments, which declines the RuBisCo function accompanied by disruption of the photosynthetic apparatus . Disruption of the chloroplast structure also negatively affects photosynthetic performance of plants growing under drought conditions. Drought negatively influences the intercellular CO_2 levels which favors the generation of reduced photosynthetic electron transport constituents, resulting in enhanced generation of ROS, which causes disruption of the photosynthetic apparatus. Disruption of the photosynthetic apparatus due to drought leads to a reduction of the photosynthetic rate, stomatal conductance, transpiration rate, photochemical efficiency of PSII and photosynthetic electron transport rate. Stomatal closure during water deficit conditions is regulated by the enhanced endogenous levels of abscisic acid (ABA), which acts as a signaling molecule to modulate a cascade of various physiological and molecular processes. This enhanced biosynthesis of ABA is due to the up regulation of the ABA biosynthetic gene NCED3 (9-cis-epoxycarotenoid dioxygenase 3) . The expression of histone H1-S is enhanced under drought stress and this protein promotes the closure of stomata . Moreover, ABA also acts as primary messenger for cell signaling pathways which further accelerates the generation of ROS, followed by increased accumulation of cytosolic Ca2+ which acts as secondary messenger, stimulating other signaling cascades to regulate plant processes at the molecular level . Drought stress decreases water potential and the relative water content of plants . Declined water potential further causes a reduction in the uptake of various essential minerals, such as nitrogen, phosphorous and potassium . Water deficit conditions have a negative impact on the nitrogen transporters and nitrogen metabolism. It is due to the down-regulation of genes, such as AMT (ammonium transporter), NRT (nitrate transporter), NR (nitrate reductase), NiR (nitrite reductase), GS (glutamine synthetase) and GOGAT (glutamate synthase), under drought stress . Reduced nutrient uptake is also accompanied by reduced efficiency of their translocation to the target sites in plants growing under water deficit conditions . Moreover, declined root growth in soils having low water also negatively affects the efficiency of the nutrient uptake. Drought stress causes an imbalance between the production of ROS and their scavenging, leading to oxidative stress in plant cells. This ROS scavenging failure leads to the over accumulation of ROS in plant cells, resulting in oxidation of proteins, peroxidation of lipid membranes and damage to genetic material. Increased ROS levels also cause a reduction in fixation efficiency of CO_2 accompanied by enhanced photorespiration. To counterattack the negative effects of drought induced oxidative stress, plant’s enzymatic and non-enzymatic antioxidants work together to provide drought resistance. However, severe drought causes an imbalance in redox homeostasis, which is mainly due to the declined efficiency of the anti-oxidative defense system. For example, activities of enzymes, such as catalase (CAT) and ascorbate peroxidase (APX), were reported to decrease under high drought conditions. The production of various osmolytes, such as soluble sugars, proline and glycine-betaine, also become enhanced, resulting in more accumulation of these compounds which act as osmoprotectants under drought stress . These osmolytes assist in maintaining the leaf turgor which results in efficient stomatal conductance followed by better CO_2 intake by leaves and water uptake by roots. The enhanced osmoprotectant accumulation is due to the up-regulation of genes involved in biosynthesis of osmolytes, aquaporins, LEA proteins, accompanied by regulation of various important transcription factors .

2.3 Drought affected area in the World and Bangladesh

According to data from the Aqueduct project at the World Resources Institute, two European countries, Moldova and Ukraine, have the highest risk of drought globally. More affected countries, which carry a medium to high risk of droughts, can be found in the Middle East, North Africa, Asia – including India and China – and Europe.

Ukraine and its smaller neighbor have been hit by devastating droughts in the past, which majorly diminished crops. The problem of climate change disrupting temperature and rain patterns is apparent in many places in Eastern Europe that have transitioned towards hotter climates recently.

Countries at risk of drought are not always the same as those experiencing the most water stress, which is defined as a lack of enough suitable water for the use of all people in the country, including drinking. While water stress was also common in South Asia, the Middle East and North Africa, it was much less common in Europe where poor water quality poses less of a problem.(Appendix I)

The survey by the World Resources Institute collected data on 138 countries and took into account past drought intensity, water stress, drought vulnerability, population, crop and livestock density.

Normally northwestern part of the country encounters more droughts than the other parts of the country. In Bangladesh, the Barind (upland of Northwestern part) has been experiencing drought conditions for the last two to three decades. This area is designated as the severe drought-prone areas (Figure 2). It covers Barind Tract, Punarbhava floodplain and Ganges river flood plain area. This drought-prone region covers most part of the greater Dinajpur, Rangpur, Pabna, Rajshahi, Chapai Nawabganj, Bogra, Joypurhat and Naogaon district. After severely drought affected Northwestern region, Southwestern part of Bangladesh is also facing drought impacts. But the severity of drought in this region is moderate. Among South western region, mainly Jhenaidah, Jessore and Satkhira districts experiences drought during the dry season (Habiba *et al.*, 2011).(Appendix II)

This has an enormous impact on the crop production as the production of all winter crops goes down with the arrival of droughts. Droughts also come with land degradation, low livestock population, unemployment, and malnutrition (Chowdhury, 2010).

Bangladesh is at higher risk from droughts. Drought conditions due to deficiency

In rainfall affect different parts of Bangladesh mostly during the pre-monsoon and postmonsoon periods. One study has shown (Figure 2) that from 1949 to 1979, drought conditions had never affected the entire country and total population in any drought year. The drought of 1979 was one of the most severe in recent times. The percentage of drought-affected areas was 31.63 percent in 1951, 46.54 percent in 1957, 37.47 percent in 1958, 22.39 percent in 1961, 18.42 percent in 1966, 42.48 percent in 1972, and 42.04 percent in 1979 (Ha and Ahmad, 2015).

A study reported that the percentage of areas in each district of the north-west region facing combined drought risk (Table 1). Sirajgong and Naogaon are two districts free from drought risk. Slight and moderate risk areas encompass 22.71% and 29.72% of total geo-graphical area. Severe and very severe risk prevails in nearly 21.29% and 9.42% of the area which includes the districts that are major producers of food grains and different vegetable, (Murad and Islam, 2011).

2.4 Events of drought

Although droughts were not continuous, they did affect the low rainfall zones of the country. Droughts are associated with the late arrival or early withdrawal of monsoon rains and with intermittent dry spells. Between 1949 and 1991, droughts occurred in Bangladesh 24 times. Very severe droughts hit the country in 1951, 1957, 1958, 1961, 1972, 1975, 1979, 1981, 1982, 1984 and 1989, 1994, and 2000, 2006 and 2009. Past droughts have typically affected about 47% area of the country and 53% of the population (Selvaraju *et al.*, 2006). Every five to ten years, Bangladesh is affected by the major country-wide droughts. It causes huge loss to the agricultural crops, livelihood, health and fisheries etc. which are directly related to food security of the country.

Table 1. Chronology of major drought events and its impact in Bangladesh

Year	Details
1874	Extremely low rainfall affected Bogra, great crop failure.
1951	Severe drought in Northwest Bangladesh substantially reduced rice production.
1973	Drought responsible for the 1974 famine in northern Bangladesh which is one of the most severe of the century.
1975	Drought affected 47 percent of the country and more than half of the total population.
1978-79	One of the most severe droughts with widespread damage to crops reducing rice production by about 2 million tons and directly affecting about 42 percent of the cultivated land and 44 percent of the population.
1981	Severe drought adversely affected crop production.
1982	Drought caused a loss of rice production of about 53000 tons while, in the same year, flood damaged about 36000 tons.
1989	Drought dried up most of the rivers in Northwest Bangladesh with dust storms in several districts, including Naogaon, Nawabganj, Nilpahamari and Thakurgaon.
1994-95	The most persistent drought in recent times which caused immense crop damage, especially to rice and jute, the main crops of Northwest Bangladesh and to bamboo clumps, a leading cash crop.
1995-96	Crops in the northwestern region affected.
2006	A reduction of Aman crop about 25-30 percent in northwestern part.

(Source: Selvaraju *et al.*, 2006)

In 2009, Bangladesh is experienced with serious agricultural drought particularly in the northwestern region.

2.5 Physiological Changes in Cereals During Drought

Water scarcity occurs during drought, where water is simply not present in the soil. Physiological drought is not necessarily caused only by a lack of water in the soil, but it can also occur when there is an excess of water (the soil water potential is lower than in the plant) or high soil drought (ions also affect the water potential). Thus, physiological drought is a condition where the plant cannot receive water (Lisar et al. 2012, Osakabe et al. 2014). The responses of plants to water stress are diverse and may involve the contribution of various defence mechanisms or modification of physiology, morphology, anatomy, biochemistry, as well as short and long-term developmental and growth-related adaptation processes (Abobatta 2019). Physiological responses to drought and heat stresses providing an escape to the water or heat stress comprise morphological and physiological adjustments (Lamaoui et al. 2018). Enlarged root system (Gregorová et al. 2015, Abobatta 2019), reduced stomatal number and conductance, decreased leaf area, increased leaf thickness, and leaf rolling or folding (Earl & Davis 2003) to lessen evapotranspiration (Anjum et al. 2011, Lamaoui et al. 2018, Kapoor et al. 2020) are strictly associated with an adaptive response. Reduced plant growth and productivity under drought are caused by altered plant water relations, decreased CO2 assimilation, cellular oxidative stress, membrane damage of affected tissues, and in some cases, inhibition of enzymes activity. Under drought conditions, plants can alter water relations to maintain cellular functions. For example, plants exhibit osmotic adjustment by synthesizing and accumulating compatible solutes such as free amino acids, sugars, and proline (edit some references) (Izanloo et al. 2008, Tatar & Gevrek 2008). Osmotic adjustment allows the plant to maintain turgor pressure and cell volume at low water potential which is important for maintaining metabolic functions. In addition, osmotic adjustment facilitates the recovery of metabolic activities after relief from stress (Izanloo et al. 2008). Although investigations have been made to study the recovery of photosynthesis from drought stress (Osakabe et al. 2014) in different crop species including wheat, studies addressing membrane stability, oxidative stress, antioxidative process, and osmolyte dynamics during drought recovery are limited. Studies quantifying the impact of plant metabolic changes during drought indicate, that stress conditions during vegetative growth periods can significantly influence grain yield of wheat (Araus et al. 2002, Souza et al. 2004, Siddiqui et al. 2017). After drought stress is removed, the availability of even a small amount of rainfall can have a significant effect on plant physiological functions, ranging from whole-plant physiological and morphological responses to biochemical responses (Abobatta 2019). Therefore, it is of particular importance to investigate the underlying mechanisms contributing to drought tolerance (Izanloo et al. 2008). Water deficit in plants decreases or suppresses the process of photosynthesis (Earl & Davis 2003, Anjum et al. 2011, Kapoor et al. 2020). The decrease in the photosynthetic process under drought is mainly attributable to the decline in CO2 conductance via stomata and mesophyll limitations, however, the decrease in photosynthetic activity due to drought may also be due to reduced ability of stomatal movement (Abid et al. 2018). The loss of CO2 uptake affects Rubisco activity and decrease the function of nitrate reductase and sucrose phosphate synthase and the ability for ribulose bisphosphate (RuBP) production (Singh & Thakur 2018). The chlorophyll content is another photosynthetic attribute strongly influenced by water deficit (Gregorová et al. 2015, Alghbari & Iksan 2018). For example, leaf chlorophyll synthesis and chlorophyll a/b proportion is altered by drought stress. A decline in photosynthetic activity, amount of chlorophylls, loss of photosystem II photochemical efficiency, alteration in stomatal movement, and disturbance in the water status of plants resulted in declined plant productivity. Among others, a major cause for the decline in the amount of chlorophyll due to drought stress is the drought-promoted O_2- and $H_2 O_2$ production, which results in lipid peroxidation and significant chlorophyll degradation. Drought stress also causes a reduction in the abundance of several Calvin cycle proteins, including Rubisco (Anjum et al. 2011). In terms of metabolites, a reduced rate of photosynthesis disrupts carbohydrate production, lowers the level of sucrose in leaves, and prevents the transport of sucrose into organs. Ultimately, reproductive development is limited. In addition, drought induces vacuolar inverse of sucrose mediated by hydrolysis and modulation of osmotic potential. Drought inhibits the cell division of the developing embryo/endosperm resulting in the poor intensity of cell division and ultimately leading to germ abortion (Andersen et al. 2002).

2.6 Effect of drought on plant growth

Drought stress is well recognized as a limiting factor that alters multiple aspects of plant growth and development (Alghbari & Iksan 2018, Kapoor et al. 2020). Germination of seeds, health, coleoptile length, and leaf area are foremost for the plant progression. Seed germination is the primary aspect of growth that is sensitive to drought (Kapoor et al. 2020). Visible symptoms of the plant exposed to water scarcity in the initial vegetative stage is besides seed germination reduction also leaf wilting. Plant growth is also in term of reducing shoot length and fresh weight of the hypocotyls negatively affected by lack of water (Abobatta 2019). The interruption in establishment of buds and flowers is also observed due to a lack of nutrients from the dried soil (Abobatta 2019, Kapoor et al. 2020). The root system is the main plant organ for adaptation to drought stress conditions. In conditions of water deficit, plants seek to extract water from deeper soil layers by boosting their root architecture (Mitra 2001, Lisar et al. 2012, Abobatta 2019). The main basis of variation appears to be constitutive, therefore, a root system architecture that allows reserve of more water quantity is the most important tool for drought tolerance (Abobatta 2019). Moreover, water availability is primarily recognized by roots, which in turn regulates its growth (Kudoyarova et al. 2011). The plant leaf is an important part of the plant because of the process of photosynthesis, which in turn is the main driver of plant growth. Decrease in leaf area is a drought avoidance strategy because declining leaf area results in a decreased water loss by the process of transpiration (Earl & Davis 2003, Anjum et al. 2011, Kapoor et al. 2020). This reduction in leaf area is attributable to the inhibition of leaf expansion by the declined rate of cell division, which results in loss of cell turgidity (Xu et al. 2010). Abiotic stress will typically cause upper and lower spikelets and distil florets to either abort or produce small grains (Nuttall et al. 2017). A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes (Farooq et al. 2009), However, the period and duration of the stress factor is important. Following heading, drought had little effect on the rate of kernel filling in wheat, but its duration (time from fertilization to maturity) was shortened, and dry weight reduced at maturity (Wardlaw & Willenbrink 2000). In barley, drought stress reduces grain yield by reducing the number of tillers, spikes, grains per plant, and individual grain weight. Post-anthesis drought stress was detrimental to grain yield regardless of the stress severity (Samarah 2005, Alghbari & Iksan 2018). In durum wheat, thousand-grain weight, grain protein yield, and test weight reduced significantly under both drought and drought stress conditions, whereby drought stress was greater than drought stress (Houshmand et al. 2014).In summary soil water deficit condition reduces crop yield by reducing the plant growth according to the following three main mechanisms: i) reduction in canopy absorption of photosynthetically active radiation, ii) decreased radiation use efficiency, and iii) decreased harvest index (Earl & Davis 2003).

2.6.1 Effect of drought on seed quality

Drought stress leads to the yield losses of major crops worldwide every year (Wang et al. 2003, Saradadevi et al. 2017, Siddiqui et al. 2017). Reducing the availability of water during drought results in a reduction in total nutrient intake and nutrients concentration in plant tissues. A significant impact of the water deficit is manifested in the transport from the perception point, namely from the root system to the above-ground parts (Garg 2003). In general, drought stress induces an increase in nitrogen content (Li et al. 2013, Alghabari et al. 2018, Magallanes-López et al. 2017, Kapoor et al. 2020), a definite decrease in phosphorus levels, and does not have significant end effects on potassium content (Garg 2003). During booting and anthesis stages in winter wheat cultivation in a pot experiment under drought stress the concentration of nitrogen and sulphur were observed higher for dwarf cultivars, whereas no significant differences were observed between tall and semidwarf wheat cultivars (Alghabari et al. 2015). Starch synthesis is highly sensitive to high temperature and drought stress. Its accumulation in wheat grains can be reduced by over 30% after heat treatment, at temperatures between 30°C and 40°C. Thus, the ability to synthesize, store, and remobilize starch at high temperature is crucial for the determination of grain sink strength (Ni et al. 2018). The effects of abiotic stress on the dietary fibre content of wheat and barley grains appear to be variable as one report suggests that βglucan content of barley decreases under high temperature and drought stress (Savin et al. 1997), whereas another reports an increase in β-glucan content under drought stress (Jansen et al. 2013). In durum wheat (Triticum turgidum L. var. durum) drought stress affect grain yield, which led to an increase in protein content by linking with better gluten strength and better bread-making quality in the drought environment, although other traits related to gluten quality and content as sodium dodecyl sulfate- (SDS)-sedimentation or mixograph mixing time were somewhat lower in that environment, which indicates probably qualitative changes at the protein level (Li et al. 2013, Magallanes-López et al. 2017). The analysis of the glutenins composition confirmed different effects of some alleles (Magallanes-López et al. 2017). Drought and drought stress in another work with durum wheat grown for two years on the field experiment caused the significant increase in grain protein content, wet and dry gluten contents, and SDS-sedimentation volume, whereby the impact of drought stress was greater than drought stress (Houshmand et al. 2014). Flour protein content and SDS sedimentation volume increased, but not significantly, under drought stress conditions in durum wheat in the work of Li et al. (2013). In contrast, the gluten strength-related parameters such as lactic acid retention capacity and mixograph peak time increased significantly under drought. Drought also significantly enhanced flour yellowness (Li et al. 2013).

2.6.2 Effect of Drought Stress in Rice

Water stress is most severe limitation to the productivity of rice (Widawsky and O'Toole, 1990). Drought is a meteorological term and is commonly defined as the inadequacy of water availability including period without significant rainfall that affects the crop growth (Hanson, et al., 1995) and soil moisture storage capacity and it occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought has been recognized as the primary constraint to rainfed rice production (Datta, et al., 1975). Drought stress tolerance is seen in almost all plants but its extent varies from species to species, even within the species. Water deficit and drought stresses are global issues to ensure survival of agricultural crops and sustainable food production (Jaleel, et al., 2007b). Conventional plant breeding attempts changed over to use physiological selection criteria since they are time consuming and rely on present genetic variability (Zhu, 2002). Drought stress in rice affects the crop in different ways. Drought stress is considered to be a loss of water, which leads to stomatal closure and limitation of gas exchange. Drought stress is characterized by reduction of water content, diminished leaf water potential, turgor pressure, stomatal activity and decreasement in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance in metabolism and finally the death of plant (Jaleel, et al., 2008c). It reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq, et al., 2008, Jaleel, et al., 2008a, Razmjoo, et al., 2008). Water stress is a limiting factor in agriculture production by preventing a crop from reaching the genetically determined theoretical maximum yield
(Begg and Turner, 1976). In plants, a better understanding of the morphological and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Nam, et al., 2001 and Martinez, et al., 2007). The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity, duration of stress, plant species and its growth stages (Chaves, et al., 2002, Jaleel, et al., 2008b).

2.6.3 Effect of drought stress on morphological and physiological traits of Rice

Drought stress is very important factor for plant growth and affects both elongation and expansion growth (Anjum, et al., 2003a, Kusaka, et al., 2005, Shao, et al., 2008). Water deficit is one of the most environmental stresses affecting agricultural production and productivity around the world and may result in considerable yield reduction. Among the crops, rice is probably more susceptible to drought as compare to other crops. Water stress reduces the leaf area, cell size and intercellular volume (Kramer, 1969). A variety that is more resistant to water flow from the stomata into the atmosphere is considered as good for drought tolerance. The reduction in soil moisture may have led to lower water content in the leaves causing guard cells to loose turgor pressure and hence the size of stomatal pores are reduced (Tezera, et al., 2002) and/ or causing stomatal closure. In addition, increased stomatal resistance may have led to reduced water transport in the leaves further causing a decrease in stomatal conductance. Reduction in stomatal conductance decreases transpiration by closing of the stomata, resulting prolong the plant survival by extending the period of availability of essential soil water reserves in the root zone. Stomatal closure also helps to maintain high leaf water content and thereby a higher leaf water potential, which leads to a reduction in photosynthetic activity (Hsiao, 1973). Higher photosynthetic rates could in turn favor a higher biomass and crop yields. The presence of cuticular wax is also important for water stress and is more in dry land adopted rice as compare to irrigated rice, resulting the leaves are thick and leathery which prevent the water loss from the surface of the rice plant. In rice, leaf rolling character and death of leaves are good criteria found useful in assessing levels of drought tolerance in a large scale screening (Chang, et al., 1974). Leaf of any crop plant frequently rolls when plants is suffering from water stress condition. When leaf temperature is increases, the stomata become close and transpiration rate decreased sharply with leaf rolling (Sobarado, 1987). Leaf rolling scored visually in rice either in the morning or mid day. Delay leaf rolling is used as an important selection criteria for drought tolerance in rice, which could be improved by incorporating the gene(s) into those lines/ varieties, that perform better under irrigated condition but not well under water stress condition. A plant having the characteristics of delay leaf rolling under water stress and faster recovery rate after removing the water stress in rice (Singh and Mackill, 1991) was considered as good trait because flag leaf in rice crops plays the important role in grain filling and development (Evans, et al., 1975). Therefore, the selection proceeds to identify genotypes which had almost erect flag leaf enabling photosynthesis for longer duration (Yoshida, et al., 1976, Khus, 1995). Leaf attributes can be incorporated for improvement of drought tolerance in crops. Type of root system is also a good selection criterion for selecting the drought tolerance line or varieties. Deep root system has been identified as the targeted for drought tolerance improvement (Boyer, 1996). Production of root system under drought is very important and had good correlation with yield under moisture stress (Darofeev and Tyselano, 1982). Studies on root related characteristics have elicited much interested from scientist because it is through roots that rice plant up take moisture and show a wide range of varietal differences existing in the rice root system (Mackill, et al., 1996). Furthermore, drought stress caused pronounced changes in root structure such as increased branching and density (Eghball and Maranville, 1993). Cultivars having deep and thick roots are good for drought stress condition and are positively correlated with xylem vessel area, which are vital to the conductance of water from soil to the upper parts of the plants to meet the evaporative demand. Large and vigorous root system and the continued production of new root hairs are required for maximum response to nutrients supply and optimum environmental conditions and that this positively correlates with the dry matter accumulation within the shoot (Willumsen, 1993). Drought affected plants generally exhibit small root system configuration and in many causes the reduction in size of root system is directly proportional to the magnitude of water storage. According to Slayter, 1973 two types of effects of water deficit on root development can be exposed, first is reduction in rate of meristmetic activity and root elongation directly associated with the level of internal water deficit and second effect of suberization on the water and nutrients uptake proportional of the root system as whole. High dry weight under water stress conditions is a desirable characteristic for survivability of the plant under water stress condition. A common adverse effect of water stress on crop plant is reduction in fresh and dry biomass production (Farooq, et al., 2009). Plant production under drought stress SINGH, et al., Effect of Drought Stress in Rice: A Review on Morphological and Physiological Characteristics 263 is strangely related to the process of the dry matter portioning and temporal biomass distribution (Kage, et al., 2004). Morphological characters viz., early maturity, early vigor, rapid growth and physiological characters viz., diffusive resistance of stomata, osmotic adjustment, leaf rolling, closing and opening of stomata, position of stomata, leaf water retention and leaf senescence were associated with drought tolerance (Singh, 1993). Increased seed size, early maturity and reduced plant height at the drought prone location was prime importance in increasing seed yield (Singh, et al., 1995).

2.6.4 Effect of drought stress on yield and its associated traits of Rice

The ultimate aim of any breeding programme is to get the high grain yield. Plant breeders emphasized on yield performance under moisture stress condition. The high yield potential of a cultivar under favorable conditions is important in determining the yielding ability under water stress. Drought index is an important criterion for selection for stress environment, which provides a measure of drought based on loss of yield under drought condition in comparison to moist condition, has been used for screening of drought tolerance genotypes (Brukner and Frohberg, 1987). Dey and Upadhyaya, 1996 suggested three different critical stages of growth – seedling, vegetative and anthesis, which are highly affected by water stress and reduces the estimates of component characters finally grain yield. Water deficit during vegetative growth tended to delay panicle initiation (Lilley and Fukai, 1994b). Water stress at flowering is most serious and devastating to yield because it has diverse effect of pollination and causes flower abortion, grain abscission and increasing of percentage of unfilled grain (Hsiao, et al., 1976). Several workers viz., Kumar, et al. 2006 and Davatgar, et al., 2009 observed that the percentage of unfilled grains were significantly higher in sites that were affected by drought at reproductive stage. It indicated the high sensitivity of rice to water stress with any intensity (mild or sever) during the reproductive stage (booting, flowering and panicle initiation). This effect might be due to decrease in translocation of assimilates towards reproductive organs (Rahman, et al., 2002). In a study of 20 early maturing rice cultivars, it is reported the severe drought stress prolonged the maturity period (Dikshit, et al., 1987) and found significant correlation with yield reduction. Sarkarung, et al., 1995 reported the yield looses are more severe when drought occurs during the reproductive phase by slow growth during panicle development, which reduces grain number and grain size but cultivars which having high recovering ability after removing the water stress condition had relatively higher grain yield. Swain, et al., 2010 evaluated eighteen rice genotypes and they found the reduction in panicle number (72%) and grain yield (12%). Singh, et al., 2010 evaluated the six generations (P1 , P2 , B1 , B2 , F1 and F2) of six crosses of rice under drought and irrigated conditions. They observed the reduction in several characters including grain yield under drought condition. They found the 107.31 cm for plant height under well irrigated condition while only 92.00 cm average mean recorded for this character under drought condition. Similarly they observed the value for several characters viz., number of ear bearing tillers (7), panicle length (24.31 cm), number of grains per panicle (94), number of spikelets per panicle (113), spikelet fertility (84.04%), test weight (21.48 g), grain yield per plant (19.64 g), biological yield per plant (45.24 g) and harvest index (43.33%), while under drought condition these value are reduced and observed as 5 ear bearing tillers, 21.36 cm for panicle length, 59.08 g for number of grains per panicle, 95.59 for number of spikelets per panicle, 61.80% for spikelet fertility, 18.97 g for test weight, 8.77 g for grain yield per plant, 25.66 g for biological yield per plant and 34.28% for harvest index, indicating the effect of drought stress on grain yield and its component characters in rice crop. Audebert, 2000 also observed reductions in height (Laude, 1971), leaf area and biomass production, tiller abortion, changes in rooting pattern and delay development. Pantuwan, et al., 2000 conducted the four sets of field experiments under lowland conditions to investigate genotypic variation for grain yield and putative droughttolerant traits. Different types of drought stress were imposed in each set of experiments and they observed a larger variation existed for grain yield under both irrigated and drought stress conditions. Depending on timing, duration, and severity of water deficit, grain yield of genotypes under drought stress condition as compare to irrigated condition was reduced and reduction observed from 18 per cent to 52 per cent. They also found a delay in flowering time and was associated withgreater reduction in grain yield, harvest index, and filled grain percentage.

2.7 Melatonin: A Multifunctional Factor in Plants

A great literature review of melatonin has shown its importance in plants as a growth promoter with both in vitro and in vivo effects. In vitro effects refer to the growth of plants in response to exogenous treatments of melatonin (externally provided). Whereas, the in vivo effects refer to the growth and development of plants in response to melatonin produced endogenously. It was proved that melatonin is naturally produced endogenously in various plant species, including medicinal herbs, crops and fruit, although melatonin content was found to vary in different plants, as summarized by Fan and his co- workers in their study in 2018. The antioxidant nature of melatonin makes it useful as a bio -stimulant in agricultural processes by developing improved resistance against oxidant, biotic and abiotic stress response caused by scavenging ROS, and protection against bacterial pathogens. All the defense mechanisms discussed in the above section have resulted in improved plant traits, quality and increased biomass. Melatonin helps in enhancing seed and root germination, flowerformation and fruit development, with increased survival rates under harsh conditions. Higher levels of melatonin present in plants delay leaves' senescence, preventing degradation of chlorophyll, and also help in increased photosynthetic activity by maintaining homeostasis levels. All of these functions of melatonin in plant growth and development are discussed below in detail.

2.8 Melatonin as Growth Promoter in Plants

In the growth and development process of plants, various phytohormones are involved, especially the auxin. As a kind of indoleamine, melatonin shares the same initial compound, which is tryptophan with indole- 3 acetic acid (IAA), so melatonin should play a role in the regulation of growth and development in plants . Melatonin is regarded as a growth promoting molecule due to its dual nature of promoting plant growth by increasing various growth parameters with both in vitro and in vivo responses and enhancing plants' yield by regulating ion homeostasis . Melatonin is an independent plant growth regulator that can regulate its own biosynthesis and that of several other natural and synthetic plant growth regulators, such as auxin, abscisic acid, gibberellins, cytokinins, ethylene, polyamines, jasmonic acid and salicylic acid. It is an important modulator of gene expression related to plant growth regulators and can also mediate their activities. Many studies have suggested that the growth- promoting effects of melatonin on plants are comparable to other plant growth regulators. Most of the studies performed have dealt with the auxin like activity of melatonin, which is able to induce growth in shoots and roots and stimulate root generation in plants. Melatonin is also able to delay senescence, protecting photosynthetic systems and related subcellular structures and processes like cytokinins. Similarly, its role in fruit ripening and post-harvest processes as a gene regulator of ethylene- related factors was also described by many researchers. In fact, multiple interrelations may exist between melatonin and many other plant growth regulators, therefore, to evaluate the exact potential of melatonin on plant growth, firstly we have to develop a complete understanding of all the changes in gene expression modulated by melatonin. Melatonin has structural similarity to auxins, specifically IAA, and hence is involved in regulation of roots and shoots. So it plays the role of auxin to encourage vegetative growth in a variety of plant species. Later on it was observed that IAA stimulated root organogenesis, and cytokinin - induced shoot organogenesis was repressed with various amounts of endogenous melatonin and repressors of the transport of serotonin and melatonin. This proposition leads to the fact that melatonin acts as a probable regulator of plant growth and development. Numerous studies have confirmed this proposition. In one such study the etiolated hypocotyls from Lupinus albus L. (lupin) were incubated in the presence of a great range of melatonin and IAA. Both of these composites were seen to be dispersed in lupin tissues in a similar concentration gradient and produced an active elevation of growth at concentrations in the micro–molar range but the plant showed inhibitory growth effect at higher concentrations in integral and de rooted lupins. The reversal effects on the application of melatonin and IAA were seen because the meristematic regions were removed.

2.8.1. In Vivo Effects of Melatonin on Growth Promotion

Plant growth promotion is seen by in vivo effects on the presence of a plant's internal levels of melatonin and other regulating hormones. Melatonin has different levels of expression, depending on plant species. Many studies on in vivo effects on plant growth have been done for which researchers credited the detected hypocotyl growth to cell expansion in tissues where melatonin in combination with IAA played a functional role. Additionally, both of these indoles stimulated the formation of root primordial from pericycle cells with resulting modification in the pattern of spreading of adventitious or lateral roots, the time course, the number and length of adventitious roots and the number of lateral roots. According to one study melatonin formed the maximum number of hypocotyls with analogous values of IAA for root length in the entire range of tested concentrations. This same group of researchers stated analogous effects of melatonin, such as an active elevation of growth and a development of inhibitory effect at high concentrations, probably due to auxin induced ethylene biosynthesis in monocotyledons, e.g., wheat, oat and canary grass. Predominantly the optimum level of growth elevation attained in coleoptiles with IAA was 100%, the optimum growth-enhancing effect of melatonin was 10% for oat, 20% for wheat, 32% for the canary grass, and 55% for barley coleoptiles, which the researchers viewed as a substantial auxinic effect. In the same way, the root growth fluctuated between 56% to 86% for the canary grass and wheat, respectively, with the growth- enhancing effect in lupin tissues up to 63% in different bioassays. Melatonin also promoted the extension of etiolated cotyledons of *Lupinus albus* L. to the same level as it was noticed for IAA earlier.

2.8.2. In Vitro Effect of Melatonin on Growth Promotion

Along with in vivo effect, in vitro melatonin mechanism has also been involved in plant growth and development. In in vitro mechanism, externally provided melatonin plays the same role as that of the plant's already existing phytohormones, such as IAA and auxin. Melatonin and indoleamine share the same initial compound, which is tryptophan along with IAA, and so they behave the same as auxin for plant development and growth. As melatonin and IAA have structural similarity to indole derivatives, so melatonin can be processed to IAA or as an agonist of IAA in plant tissues due to the fact that melatonin can be transformed into 5 methoxyindolacetic acid (a compound that exhibits low auxin activity) in animals. Externally coating the plant's seeds with different amounts of melatonin has been studied in the literature. In one such report soybean seeds were coated with a range of melatonin amounts, which significantly improved plant height, leaf growth, seed amounts and increased vegetative growth regeneration of adventitious and lateral roots in soybean. In another experimentation, Afreen along with her co- workers explained the in vitro effect of melatonin that stimulated the vegetative growth and expansion of *Glycyrrhiza uralensis* Fischer in a dose dependent manner. The outcomes achieved by this group of researchers exposed evidence that the amount of melatonin in their model plant elevated the plant growth as it grew older, with values that were four times higher for a 6- month- older plant than for the 3- month- old plant. They also observed that the concentration of melatonin in the plant providing the highest growth rate was in those that were grown under the red light effect, signifying that there was a relationship between the concentration of indole and the growth and development of the plant. The same effects were also mentioned in another study, in the wild leaf mustard, i.e., *Brassica juncea* (L.) Czern. They observed that lesser concentrations of melatonin,

i.e., 0.1 µM, showed a root growth enhancing effect whereas higher concentrations, i.e., 100 µM, showed an inhibitory effect while a stimulatory effect was only measurable in younger seedlings. Endogenous free IAA levels also increased at low levels of melatonin, whereas at higher levels of melatonin there was no predominant enhancement in the levels of IAA, and as a result there was strong inhibition in the root elongation. This caused researchers to propose that the inhibitory effect of melatonin on root elongation and growth actually involves a mechanism that is irrelevant to the mechanism of IAA. Like the abovementioned experiments, many others have been documented with different levels and conditions of melatonin. Interestingly, the concentrations of serotonin and melatonin are high in thidiazuron (TDZ)- treated leaf explants with auxin- transported action suppressors. The augmentation of the TDZ medium with lidocaine, a sodium channel blocker, led to elevated levels of serotonin and melatonin but not auxin and considerably reduced the rate of TDZ -induced regeneration in the explants . Taking this into consideration, the levels of serotonin and melatonin as well as auxin elevated by exposure to TDZ stimulated the regeneration of explant, explaining the fact that melatonin acts either as a hormone autonomously or in combination with auxin and its own precursors and metabolites. In plants the activity of melatonin through Ca^{2+} - calmodulin can be a probable mechanism of signaling, considering the Ca^{2+} dependent action of auxin in numerous physiological responses.

Melatonin has a noteworthy influence on the cytoskeleton in plants and, additionally, it has great affinity for the calmodulin. It is also responsible for the protein kinase Ca2+ - dependent inhibition, via the interaction of calmodulin–kinase being related to reorganizations of the cytoskeleton, which denotes some of the most primitive effects that have been described for melatonin. So, the literature on Echinacea purpurea (L.) explants explained that activation of calcium channels (that change polarity of cells) enhanced the levels of melatonin, along with an inhibition in TDZ- induced callus induction. This explains the growth enhancing in vitro effects of melatonin at low concentrations, while inhibitory results occur at higher concentrations.

2.9 Role of Melatonin in Crop Improvement

Melatonin plays a pivotal role in crop improvement, as it is used as a potential bio-stimulator for enhancement of crop yields, safety, and is ecofriendly. It is biodegradable and safer to be used in organic farming. The excessive intake of melatonin in plants can prevent peroxidation of plant products due to its antioxidant nature and thus increase the shelf life of plants and improve crop yields. The main component of plants is chlorophyll, so its preservation and photosynthetic stimulation is of great importance in crop improvement. Horticultural crops are major sources of food and feed, which face a number of environmental challenges from fungal, bacterial or viral infections. Melatonin regulates anti stress mechanisms to make these horticultural crops stress free with high quality production rates. It also improves the post- harvest preservation of several types of fruits and vegetables. Exogenous melatonin can significantly reduce H_2O_2 content in roots, resulting in delayed post- harvest physiological deterioration (PPD) symptoms caused by damage during harvest and treatment, ultimately prolonging the preservation period of vegetables (onion, cabbage, cucumber, cauliflower, beans, carrot and pepper) and fruits (apple, banana, cherry, olive, grape, cranberry, kiwi, mulberry, pineapple, pomegranate, and strawberry). Gray mold is one of main diseases caused in apples by Botrytis cinerea during the post- harvest time, which significantly reduces the shelf life of the apple. So Cao and co - workers experimented on this and used 200 μM exogenous melatonin on an apple plant for 72 h incubation in order to inhibit gray mold. Melatonin significantly increased the shelf life of apples by inhibiting gray mold fungal infection.

Literature reviewed on post harvest preservation activity of melatonin showed significant results in soaked bananas and other fruits. These findings provide a valuable scientific basis for future research aiming at extending the shelf life of fruits and vegetables. Although exogenous melatonin can be used to increase the preservation period of post- harvest fruits and vegetables, it can also be of great interest to determine whether the shelf life of fruits and vegetables can be prolonged by increasing endogenous melatonin via a transgenic approach. A lot of different case studies show the antioxidant and preservative effects of melatonin in plants under stress conditions, which directly contributes to an increase in crop yield.

2.9.1 Role of Melatonin in Chlorophyll Preservation

As described earlier, chlorophyll is a major component of photosynthesis in plants and it needs to be protected and preserved. There are two photosystems—photosystem I and photosystem II—that generate energy in the form of photons that are captured by chlorophyll and are used in the synthesis of carbohydrates. Environmental stress factors such as temperature and UV radiation generate ROS in plants and trigger damage in reaction center D1 protein binding and also damage chlorophyll. Another major cause of chlorophyll damage is leaf senescence, which degrades chlorophyll and causes changes in plant hormone levels, damages molecular integrity, degenerates the cell wall and ultimately leads to plant death. Melatonin serves as an antioxidant in plants and protects chlorophyll against degradation. It protects the plants against senescence (loss of chlorophyll), which occurs due to ROS/RNS, and regulates SAGs. Different experiments and studies have evolved its chlorophyll preservation activity in many plants. An experiment on barley leaves treated with a solution containing melatonin (1 mM) for 48 h showed a 2X higher chlorophyll content in melatonin- treated leaves than in control. Similarly, cucumber plants that come under heat stress are preserved by the effect of melatonin. Exogenous melatonin may preserve plants under heavy metal (Zn, Cd and Pb) stress. It preserves chlorophyll content in plants by the radical rummaging activity of indoleamine of melatonin. Indoleamine of melatonin not only preserves chlorophyll under stress response but also increases the efficiency of photosystem II and elevates levels of ascorbic acid. Another study showed the role of melatonin in transgenic rice plants. SNA (serotonin N acetyltransferase) is a precursor of N acetyl serotonin, which is further converted to melatonin. Transgenic plants are treated and expressed with the human SNA gene under the ubiquitin promoter with the Agrobacterium - mediated method. The transgenic plants that were used had both serotonin and senescent detached leaves. Melatonin causes a delay in the senescence condition of leaves under abiotic stresses in both monocots and dicots. Further research has been done on the biological functions of melatonin in post- harvest senescence and in chlorophyll preservation that can be of great significance in agricultural science.

2.9.2 Role of Melatonin in Photosynthetic Activity

In plants melatonin is not only helpful in the preservation of chlorophyll but has also been found efficient in the increased photosynthetic activity of chlorophyll with maintained redox homeostasis. It protects many horticultural crops, such as wheat, barley, sweet cherry and many others, by preventing damage in the photosynthetic apparatus through the elevated levels of melatonin. Many experiments have been devised in order to demonstrate this statement. In one such experiment, cucumber seedlings of 25–100 μM were sprayed with a variable range of melatonin. It increased the photosynthetic rate in both seedlings under normal and heat stress conditions, respectively. However the CO2 concentration was reduced due to the conversion by carbohydrates for increased photosynthesis. Besides horticultural crops, many reports have been published recently on the effect of melatonin on increased photosynthetic activity of transgenic plants. In one study, a group of researchers increased the production of endogenous melatonin in the chloroplasts of various transgenic lines of A. thaliana. As a result, their chloroplasts exhibited an improved redox state with lower ROS levels under drought stress, compared to the wild type. The high melatonin content and low levels of ROS resulted in all the transgenic plants being more tolerant to drought stress.

This was indicated by the enhanced photosynthetic efficiency and higher biomass of the transgenic plants, compared to the wild - type under drought - stressed conditions. Similarly, in another study, transgenic Bermuda grass treated with melatonin upregulated the expression of photosynthesis- related genes under drought stress, which resulted in the maximum increase in the photochemical efficiency of PSII and the total chlorophyll content by enhancing the biosynthesis of chlorophyll and slowing the rate of its decomposition. Therefore, melatonin plays a key role in protecting PSII and ameliorating the decrease of chlorophyll content under drought stress. In line with the above findings, the influences of foliar- sprayed melatonin on maize seedling growth during drought stress were investigated in another study. Results showed that the melatonin- treated plants showed higher photosynthetic rates, stomatal conductances and transpiration rates as compared to untreated plants. In addition to the abovementioned example many other experimentations and studies were reviewed for increased photosynthetic activity by melatonin.

2.9.3 Role of Melatonin in Increases in Biomass

Since melatonin is being used in crop improvement, another main factor is whether melatonin can increase the biomass of plants. Different modifications were made to the metabolic indoleamine enzyme of melatonin to develop transgenic plants. Melatonin has the ability to increase the biomass of plants by improving efficiency in the growth of plants, developing defenses against stress response and improving the germination of seeds and seedlings. One such experiment was performed on red cabbage seedlings (Brassica oleracea rubrum) in which its seeds were incubated with melatonin at concentrations of 1, 10 and 100 µM with the hydropriming method. Both the control (non incubated) and the pretreated seeds were allowed to germinate in darkness at 25 °C for three days. The young seedlings were then grown for five more days in light. Both the control and pretreated seedlings were tested with CuSO4 water solutions at different concentrations. The seedlings were affected by Cu water, but the rate of germination in the non- treated was 53.5%, however, it was 73% in pretreated seeds. So the pre -treatment with melatonin of seeds increased the rate of germination in plants. Corn plants treated with exogenous melatonin had irreversible generated genes for larger root systems. Melatonin increased by 20% production rates in corn plants. Arabidopsis thaliana is one of the most important plants in many aspects. In A. thaliana the biomass was increased under the effect of serotonin (a precursor of melatonin) on the lateral root development system analyzed by using GC- MS (gas chromatography coupled to mass spectrometry). It increased around 10–160 µm in the root development system. The increase in concentrations of serotonin stimulated the primary root growth and root hair development. All these results show that exogenous concentrations of serotonin are able to convert into melatonin, and it can antagonize the higher levels of serotonin concentrations. Mechanisms that define how melatonin promotes root development are still under research. Melatonin is highly present in different parts of the root with an obvious gradient. Its order of presence and quantification was done by liquid chromatography along with fluorescent detection. It was highly seen in the apical, central and then at the basal portion of the root system. With the help of liquid chromatography not only was the presence of melatonin detected, but also the changes in the melatonin level due to the influence of light and dark reactions were determined. As a consequence, all of the above effects of melatonin, like chlorophyll preservation in leaves, root development, root regeneration, increase in biomass, delayed senescence in leaves, directly contribute to crop improvement and ultimately lead to increased food production. Most of the world is facing malnutrition and increased food demands with low resources, so in this scenario melatonin contributions to crop improvement will play a great role. Food shortages of the commonly used crops of rice, wheat, barley and corn are a great problem for the world. Some of the other contributions of melatonin to crop improvement are by □ Increasing the rate of plant germination, □ Making plants resistant to environmental stresses, Gene manipulation of synthetic enzymes of melatonin, such as AANAT/ASMT. Manipulation in melatonin genes of synthetic enzymes can be beneficial to the enhanced production of endogenous melatonin in plants. Moreover, the uses of agrochemicals such as BTH (benzothiadiazole) and chitosan, which activate the plant's own defense mechanism, are also greatly contributing in melatonin production. Therefore, any method to meet the shortage of food with enhanced production will be a massive ramification. In this regard, melatonin modifies transgenic seed or seedlings with indoleamine and enhances crop production, while also improving the nutritional values of crops.

2.9.4 Role of Melatonin in Regulation of Plant Physiology

Melatonin is a growth regulator known for its important roles in the regulation of plant growth and development . It regulates plant's developmental processes starting from the seed germination and has been considered to show similar effects, such as auxins, during the process of etiolation . It is also believed that melatonin and auxins can have a coregulatory impact on plant growth . The concentration of melatonin also acts as a rate limiting step in the regulation of physiological processes. At lower concentrations, it promotes the growth, whereas at higher concentrations, it has inhibitory effects . Melatonin also regulates the important plant processes such as morphogenesis, rhizogenesis and caulogenesis. Melatonin positively regulates the growth of roots. This was supported by studies carried out on genetically modified rice in which gene encoding serotonin-Nacetyltransferase (SNAT) was over expressed. The over expression of SNAT resulted in a manifold enhancement of endogenous melatonin levels accompanied by a significant increment of rice seedling root growth. In plant roots, melatonin also induces the formation of root primodia from pericycle cells. Transcriptomic studies carried out on cucumber roots established that melatonin treatment up-regulated 121 genes, and downregulated 196 genes. This transcriptomic regulation enhanced the plant growth by increasing the total count of lateral roots. This melatonin mediated root growth in plants is supposed to be regulated in an auxin dependent manner. Melatonin also promotes plant growth by enhancing the efficiency of carbon assimilation. Moreover, another fact favoring the improved photosynthesis is the stimulated stomatal conductance after melatonin application. Photochemical efficiency of PSII is also stimulated by melatonin, enhancing the overall photosynthesis. Moreover, melatonin also boosts the accumulation of RuBisCO along with enhanced total nitrogen and protein content. Melatonin mediated enhancement in photosynthesis is also accompanied by the reduced catabolism of chlorophyll molecules and down-regulation of genes favoring the process of senescence. A delay in senescence in melatonin treated plants is favored by low H_2O_2 levels accompanied by high APX activity. Additionally, melatonin regulates the ascorbateglutathione cycle, resulting in more accumulation of ascorbate and glutathione, accompanied by low levels of dehydroascorbate and oxidized glutathione . Seeds treated with melatonin before sowing resulted in improved germination and vigor plants . This melatonin seed priming has been followed by overall better vegetative and reproductive growth of plants leading to improvement in yields . In addition to other physiological processes, melatonin also regulates fruit ripening. In tomatoes, melatonin has been observed to trigger fruit ripening by stimulating ethylene biosynthesis accompanied by the up-regulation of transcripts involved in ethylene signalling pathways . Moreover, melatonin also regulates the biosynthesis of anthocyanin and proteins related to the process of fruit ripening .

2.9.5 Other Functions of Melatonin in Plants

As explained above some of the other important functions of melatonin have also been interestingly reviewed and discussed. Melatonin in plants produces dark and light signals, which synchronize with the photoperiodic ecological that signals the daylight responses of flowering that only proceed in daylight with a short time tenure. *Chenopodium Renbur* L. is one such plant that has the ability of diurnal cadence that takes place at night. Exogenous melatonin regulates the transition of flowering in many plant types such as *Chenopodium rubrum* L., *Pterygpra californica Ruprecht*, and *Arabidopsis thaliana*. It plays an essential role in the maintenance of reproductive physiology and flower development of the family *Hypericum perforatum* L. (St John’s wort). As during the uninucleate microsporogenesis stage, there are high concentrations of indole and melatonin contents, which lead to an increased regenerative potential of isolated anthers. It plays a key role in the conservation of dormancy germs and in the preservation of different states in fruit tissues. Increased rates of melatonin in juicy fruits indicate its role in the ripening of fruits. Melatonin is essential in the regulation of growing stages that may lead to the conservation of dormancy. It has some specific actions on the chlorophyll degrading enzyme, named as chlorophyllase, pheophorbide that are oxygenase or red chlorophyll catabolite reductase and restricts the generation of free radicals, thus leading to a suspension in the senescence process. Melatonin and other hormones in plants have hermetic biphasic expression of molecules and hormones, which are interrelated with the stress system of the plants. The adaptive significance of circadian rhythms displays high plasticity for managing the endogenous clock with the environment, which can be day or night duration. Cardiac rhythms and hermetic type doses have dependent mechanisms. Other than that, plasma melatonin and cardiac rhythms present a U shape pattern that gives a fully synchronized function of time. Another major function of melatonin relates to the emission of green lead volatiles with biosynthetic enzymes regulated by the cardiac clock.

Melatonin can be extracted from food or generated endogenously. It has antioxidant properties in both food and endogenous compounds and so can be taken in appropriate amounts from diet and other different living organisms. Excess production of melatonin can cause aging in plants as well as animals. Daily intake of melatonin rich foodstuffs such as coffee, corn, rice, tea, etc., can improve the health of consumers.

2.10 Role of Melatonin in Alleviating Drought Stress

Drought stress damages seedlings' growth through the over-excessed accumulation of ROS , which can also lead to the disruption of the antioxidant system and photosynthetic capacity . Applications of MT can effectively alleviate the drought damages in many agricultural crops, such as rice, wheat , maize , soybean , rapeseed , and cotton . However, limited reports have been shown in rice. We here reported that drought stress induced the retarded growth of seedlings or even lethal phenotypes, whereas foliar spray applications of 100-µM MT greatly mitigated the severe drought stress-induced damages to rice seedlings Consistent with previous reports, the alleviated effects of melatonin on drought stress in this study were mainly focused on the following aspects, including (i) the improved capacity of the photosynthetic system (ii) the restored stomatal phenotype and conductance and water utilization (iii) the enhanced antioxidant system and (iv) the adjusted osmotic substances, i.e., ABA, SS and free proline Inhibited growth of the plant was largely attributed to the essential role of water in cell turgor . Melatonin can enhance the stomatal conductance to improve the availability of water to ameliorate drought adaption . We also found that melatonin significantly increased the RWC of seedlings and maintained the stomatal conductance), suggesting that the alleviated effects of melatonin on drought stress was associated with stoma movement.

Accumulation of osmolytes, such as SS and proline, was expected to adjust osmotic stress during drought stress . Subsequently, some studies also reported that enhanced soluble sugars were detected after the treatment of melatonin . Others demonstrated that accumulated osmolytes caused osmotic stress under abiotic stress, which was related to the redox homeostasis by exogenous melatonin . The current results were aligned with the previous studies, showing decreased accumulation of SS and proline after the MT treatment under severe drought stress.(Lei et al). proposed that such MTalleviated effects were not associated with osmotic adjustment. Besides, another possible explanation was that the disruption of ROS homeostasis under severe drought stress caused depressed functioning of cell metabolism.

The enhanced effects of melatonin on drought adaption have been well-documented via the enhanced antioxidant system in many plants. Aside from enzymatic antioxidants, i.e., SOD, APX, CAT, and GR, the nonenzymatic ASA–GSH biosynthesis was also significantly increased after the melatonin treatment. ASA– GSH is associated with abiotic tolerance in higher plants , and the improved tolerance of wheat seedlings to drought stress has been reported to largely depend on the regulated ASA–GSH biosynthesis. Our study validated these findings , and we confirm that melatonin can mitigate the damages of drought stress to rice seedlings.

CHAPTER III

MATERIALS AND METHODS

The pot experiment was conducted from December 2020 to May 2021 comprising of collection of seed, raising of seedlings, growing and experimentation, data collection, compilation, etc. to study the effect of Melatonin in improving morpho-physiological , growth and yield performance of rice under drought stress. A brief of soil, climate, materials and methods used for conducting the experiment is presented below.

3.1 Location of the experimental site

The experiment was set at the Net House and Plant Physiology Laboratory of the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The location of the pot experiment at 24^0 75′ N latitude and 90^0 50′ E longitude at the elevation of above 18m of sea level and it was under the Agro Ecological Zone-28, namely Madhupur Tract. For better understanding the experimental location, the Map of AEZ of Bangladesh has been added in Appendix III.

3.2 Characteristics of Soil that used in Pot

The soil used in pot preparation was collected from the experimental field of Department of Agricultural Botany, SAU, Dhaka. The pot experiment was conducted by using typical rice growing silty loam soil having noncalcarious properties. The soil was Deep Red

Brown Terrace Soil under Tejgaon Series belonging to the Agro-Ecological Zone of Madhupur Tract. The soil for the pot was collected from 0-15 cm depth. The collected soil was pulverized followed by the removal of weeds, stubble, brick pieces, insects, etc. The collected soil was sun dried, crushed and passed through a 2 mm sieve. After that the soils were mixed up properly and 400 g soil was taken for initial physical and chemical analysis. The morphological properties of this soil have been presented in Appendix IV and the physiochemical properties in Appendix V.

3.3 Climate

The site of the study was characterized by a subtropical monsoon climatic zone. Moderately low temperature along with moderate rainfall prevailed during the period from December to May. The cool and dry weather prevailed during December to January with the mean temperature 22.67^{o}C. Temperature during February to May was moderately hot but highly humid along with moderate to high rainfall. Cyclone Fani, the strongest storm has barrelled into Bangladesh after leaving a trail of deadly destruction across the eastern coast of India in the month of April. The detailed meteorological records (monthly) of air temperature, relative humidity, rainfall from December, 2020 to May, 2021 have been presented in Appendix VI.

3.4 Planting Material

Oryza sativa L. cv. BRRI dhan29 was used as test crop which is a drought tolerant rice variety recommended for cultivation in *Boro* season. This variety was developed at Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur and were released for farmers use in 1994. It’s life cycle is 160 Days. Plant height is almost 95 cm. Yield is almost 7.5 t ha^{-1} depending on various drought level.

3.5 Treatments

The pot experiment consisted of two factors as shown below:

Factor A: Different levels of drought	**Factor B**: Different doses of melatonin (M)
i. D_0 = No drought / without drought	i. $M_{0=}$ 0 µM Melatonin
ii. D_1 = withholding of water for 10 days	ii. M_1 = 100 µM Melatonin
iii.D_2=withholding of water for 20 days	

There were following 6 treatment combinations:

D_0 M_0 / C = Without Drought + 0 µM Melatonin
D_0 M_1 = Without Drought + 100 µM Melatonin
D_1 M_0 = withholding of water for 10 days + 0 µM Melatonin
D_1 M_1 = withholding of water for 10 days + 100 µM Melatonin
D_2 M_0 = withholding of water for 20 days + 0 µM Melatonin
D_2 M_1 = withholding of water for 20 days + 100 µM Melatonin

3.5.1 Application of Drought treatment

There were three drought levels including control. The drought treatments were D_0 (Control), D_1, and D_2. The control plant was watered as per requirement while the others were subjected to drought stress by withholding water. Treatments were applied 15 days after transplanting. The treatments were: Control (C), withholding of water for 10 days (D1) and withholding of water for 20 days (D2).

3.5.2 Melatonin treatment application

Melatonin (M) was used to test the role of M in improving morpho-physiological and yield performance of rice under drought stress. There were two melatonin doses including control. The melatonin treatments were M_0 and M_1. When no melatonin added it termed as control (C) and for M_1, 100 µM melatonin was added to the pot.

3.6 Experimental Design and Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) as two factorial arrangements with three replications. The experimental area was divided into three equal blocks. Each contain 6 pots where 6 treatment combinations were allotted randomly. There were total 18 (6×3) pots in the experiment. The layout of the experiment has been shown in Appendix VII.

3.7 Plant Material

Seeds of *Oryza sativa* L. cv. BRRI dhan29 was used as plant material, which were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

3.8 Pot Preparation

Plastic pots were used in this experiment. The diameter of each pot was 35 cm (14 inches) at the top and 15 cm (6 inches) at the bottom. The depth of each pot was 30 cm (12 inches). The collected soil was sun dried, crushed and passed through a sieve to remove weeds, stubble, brick pieces, insects, etc. The dry soil was then thoroughly mixed up with well rotten cow dung (75 g for 12 kg soil) before filling the pots. Each pot was filled up with 12 kg soil on 20 January, 2021 and all experimental pots received recommended doses of N, P and K fertilizers. After that the pots were prelabeled for each treatment combination and placed at the net house of the Department of Agricultural Botany. At last, measured water was added to bring soil at field capacity condition.

3.9 Manure and Fertilizer Application

Well rotten cow dung at the rate of 12.5 t ha^{-1} mixed up with soil before filling the pot. The following fertilizers i.e., urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and $ZnSO_4$ were used as sources of nitrogen, phosphorus, potassium, melatonin and zinc were applied at a rate recommended by BARI for the variety BRRI dhan29 shown in tabular form below.

Table 2. Manures and Fertilizers applied for the experimental pot

Manures and Fertilizers	**Dose ha^{-1}**	**Dose ha^{-1}**	**Dose Pot^{-1}**	**Application (%)**			
				Basal	**15 DAT**	**35 DAT**	**55 DAT**
Cow dung	---	12.5 Ton	75 g	100	---	---	---
Urea	36 kg	269 kg	1.62 g	---	33.33	33.33	33.33
TSP	13 kg	97 kg	0.66 g	100	---	---	---
MoP	16 kg	120 kg	0.72 g	100	---	---	---
Zypsum	13 kg	97 kg	0.58 g	100	---	---	---
$ZnSO_4$	1.5 kg	11 kg	0.066 g	100	---	---	---

The weight of 1 ha soil at the depth of 15 cm is considered approximately 2 million kg of soil. According to the above rate, manures and fertilizers were calculated as per pot that contained 12 kg soil. The whole amounts of TSP, MoP, Zypsum and $ZnSO_4$ were applied during the final pot preparation. Urea was applied in three equal splits at 15, 35 and 55 days after transplanting (DAT).

3.10 Seedbed Preparation

Wet seedbed was prepared (Plate I) by December 7 , 2020 and sprouted seeds were sown on December 8, 2020 following the recommendation of BRRI (BRRI 1995).

3.11 Seedling Raising

A very common procedure was followed in raising of seedlings i.e., the seeds were soaked for 48 hours and then washed properly in fresh water and after that incubated for sprouting. The sprouted seeds were sown in the wet seedbed on December 8, 2020 (Plate II).Netting was also done.(plate III)

3.12 Uprooting and Transplanting of Seedlings

Healthy and uniform seedlings of thirty days old were uprooted carefully from the seedbed (Plate IV) and were transplanted in the experimental pots (Plate V) at the rate of single seedling hill^{-1} on January 27, 2021 maintaining three seedlings in each pot. The seedbed was watered before uprooting the seedlings from the seedbed to minimize the root damage. The seedlings were watered after transplanting in the pot for their better establishment. After one week of transplanting all the experimental pots were checked for any missing hill, which was filled up with extra seedlings.

3.13 Intercultural Operations

After transplantation of seedlings, different intercultural operations like weeding, irrigation, plant protection measures etc. were accomplished for better growth and development of the seedlings.

3.13.1 Weeding and Irrigation

The hand weeding was done as when necessary to keep the experimental pots free from small aquatic weeds. Irrigation was done whenever necessary but the frequency of irrigation became less in harvesting stage. Irrigation was done at evening as drought was applied with irrigation water.

3.13.2 Plant Protection Measures

Leaf blast disease was observed and Trooper 75 WP@ 8g/10 L water was sprayed to effectively control the disease. Beside this, about 1-2inch depth of water was maintained and Muriate of potash (MoP) was also top dressed. During the conduction of experiment there was a severe attack of rats which were effectively controlled by using Zinc Phosphide (2%) and rat trap, where dry fish was used. The holes made by the rats was poured off with water.

3.14 General observation of the experimental pots

The plants were under regular observation and the plants looked normal green except the plants treated with drought. No lodging was observed but the maximum tillering, panicle initiation, and flowering stages were not uniform.

3.15 Detection of maximum tillering and panicle initiation stage

Maximum tillering and panicle initiation stages were detected through regular inspection. When the number of tillers hill^{-1} reached the highest number and after that decreasing in trend, was considered as maximum tillering stage. When a small growth at the top of upper most nodes of main stem was noted like a dome was considered as an indication of the beginning of panicle initiation stage. But these stages were not uniform and were varied with treatments.

3.16 Harvesting

The crops were harvested at maturity when 80-90% were turned into straw colored on May 28, 2021 . The crop was cut at the ground level and pot wise crop was bundled separately, tagged and brought to the threshing floor. The grains were then sun dried to a moisture content of 12% and straw was also sun dried properly. The grain and straw yields and different plant physiological parameters were recorded after harvesting.

3.17 Data Collection

The data on the following parameters were collected from each treatment.

Morphological parameters

- Plant Height (cm)
- No. of Tillers Plant^{-1}
- Leaf Area Index (LAI)

Physiological parameters

- Chlorophyll Content (SPAD Value)
- Leaf Membrane Stability Index (MSI)
- Relative Water Content (RWC)
- Dry Weight of Root
- Dry Weight of Stem
- Dry Weight of Leaf
- Total Dry Matter (TDM)
- Proline Content

Yield contributing and other parameters

- Panicle Length (cm)
- No. of Effective Tillers Plant^{-1}
- No. of Non-Effective Tillers Plant^{-1}
- No. of Filled Grains Panicle^{-1}
- No. of Unfilled Grains Panicle^{-1}
- 1000 Grain Weight (g)

E. Yields

- Grain Yield Plant^{-1}

3.18 Detailed Procedures of Recording Data

A brief outline of the data collecting procedure followed during the experiment is given below:

3.18.1 Plant Height (cm)

Plant height was measured in centimeter from 30 days after transplanting (DAT) at 15 days interval up to 120 DAT, beginning from the top surface level of the pot to the tip of the longest leaf at booting and flowering stage and at maturity stage, from the top surface level of the pot to the tip of the tipper end of the longest panicle.

3.18.2 Number of Tillers Plant^{-1}

Tillers, which had at least one visible leaf were counted from 30 days after transplanting

(DAT) at 15 days' interval up to 120 DAT.

3.18.3 Leaf Area (cm^2)

Leaf area was measured in centimeter^2 by non-destructive method at heading stage.Leaf length and width were multiplied to calculate leaf area.

3.18.4 Leaf Membrane Stability Index (MSI%)

The plasma membrane stability or intactness was estimated through the leakage of electrolytes. Fresh leaf trips (0.2 g) of uniform size were placed in test tubes, containing 10 ml distilled water and kept for 30 minutes in water bath (Plate VIII) at 40 °C for measuring the initial electrolyte conductivity (C_1). The final electrolyte conductivity (C_2) was measured after boiling the plant samples for 15 minutes at 100 °C. MSI was calculated as-

$$MSI = (1- c1 / c_2) \times 100$$

3.18.5 Relative water content (RWC)

Relative water content (RWC) was measured according to the following method suggested by Barrs and Weatherly (1962). From each experimental pot three leaves were randomly selected and cut with scissors (Plate VII) . Fresh weight (FW) of leaf laminas were taken and then immediately floated on distilled water in a Petri dish for 4 hours in the dark. After drying excess surface water with paper towels turgid weights (TW) were measured. Then the sample was oven dried at 80 ℃ for 48 hours and dry weights (DW) were measured.

RWC% was calculated by the following formula:

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

3.18.6 Chlorophyll Content (SPAD Value)

Leaf chlorophyll content was measured by using a hand-held chlorophyll content SPAD meter (SPAD 502, Konica Minolta, Japan). At each evaluation the chlorophyll content was measured five times from three randomly selected leaves at different positions plant^{-1} and the average was used for analysis.

3.18.7 Proline Content

Following the method of Bates et al. (1973) Proline content was quantified where leaf specimens were extracted by using sulfo-salicylic acid and then centrifuged. Extracted supernatant was assorted with glacial acetic acid and ninhydrin and heated at 100℃ for 1 h. After being cooled, toluene was added to discrete the chromophore and measured the absorbance at 520 nm.

3.18.8 Dry Weight of Root

After harvesting, roots of the plants were very carefully separated from the soil by washing and then sun dried. Then they were sliced into small pieces to put into pre-labeled envelop and placed in oven for 72 hours at 70 ℃. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of root was taken.

3.18.9 Dry Weight of Stem

After harvesting, stems of the plants were separated by removing leaves and then sun dried. Then they were sliced into very thin pieces to put into pre-labeled envelop and placed in oven for 72 hours at 70 ℃. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of stem was taken.

3.18.10 Dry Weight of Leaf

After harvesting, leaves of the plants were collected and sun dried. Then they were sliced into small pieces and were put into pre-labeled envelop and placed in oven for 72 hours at 70 ℃. After oven drying the samples were put into desiccators to cool down at room temperature. Then dry weight of the sample was taken.

3.18.11 Total Dry Matter (TDM)

The plant parts i.e., roots, stems, leaves and panicles were detached from each other and were kept separately in oven for 72 hours at 70 ℃ . The oven dried samples of these plant parts were weighted for dry matter production. The total dry matter production was calculated from the summation of dry matter produced by the abovementioned plant parts and grain weight per plants in gram.

3.18.12 Panicle Length (cm)

Panicle length was measured in centimeter from the basal nodes of the rachis to the apex of each panicle. Each observation was actually an average of 5 panicles.

3.18.13 Number of Effective and Non-Effective Tillers Plant^{-1}

The total number of tillers plant^{-1} was counted from the experimental pots at maturity and were grouped into effective (panicle bearing tillers) and non-effective tillers plant^{-1}.

3.18.14 Number of Filled Grains and Unfilled Grains Panicle^{-1}

Each grain was tested for whether it was filled or not by pressing the grain between the forefinger and the thumb. In case of more than 5 effective tillers plant^{-1}, grains of 5 randomly selected panicles of each experimental pot were counted and then the average number of filled and unfilled grains for each panicle was determined. In case of less than 5 effective tillers plant^{-1}, grains of all the panicles plant^{-1} were counted and then the average number of filled and unfilled grains for each panicle were determined.

3.18.15 Thousand Grain Weight (g)

200 clean sundried grains were counted from the seed stock obtained from the sample plants and weighed by using an electronic balance and then multiplied by 5.

3.18.16 Grain Yield Plant^{-1}

The grains plant^{-1} was separated by threshing and then properly sun dried and weighed to get grain yield plant^{-1}.

3.19 Statistical Analysis

The recorded data of different parameters were statistically analyzed to get the level of significance using the Statistix 10 computer package program. Analysis of variance was calculated following two factors randomized complete block design. The mean differences among the treatments were compared by least significant difference (LSD) test at 5% level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

The effect of melatonin in improving morphological, physiological and yield performance of rice plant under drought stress in the present study were presented in the tables and figures and discussed. A summary of the analysis of variance (ANOVA) with regards to all the studied parameters has been shown in Appendices VIII to XVII. The results obtained in the experiment were presented and discussed under the following subheadings.

4.1 Results

4.1.1 Plant Height

Subjecting rice plants to drought stress caused a significant ($P \leq 0.01$) reduction in plant height (cm) at 30 DAT, 60 DAT, 90 DAT and, at harvest compared to the control treatment without drought stress (Figure 1 and Appendix VIII). A clear difference was noticed between the plants grown under drought stress conditions and control conditions. At 30 DAT, 60 DAT, 90 DAT and at harvest with D_1 treatment plant height was decreased by 17.91%, 16.09%, 14.37%, 9.87% and with D_2 treatment by 32.47%, 28.25%, 23.18%, 18.88% respectively, when compared to control treatment. Fig.1 showed that plant height decreased gradually with increasing drought stress.

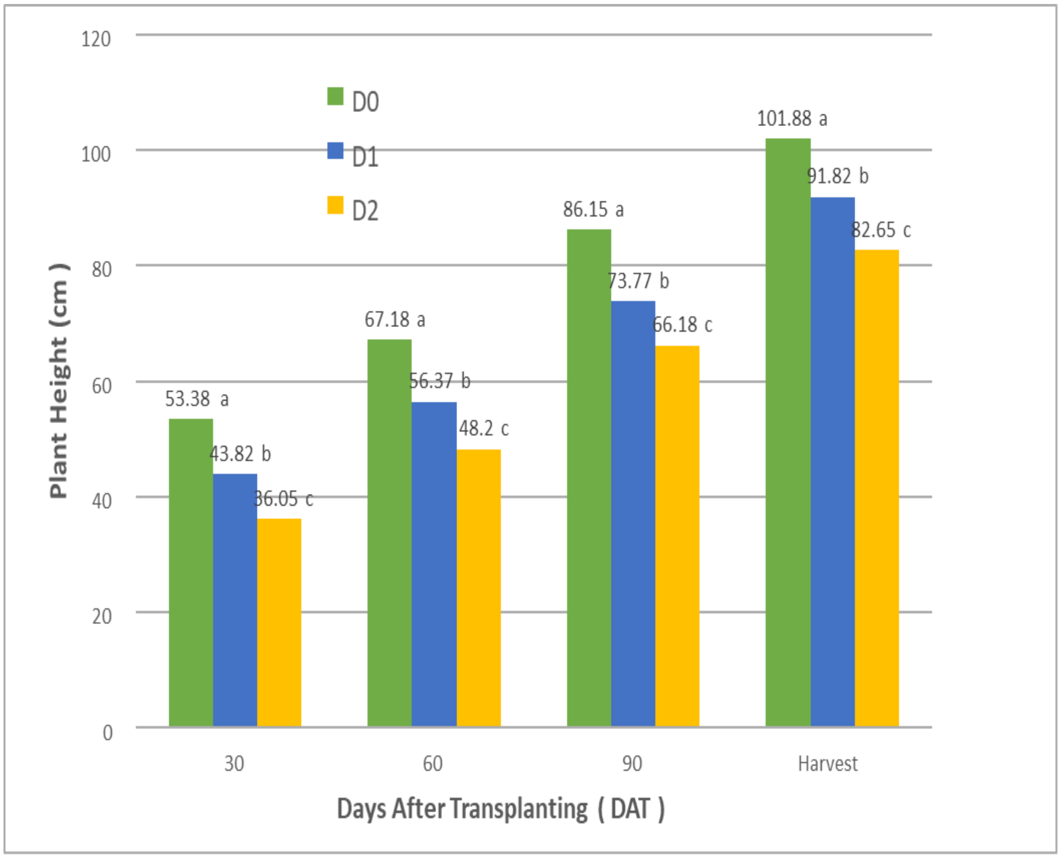

Figure 1.

Effect of different drought periods on the plant height of rice at different days after transplanting (LSD $_{(0.05)}$ = 1.24, 1.10, 2.27 and 3.83 at 30, 60, 90 DAT and at harvest, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

Supplementary melatonin application (100 µM) improved plant height significantly ($P \leq 0.01$). An increase of plant height at 30 DAT, 60 DAT, 90 DAT and, at harvest with 100 µM Melatonin (M_1 treatment) was 12.13%, 16.53%, 7.15%, 6.87% respectively, when compared to control treatment without melatonin (Figure 2 and Appendix VIII). At 30 DAT and 60 DAT, the applied 100 µM Melatonin (M_1 treatment) increased the plant height. But at 90 DAT and at harvest means were significantly different from one another. In every case, the lowest values of the plant height of rice plants were produced when rice plants did not receive any melatonin fertilizer.

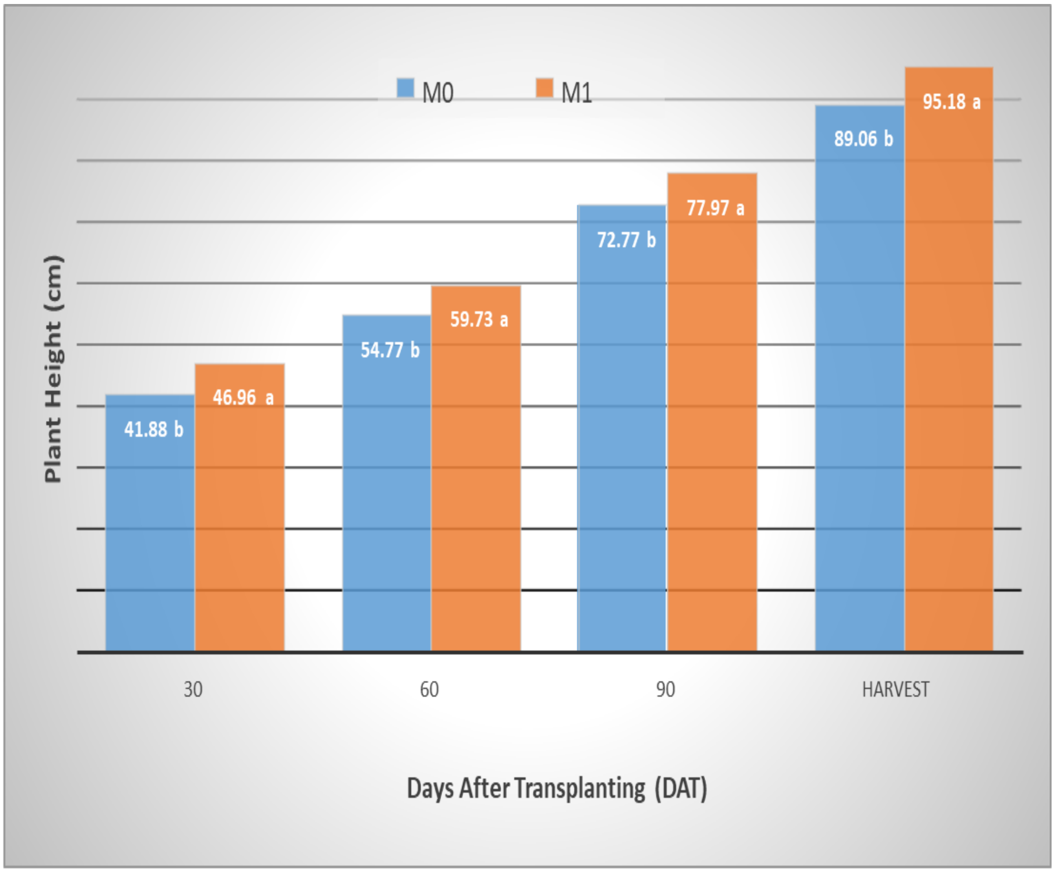

Figure 2. Effect of different melatonin levels on the plant height of rice at different days after transplanting (LSD $_{(0.05)}$ = 1.02, 0.90, and 1.86 at 30, 60, 90 DAT and at harvest, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

Plant height of rice plants decreased significantly under drought stress (Table 3 and Appendix VIII). However, the application of melatonin significantly ($P \leq 0.05$) increased the height of rice plants exposed to drought stress, in contrast to drought treatment without Melatonin. It was revealed from statistically significant Drought × Melatonin interaction (Table 3). At 30 DAT, treatment with D_1 treatment, the plant height increment at 100 µM Melatonin was 14.25% when compared to D_1 treatment alone. Similarly, at 60 DAT, 90 DAT and, at harvest treatment with D_1 , the plant height increment at 100 µM Melatonin (M_1 treatment) was 11.24%, 7.89% and, 7.57% respectively when compared to drought treatment alone. On the other hand, at 30 DAT, 60 DAT, 90 DAT and, at harvest treatment with D_2 treatment , the plant height increment at 100 µM Melatonin was 4.40%, 2.34%, 1.75% and, 2.37% respectively when compared to drought treatment alone. Generally, melatonin increased the plant height by developing drought tolerance at both D_1 and D_2level.

Table 3. Interaction effect of different drought periods and melatonin levels on the plant height of rice at different days after transplanting

Treatments	Plant Height(cm)			
	30 DAT	60 DAT	90 DAT	At Harvest
D_0M_0	51.73 b	65.57 b	84.70 a	99.77 ab
D_0 M_1	55.03 a	68.80 a	87.60 a	104.00 a
D_1 M_0	40.90 d	53.37 d	70.97 c	88.47 c
D_1 M_1	46.73 c	59.37 c	76.57 b	95.17 b
D_2 M_0	33.00 f	45.37 f	62.63 d	78.93 d
D_2 M_1	39.10 e	51.03 e	69.73 c	86.37 c
LSD(0.05)	1.76	1.55	3.22	5.41
CV(%)	2.18	1.49	2.35	3.23

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Drought affected rice plants of the present study showed reduction of plant height. There are various reasons for this growth reduction. Drought stress inhibits rice seedling growth due to lower turgor pressure, reduction in photosynthesis, and cellular damage caused by reactive oxygen species. However, our findings indicate that exogenous melatonin can alleviate the inhibition of plant growth to some extent which is caused by drought stress. This result is cirribirating with several other previous studies. Foliar application with melatonin enhanced shoot and root growth in rice seedlings under drought stress (Silalert and Pattanagul 2021). The effect of melatonin on the improvement of plant growth was also reported in maize (Ahmad et al., 2019) and kiwifruit seedlings (Liang et al., 2019). Li et al. (2022) also reported increased shoot length and root length which was imparted by exogenous melatonin application.

4.1.2 No. of Tillers Plant^{-1} at harvest

Tiller formation in rice is a very important agronomic trait for grain production and number of tillers provide valuable information about the stress profile of a plant under abiotic stress (Suzuki *et al*., 2005). The number of tillers plant^{-1} were significantly ($P \leq 0.01$) reduced by increasing level of drought (Figure 3 and Appendix IX). D_1 and D_2 treatment reduced tiller number by 9.28% and 24.14% respectively at harvest when compared to control. In respect of drought effect, the result showed that the number of tillers plant^{-1} were greatly affected even at D_1 treatment but the maximum reduction in number of tillers plant^{-1} was found at D_2 treatment.

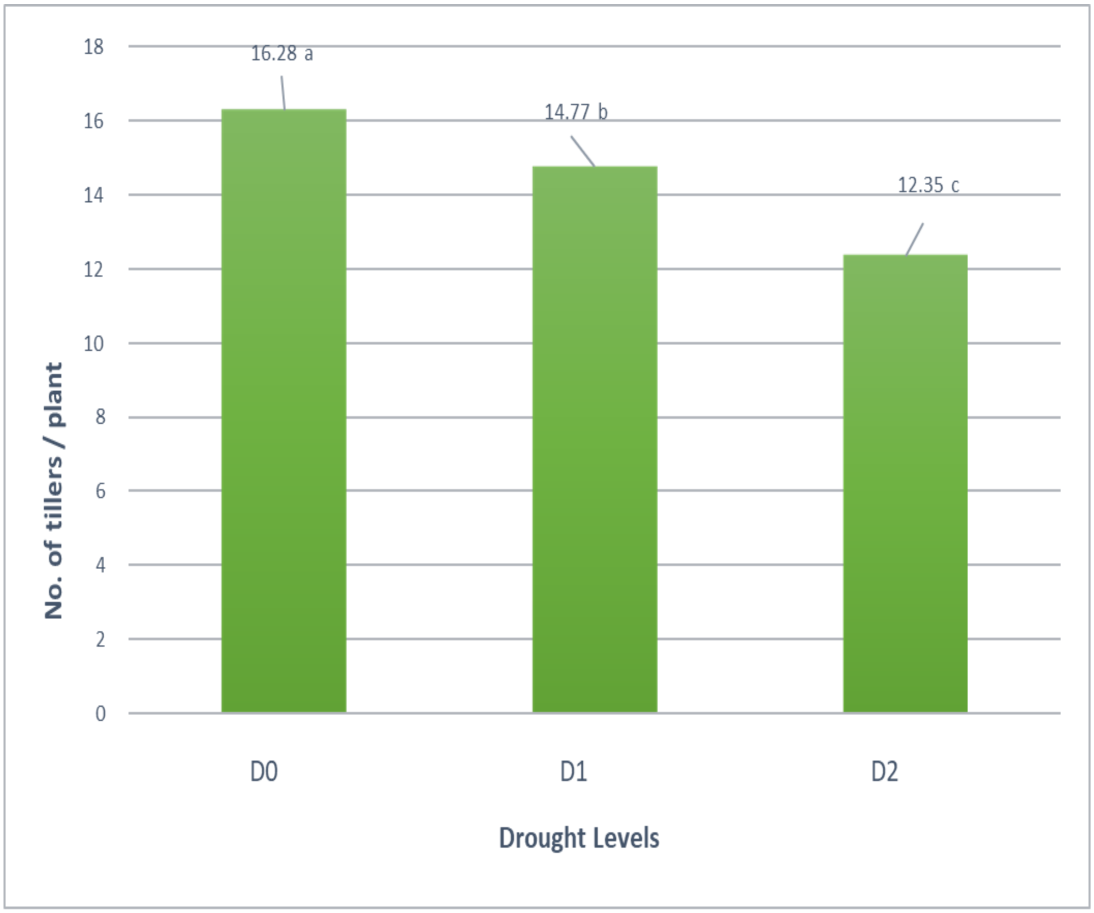

Figure 3. Effect of different drought periods on number of tillers plant^{-1} at harvest of rice after transplanting (LSD $_{(0.05)}$ = 0.72 at harvest and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

By exogenous application of melatonin, the number of tillers plant^{-1} was increased significantly ($P \leq 0.01$) at different days after transplanting (Figure 4 and Appendix IX). The number of tillers plant^{-1} enhancement over control was 12.25% by M_1 treatment at harvest. So, the parameters of plants not receiving supplementary melatonin (control) were still lower than the values of melatonin treatment.

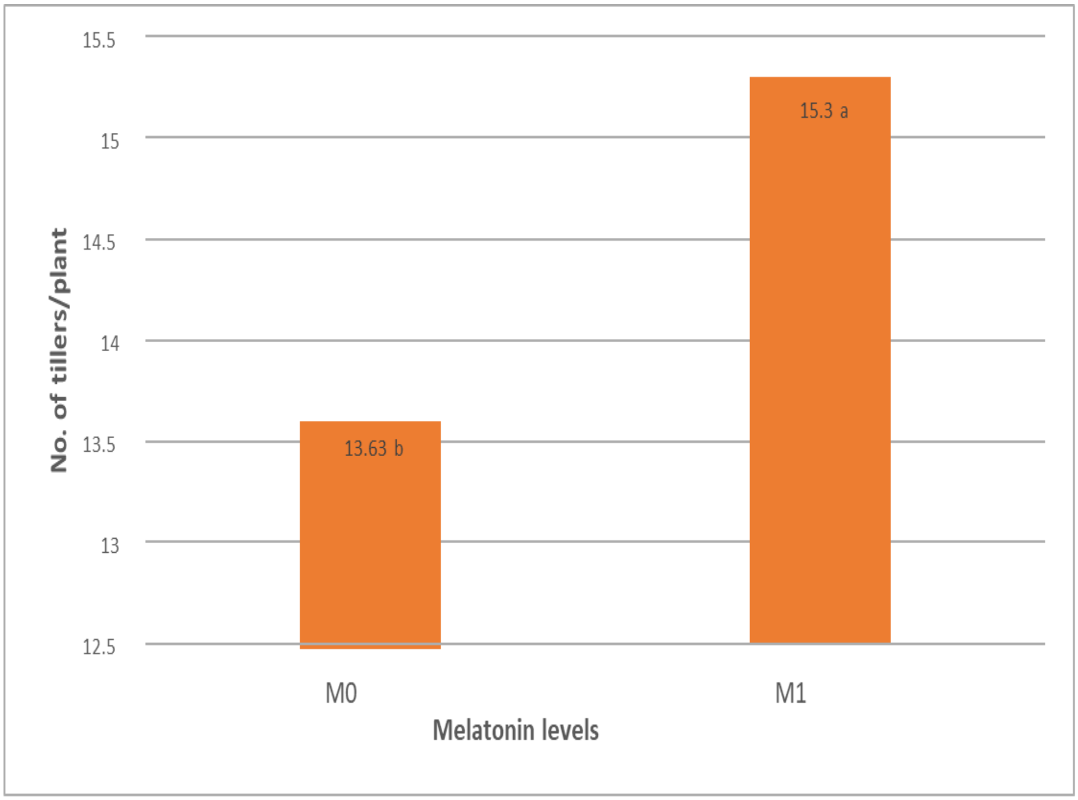

Figure 4. Effect of different melatonin levels on number of tillers plant^{-1} at harvest of rice after transplanting (LSD $_{(0.05)}$ = 0.59 at harvest and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

Results showed that total number of tillers plant^{-1} at harvest was significantly lowered by increasing the concentration of drought (Table 4 and Appendix IX). However, melatonin supplementation (M_1) improved the number of tillers plant^{-1} significantly ($P \leq 0.05$) at both levels of drought (D_1, D_2). It was evident from statistically significant Drought × Melatonin interaction (Table 4). Number of tillers plant^{-1} was increased by 18.74% after M_1 supplementation of drought stressed (D_1M_1) plant when compared to drought treatment (D_1 M_0) alone at harvest. Whereas, M_1 supplementation during drought stress (D_2) increased number of tillers plant^{-1} by 15.34% at harvest when compared to drought treatment (D_2 M_0) alone. In comparison to the group of drought stress alone (D_1M_0, D_2M_0), the number of tillers plant^{-1} increased all the way to the level of M_1 treatment, while there was no further significant difference between these two treatments.

Table 4. Interaction effect of different drought periods and melatonin levels on the number of tillers plant^{-1} of rice after transplanting

Treatments	**No. of Tillers/plant at harvest**
D_0M_0	15.93 a
D_0M_1	16.63 a
D_1M_0	13.50 b
D_1M_1	16.03 a
D_2M_0	11.47 c
D_2M_1	13.23 b
LSD(0.05)	**1.02**
CV(%)	**3.89**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

In the present study drought stress decreased the tiller number. Number of effective tillers decreased but number of non-effective tillers increased in rice plant under drought stress as observed by Pant et al. (2019). Singh et al. (2018) also reported a reduction in tiller number plant^{-1} under drought stress. The number of tillers reduces due to reduced growth and photosynthesis processes of plant (Quampah et al., 2011). However, melatonin addition with drought treatment increased the tiller number, compared to drought treatment alone. Melatonin increased productive tillers, grains per panicle, and 1000grain weights and yield of rice under that combined stress of cadmium-lead (Jiang et al.
2022).

4.1.3 Leaf Area (cm^2)

The most immediate response to drought stress was the decrease in the expansion rate of the leaf surface area. The leaf area of rice plants was significantly ($P \leq 0.01$) affected by drought stress (Figure 5 and Appendix X). The highest leaf area was observed with control (D_0) treatment while the lowest was observed with D_2 treatment. 22.17% and 40.36% reduction in leaf area was noticed in drought-affected D_1 and D_2 plants respectively when compared to control.

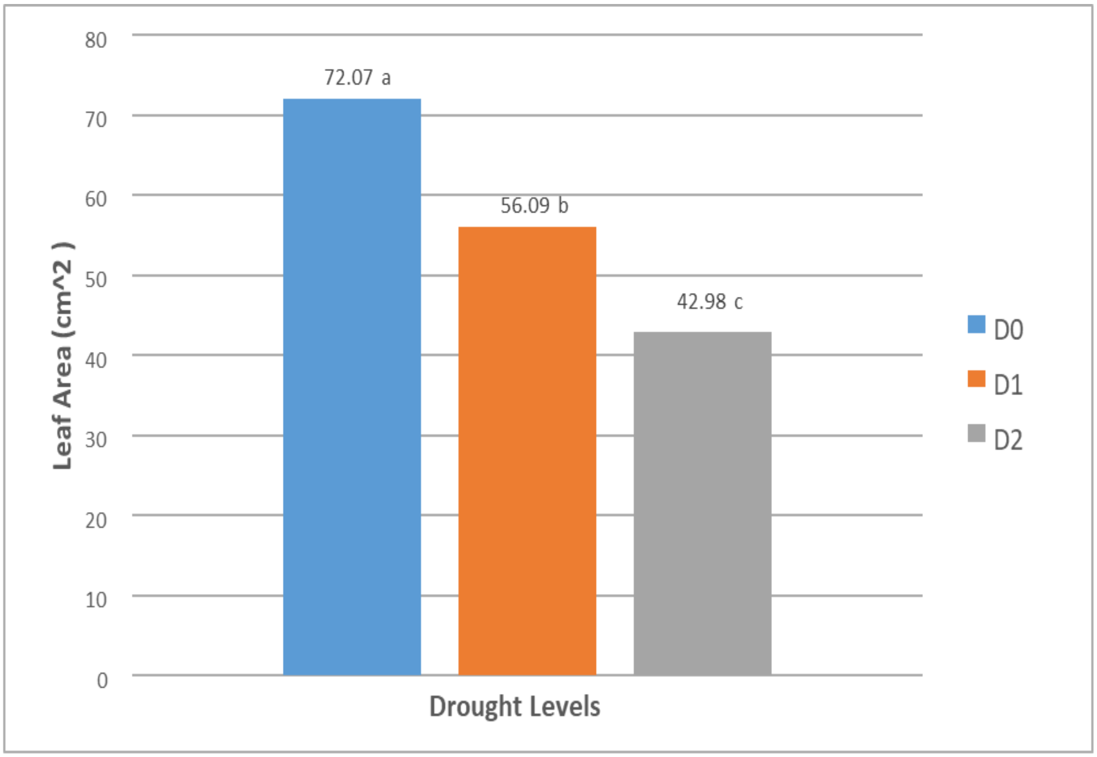

Figure 5. Effect of different drought periods on the leaf area of rice (LSD $_{(0.05)}$ = 2.54 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

Increasing melatonin fertilizer significantly ($P \leq 0.01$) increased the leaf area of rice plants (12.32% in M_1 over control). The highest values of leaf area were produced by the higher level of melatonin (M_1). On the other hand, the lowest values of the leaf area of rice were produced by control (when rice plants did not receive any melatonin fertilizer) without any significant differences with those produced by the melatonin level of M_1 (Figure 6 and Appendix X).

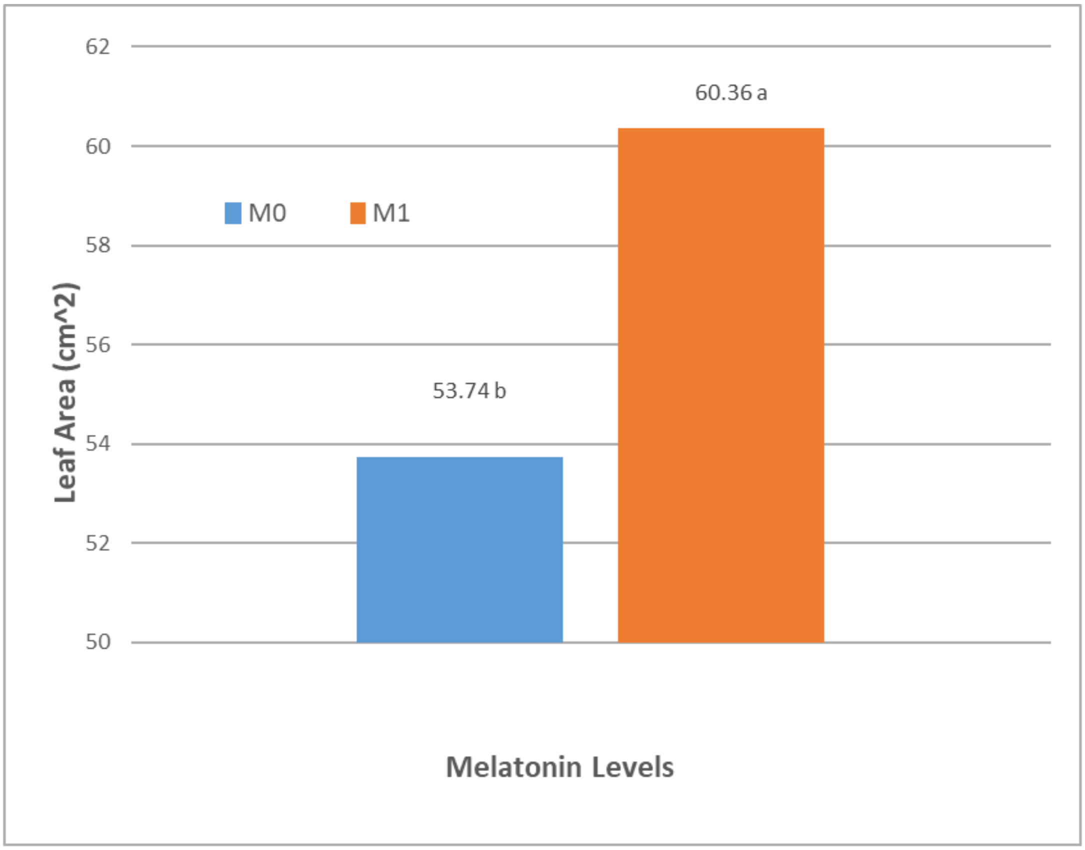

Figure 6. Effect of different melatonin levels on the leaf area of rice (LSD $_{(0.05)}$ = 2.08 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

Statistical analysis has shown that drought reduced the leaf area of rice plants significantly (Table 5 and Appendix X). Nevertheless, the melatonin application had a pronounced positive effect on leaf area enhancement as evident from Drought × Melatonin interaction (Table 5). Leaf area was increased by 13.65 % after M_1 supplementation of drought stressed (D_1M_1) plants when compared to drought treatment (D_1M_0) alone. Whereas, M_1 supplementation during drought stress (D_2) increased leaf area by 13.56% when compared to drought treatment (D_2M_0). Data regarding the leaf area depicted that treatment using M_1 recorded the statistically maximum leaf area at both D1 and D_2 level of drought. however, statistically, all the treatments were at par.

Table 5. Interaction effect of different drought periods and melatonin levels on the leaf area, membrane stability index (MSI %) and relative water content (RWC %) of rice

Treatments	**Leaf Area (cm^2)**	**Membrane Stability Index (MSI %)**	**Relative Water Content (RWC %)**
D_0 M_0	68.47 b	79.73 a	91.98 a
D_0 M_1	75.67 a	82.55 a	93.83 a
D_1 M_0	52.51 d	64.16 c	80.31 c
D_1 M_1	59.68 c	72.83 b	86.64 b
D_2 M_0	40.25 f	56.66 d	73.40 d
D_2 M_1	45.71 e	62.44 c	78.10 c
LSD(0.05)	**3.59**	**4.96**	**3.95**
CV(%)	**3.46**	**3.91**	**2.59**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Leaf area decreased under drought stress in our study. Similar reduction in leaf area of observed in rice while working with different rice genotypes of rice (Singh et al. 2018). Negative effects of water deficit on mineral nutrition and metabolism decrease the leaf area and alter assimilate partitioning among the plant organs (Zain et al. 2014). However, exogenous addition of melatonin restored the reduction of leaf area.

4.1.4 Leaf Membrane Stability Index (MSI%)

One of the major influence of drought stress include changes in membrane permeability leading to destabilization of membrane proteins. That’s why, electrolyte leakage was measured to determine the leaf cell membrane stability index (MSI%). Membrane stability index (MSI%) of plants decreased significantly ($P \leq 0.01$) with the increment of drought stress (Figure 7 and Appendix X). MSI% decreased by 15.59% and 26.61% under D_1 and D_2 drought stress respectively compared to control.

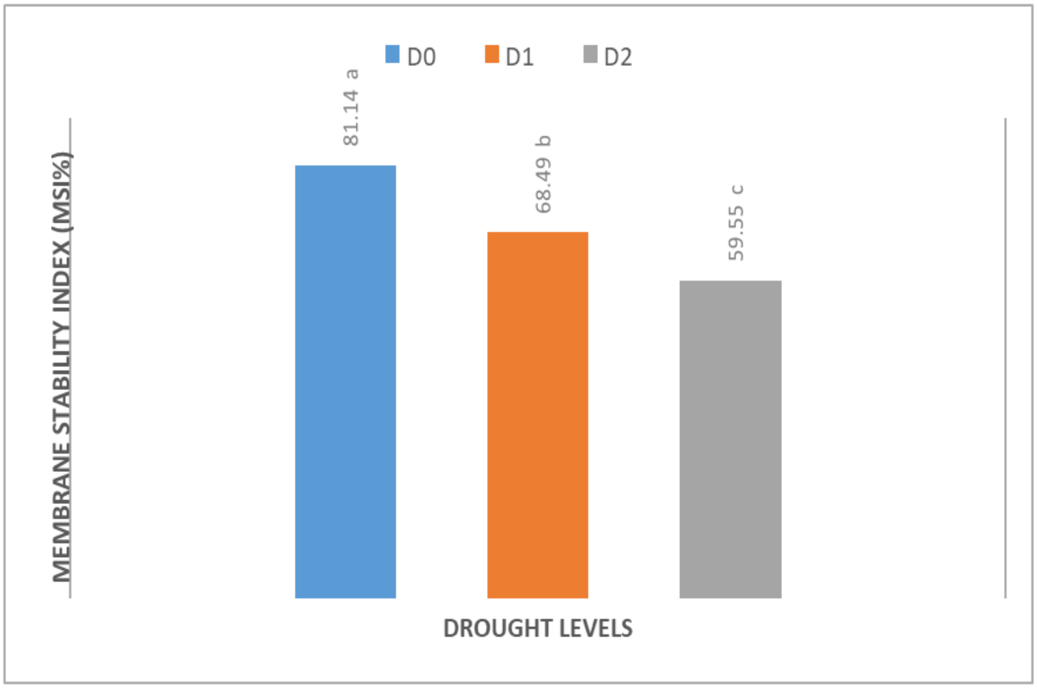

Figure 7. Effect of different drought periods on the membrane stability index (MSI%) of rice (LSD $_{(0.05)}$ = 3.51 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

However, supplied melatonin increased MSI% of rice plants significantly ($P \leq 0.01$) compared untreated plants (Figure 8 and Appendix X). The application of M_1 treatment improved MSI% by 8.60% when compared to control treatment. Thus, melatonin (M_1) were proved beneficial in improving the MSI % of rice plants.

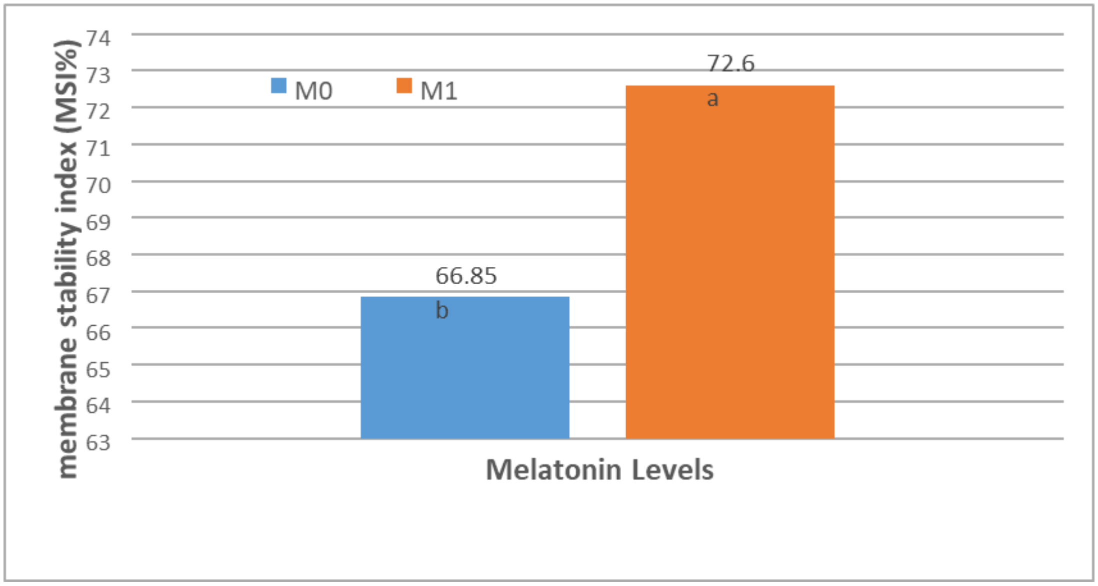

Figure 8. Effect of different melatonin levels on the membrane stability index (MSI%) of rice (LSD $_{(0.05)}$ = 2.86 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

The differences in membrane stability index (MSI%) between two melatonin treatments were significant ($P \leq 0.05$) for both level of drought (D_1 and D_2). In other words, the interaction effect of Drought × Melatonin treatment had significant effect on membrane stability index (MSI%) which was evident from statistically significant Drought × Melatonin interactive effect (Table 5 and Appendix X). Melatonin supplementation at the rate of M_1 was sufficient to improve MSI% significantly by 13.51% and 10.20% respectively at both D_1 level and D_2 level when compared to drought treatment alone. Thus, the results of interaction corresponding to membrane stability index (MSI%) provided that membrane stability index reached its maximum values when rice plants were treated with M_1 treatment for both D_1 level and D_2 level.

Drought stress causes physiological alteration and the over production of reactive oxygen species like H_2O_2, superoxide ion, singlet oxygen, all these are responsible for damaging cell membrane and different cellular organelles (Naghizadeh et al., 2019; Silalert and Pattanagul 2021). In the present study, the control and only melatonin applied plants showed higher MSI value indicating the better membrane condition. Drought stress decreased the value of MSI value indicating the damaging sign of membrane. On the other hand, while the melatonin was included with drought affected plants the MSI value increased, compared to the drought stress alone. Similar beneficial affect was demonstrated by other researchers. Drought affected rice plants with melatonin inclusion decreased the electrolyte leakage, malondialdehyde (MDA) and H_2O_2 of rice plants (Silalert and Pattanagul 2021).

Foliar application with melatonin also increased antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) which contributed the membrane protection and decreased electrolyte leakage and malondialdehyde (MDA) on rice plant (Silalert and Pattanagul 2021).

Melatonin has been shown to reduce electrolyte leakage and MDA content in *Cucumis sativus* L. and *Moldavian balm* under moderate and severe drought stress (Zhang et al., 2013; Naghizadeh et al., 2019). Melatonin also acts as a powerful scavenger of ROS in plants (Arnao and Hernández-Ruiz, 2015). Similar results were reported in rapeseed seedlings (Li et al., 2018), naked oat seedlings (Gao et al., 2018), maize seedlings (Su et al., 2019), and Chinese hickory plants (Sharma et al., 2020), which showed the capability of melatonin to enhance antioxidant enzyme under drought stress. The increased antioxidant enzyme activity may help reduce ROS accumulation, lower lipid peroxidation, and cellular damage, resulting in higher relative water content and lower membrane damage.

4.1.5 Relative Water Content (RWC%)

A characteristic symptom of drought stressed rice plants was tissue dehydration and that was exhibited as leaf relative water content (RWC %) reduction, compared with nondrought stress treatment (Figure 9 and Appendix X). Increased drought level caused significant ($P \leq 0.01$) reduction in leaf relative water contents (RWC%). RWC% was reduced from 92.91% in the control plants to 83.48% and 75.75% in the plants treated with D_1 and D_2 level of drought respectively.

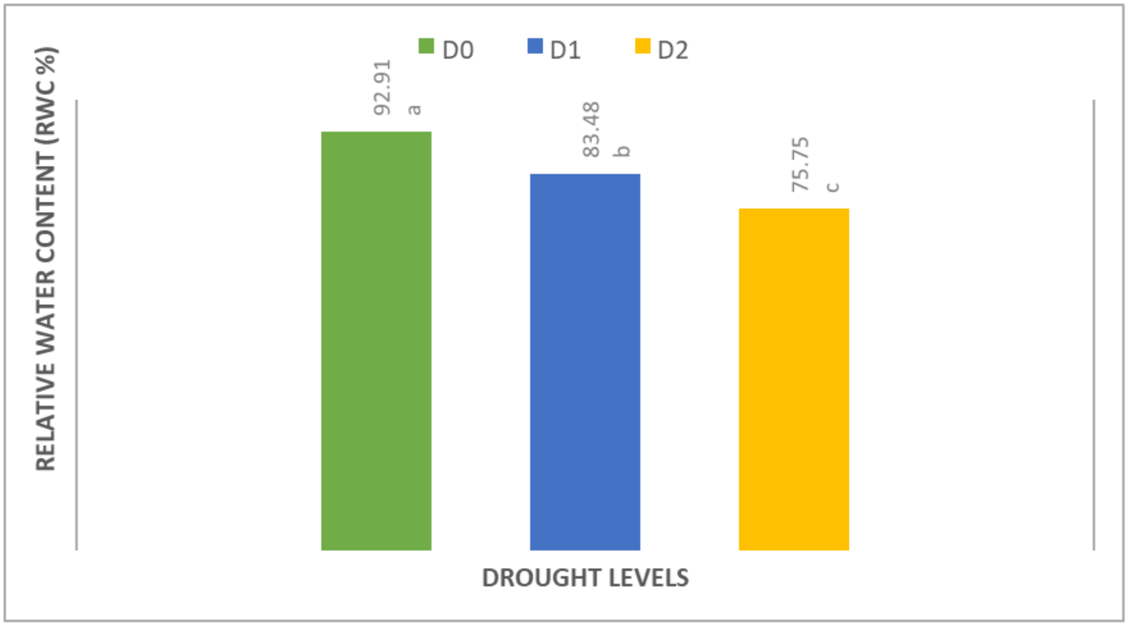

Figure 9. Effect of different drought periods on the relative water content (RWC %) of rice (LSD $_{(0.05)}$ = 2.80 and bars with different letters are significantly different at $p \leq$

0.05 applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

Melatonin addition to rice plants resulted in enhancement of the leaf relative water content (RWC %) significantly ($P \leq 0.01$).

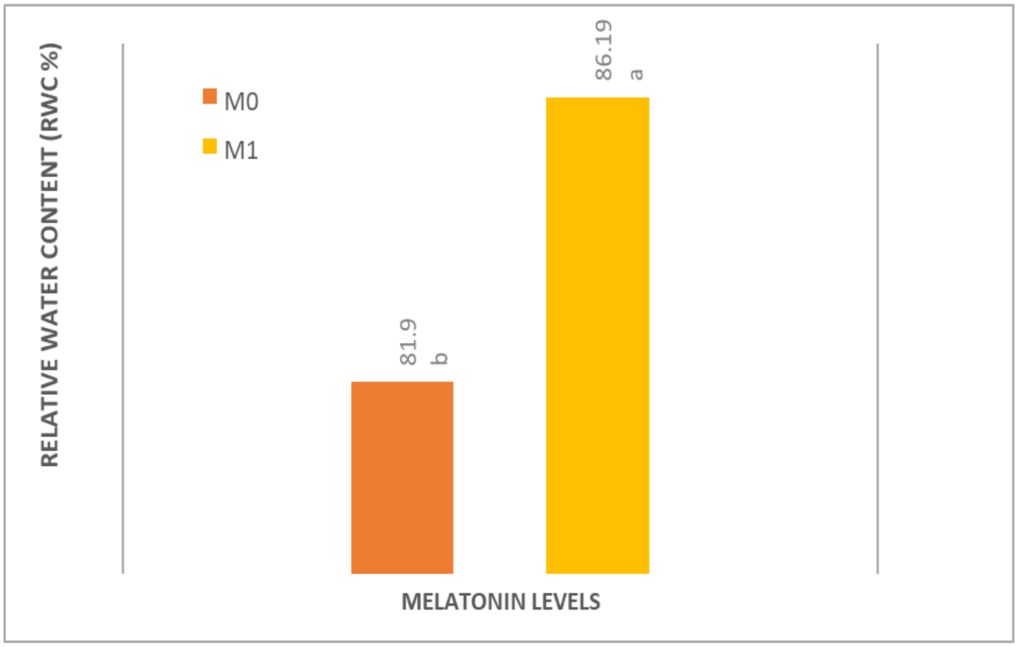

Figure 10. Effect of different melatonin levels on the relative water content (RWC %) of rice (LSD $_{(0.05)}$ = 2.28 and bars with different letters with are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin, M_1= 100 µM Melatonin)

Relative water content (RWC %) in leaf was greatly influenced by drought levels whereas melatonin application (M_1) improved the RWC % at both levels of drought (D_1 and D_2). Drought × Melatonin interactive effect supported this finding (Table 5 and Appendix X). Under D_1 drought stressed condition, RWC % increased by 7.88% at M_1 (D_1 M_1) in comparison with D_1 drought treatment alone (D_1 M_0). Whereas, under D_2 treated condition, an increase of 6.40% in RWC % was observed at M_1 treatment (D_2 M_1) compared to D_2 M_0 treatment where no melatonin was added. This result suggest that additional melatonin supply ensures more water content in leaf under drought stress condition. Though there were no statistically significant differences among mean values, a significant trend to increase in relative water content with increasing melatonin level was noticeable at both D_1 level and D_2 level.

Higher relative water content together with increased proline and sugar accumulation was recorded in melatonin added drought affected rice plants (Silalert and Pattanagul 2021). Melatonin stimulates the biosynthesis of soluble sugars and osmoprotectant

molecules like prolinewhich is responsible for maintaining the turgor and osmotic pressure of plant cells growing under drought stress and ultimately increases the water uptake capacity of plants (Chen et al., 2020). In our study, the increased proline content is corroborating the increased leaf RWC if rice plants. Ahmad et al. (2021).
Reported that 100 µM melatonin application as soil drenching enhanced relative water content, and osmo-protectants of maize seedlings (Ahmad et al. 2021). Exogenous application of melatonin increased proline and glyceine betaine content in soybean plants under salt stress (Alharbi et al. 2021).

4.1.6 Chlorophyll Content (SPAD Value)

Chlorophyll meter (SPAD) is a convenient tool to estimate the absolute values of chlorophyll per unit leaf area. Drought stress caused a substantial decline ($P \leq 0.01$) for leaf chlorophyll content (Figure 11 and Appendix XI). Leaf chlorophyll content (SPAD) was high in the control both at 60 DAT and 90 DAT. Under D_1 drought stress, SPAD values reduced by 14.18% and 14.15% at 60 DAT and 90 DAT respectively when compared to unstressed control. But under D_2 drought stress, there was a 19.77% and 18.271% reduction over control in SPAD values at 60 DAT and 90 DAT respectively. The percent reduction of total chlorophyll content proportionally increased with the increase of drought.

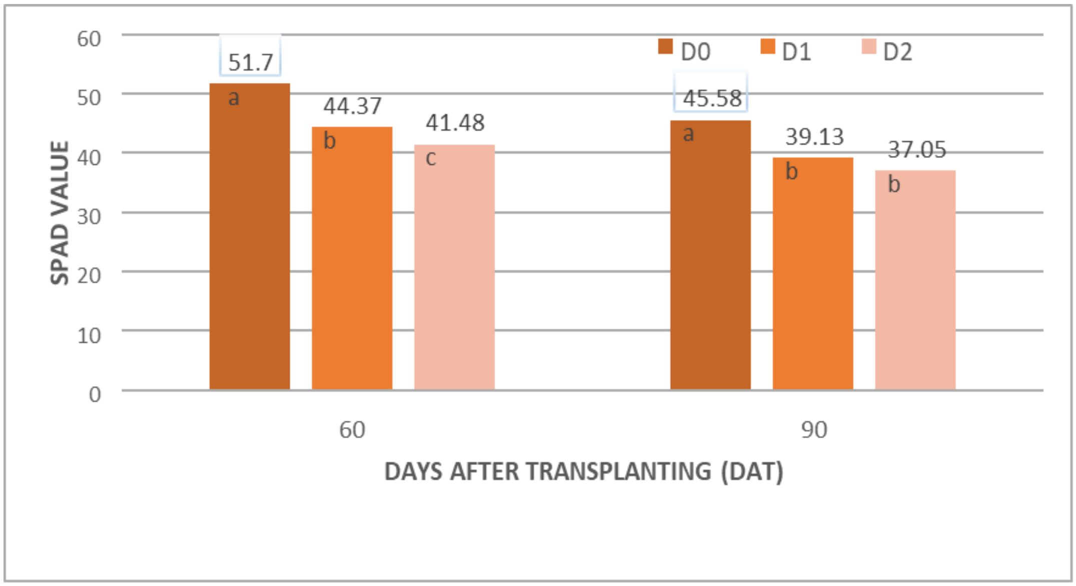

Figure 11. Effect of different drought periods on the chlorophyll content (SPAD value) of rice at different days after transplanting ($LSD_{(0.05)}$ = 1.49 and 2.56 at 60 and 90 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

When supplemental melatonin was applied, the improvements in leaf chlorophyll content
(SPAD) were significant ($P \leq 0.01$). M_1 application increased leaf chlorophyll content (SPAD) by 7.18 % at 60 DAT, whereas at 90 DAT it was by 8.74% when compared to

control (Figure 12 and Appendix XI). In comparison to the control group, leaf chlorophyll content (SPAD) was significantly increased all the way to the level of M_1 treatment, while there was no further significant difference between these two treatments.

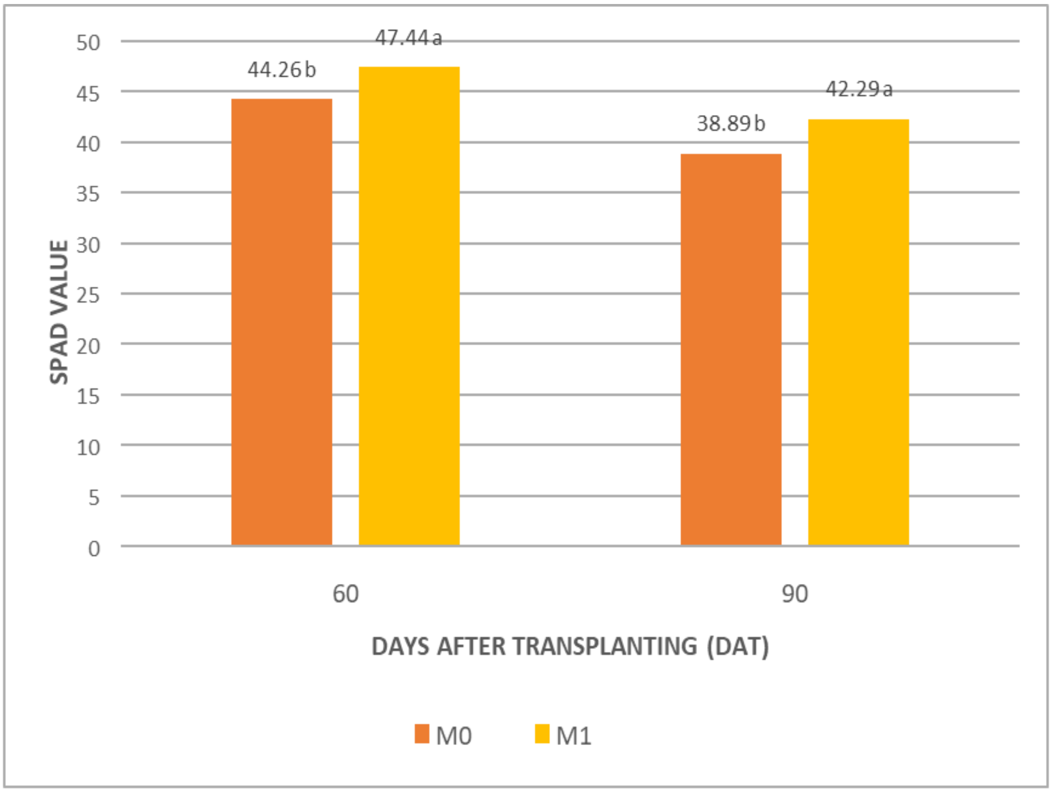

Figure 12. Effect of different melatonin levels on the chlorophyll content (SPAD value) of rice at different days after transplanting (LSD $_{(0.05)}$ = 1.22 and 2.09 at 60 and 90 DAT, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

Decrement of leaf chlorophyll content (SPAD) might suggest that drought stress enhanced the degradation of chlorophyll. Nevertheless, the Drought × Melatonin interaction significantly ($P \leq 0.01$) increased SPAD values compared to the saline conditions alone (Table 6 and Appendix XI). At 60 DAT and 90 DAT, under D_1 drought, the leaf chlorophyll content (SPAD) increment at M_1 (D_1 M_1) was 9.44% and 14.61% respectively when compared to drought treatment (D_1 M_0) alone. On the other hand, at 60 DAT and 90 DAT under D_2 drought, leaf chlorophyll content (SPAD) increased by 9.68% and 7.56% at M_1 treatment (D_2 M_1) when compared to drought treatment (D_2 M_0) alone.

Table 6. Interaction effect of different drought periods and melatonin levels on the chlorophyll content (SPAD value) of rice at different days after transplanting

Treatments	**SPAD Value**	
	60 DAT	**90 DAT**
D_0 M_0	50.83 a	44.50 ab
D_0 M_1	52.57 a	46.67 a
D_1 M_0	42.37 c	36.47 d
D_1 M_1	46.37 b	41.80 bc
D_2 M_0	39.57 d	35.70 d
D_2 M_1	43.40 c	38.40 cd
LSD(0.05)	**2.11**	**3.62**
CV(%)	**2.53**	**4.90**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

Drought stress stimulates the production of ROS in plant cells, resulting in chlorophyll degradation (Yordanov et al., 2000). Chlorophyll degradation is also accelerated by the chlorophyllase enzyme, and the transcription level of the gene encoding this enzyme is upregulated in response to drought stress (Wang et al., 2013; Ma et al., 2018). From the results of the present study, it is clear that SPAD value indicating the greenness of leaves decreased under drought stress while the exogenous melatonin addition under drought stress restored the greenness to some extent. Our findings indicate that exogenous melatonin may played role in improving chlorophyll content in the drought-stressed plant.

Silalert and Pattanagul (2021) observed the similar results in rice plant. Additionally, previous research has demonstrated that exogenous melatonin can reduce the droughtinduced chlorophyll degradation and improve photosynthetic rate in tomato, cucumber, and maize (Liu et al., 2015; Wang et al., 2016; Ahmad et al., 2019). Melatonin treatment may help prevent chlorophyll degradation by down-regulating the gene encoding the chlorophyllase enzyme. (Sharma et al., 2020).

4.1.7 Proline content

Drought stress caused a substantial decline ($P \leq 0.01$) for leaf proline content (Figure 13 and Appendix XII). Leaf proline content was low in the control. Under D_1 drought stress, Proline content increased by 45.61% when compared to unstressed control. But under D_2 drought stress, there was a 121.05% increase over control in proline content. The percent increase of proline content proportionally increased with the increase of drought.

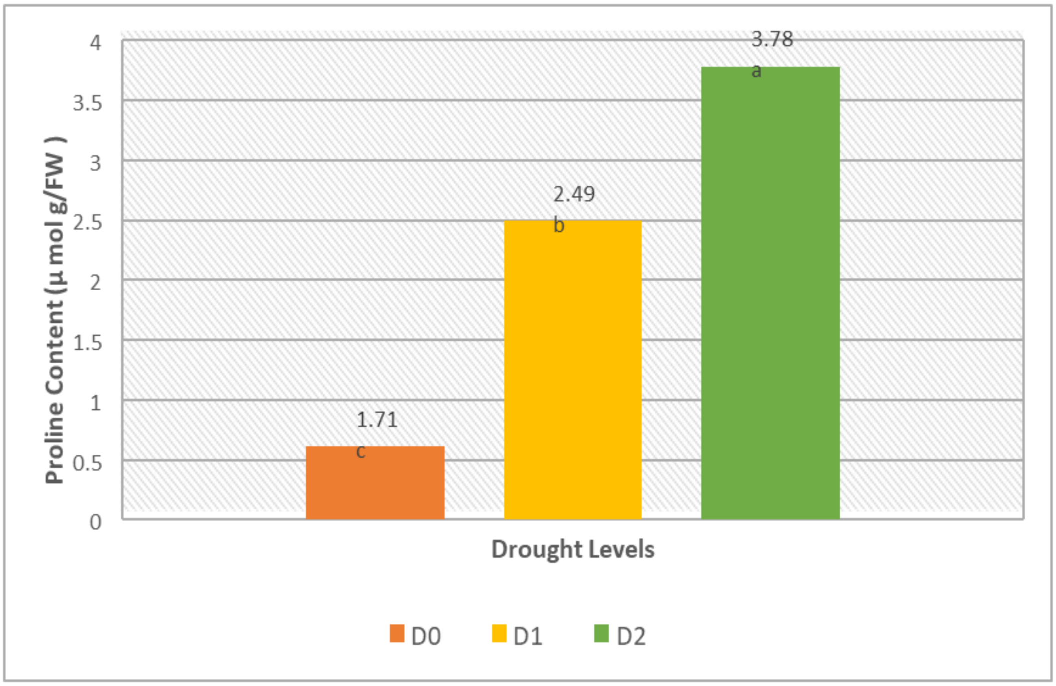

Figure 13. Effect of different drought periods on the proline content of rice after transplanting (LSD $_{(0.05)}$ = 0.62 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

When supplemental melatonin was applied, the improvements in leaf proline content were significant ($P \leq 0.01$). M_1 application increased leaf proline content by 46.77 % when compared to control (Figure 14 and Appendix XII). In comparison to the control group, leaf proline content was significantly increased all the way to the level of M_1 treatment.

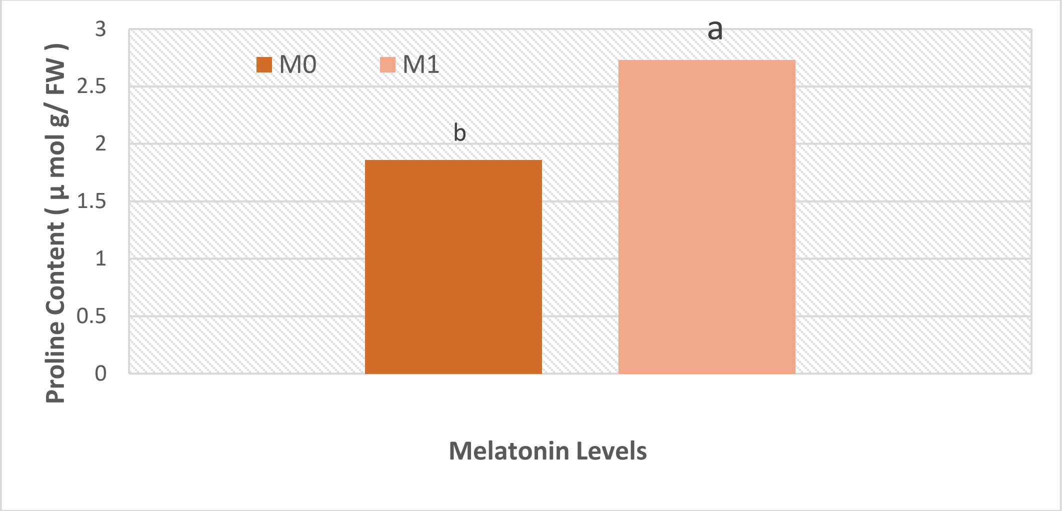

Figure 14. Effect of different melatonin levels on the proline content of rice after transplanting (LSD $_{(0.05)}$ = 0.50 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin, M_1= 100 µM Melatonin)

Increment of leaf proline content might suggest that drought stress enhanced the increase of proline (Table 7 and Appendix XII). Under D_1 drought, the leaf proline content increment at M_1 (D_1 M_1) was 45.81% when compared to drought treatment (D_1 M_0) alone. On the other hand, under D_2 drought, leaf proline content increased by 43.87% at M_1 treatment (D_2 M_1) when compared to drought treatment (D_2 M_0) alone.

Table 7. Interaction effect of different drought periods and melatonin levels on the proline content of rice at different days after transplanting

Treatments	**Proline content (µmol/g FW)**
D_0M_0	0.45 d
D_0M_1	0.77 d
D_1M_0	2.03 c
D_1M_1	2.96 b
D_2M0	3.10 b
D_2M_1	4.46 a
LSD(0.05)	**0.87**
CV(%)	**20.87**

Proline is one of the vital osmoprotectant molecules which has been reported to increase under drought stress condition. Increase of this molecule helps in osmoregulation which can increase water uptake on plants (Alharbi et al. 2021; Silalert and Pattanagul 2021). In the present study, drought stress increased the proline content of rice seedlings. Further increase of proline content was recorded with exogenous melatonin supplementation with drought treatment. In their study, Silalert and Pattanagul (2021) demonstrated that foliar application with 100 µM melatonin increased the proline level of drought stressed rice plants. The effect of melatonin on improving proline accumulation has also been reported in *Zea mays* L. (Ahmad et al., 2019) and *Coffea arabica* L. (Campos et al., 2019). Ding et al. (2018) proposed that melatonin increases the expression of P5CS, a key gene for proline biosynthesis, and stimulated pyrroline-5-carboxylate synthase (P5CS) activity in poplar leaf under methyl viologen stress, indicating that melatonin mediates the biosynthesis of proline during oxidative stress.

4.1.8 Dry Weights of Root, Shoot, Leaf and Total Dry Matter (TDM) plant^{-1}

Dry matter estimation is regarded as a valuable index for monitoring vegetative growth of the rice plant (Hakim *et al*., 2014b). Total dry matter (TDM) is defined as the sum total of root, shoot, and leaf dry weight. The total dry matter (TDM) was significantly ($P \leq 0.01$) influenced under different levels of drought (Figure 15 and Appendix XIII). Root, shoot, leaf dry weights, and TDM decreased by 20.42%, 23.40%, 19.90%, and 20.90% respectively in drought-stressed plants (D_1) when compared to unstressed control. Further, a dramatic reduction of dry weights of root, shoot, leaf, and TDM with D_2 drought stress was 34.32%, 39.53%, 28.57%, and 32.29% respectively when compared to control plants without drought stress. A maximal reduction in root, shoot, leaf dry weights, and TDM was observed at D_2 treatment.

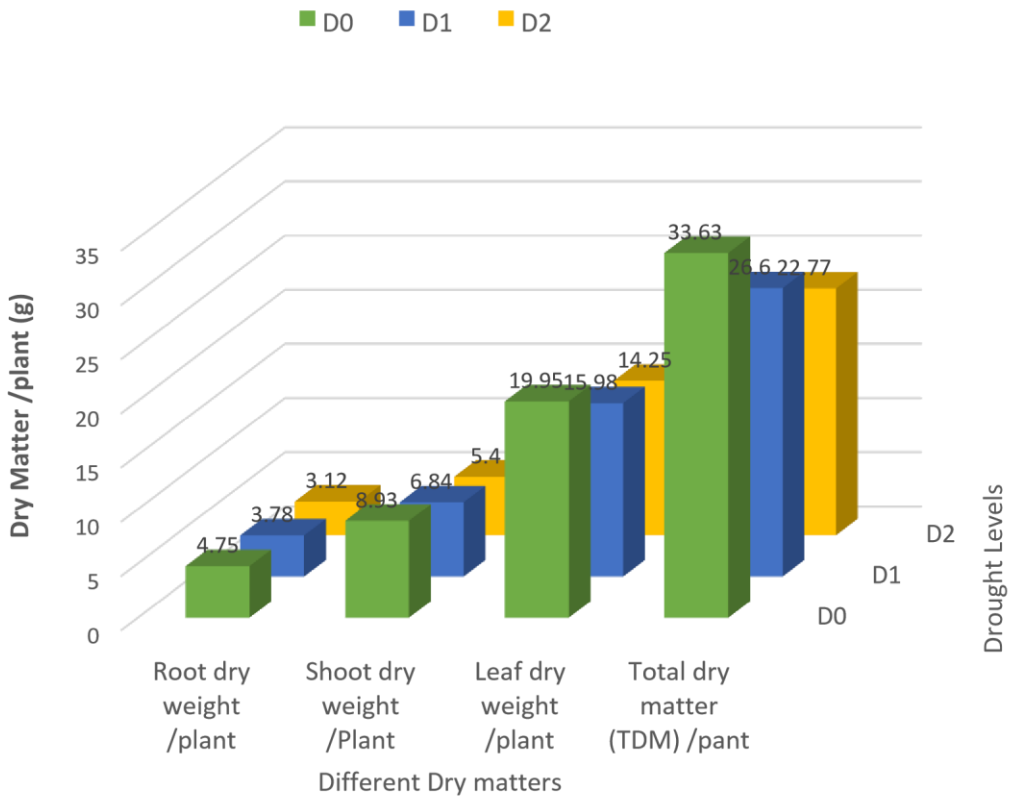

Figure 15. Effect of different drought periods on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice (LSD $_{(0.05)}$ = 0.12, 0.53, 1.40 and 1.81 for dry weights of root, shoot, leaf and total dry matter (TDM), respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

The supplementation of melatonin revealed a significant ($P \leq 0.01$) increment in root, shoot, leaf dry weights, and TDM of rice plants (Figure 16 and Appendix XIII). 21.37%, 26.12%, 12.52%, and 17.02% increment in the root, shoot, leaf dry weights, and TDM were noticed respectively in M_1 treated plants, when compared to control plants that did not receive any melatonin.

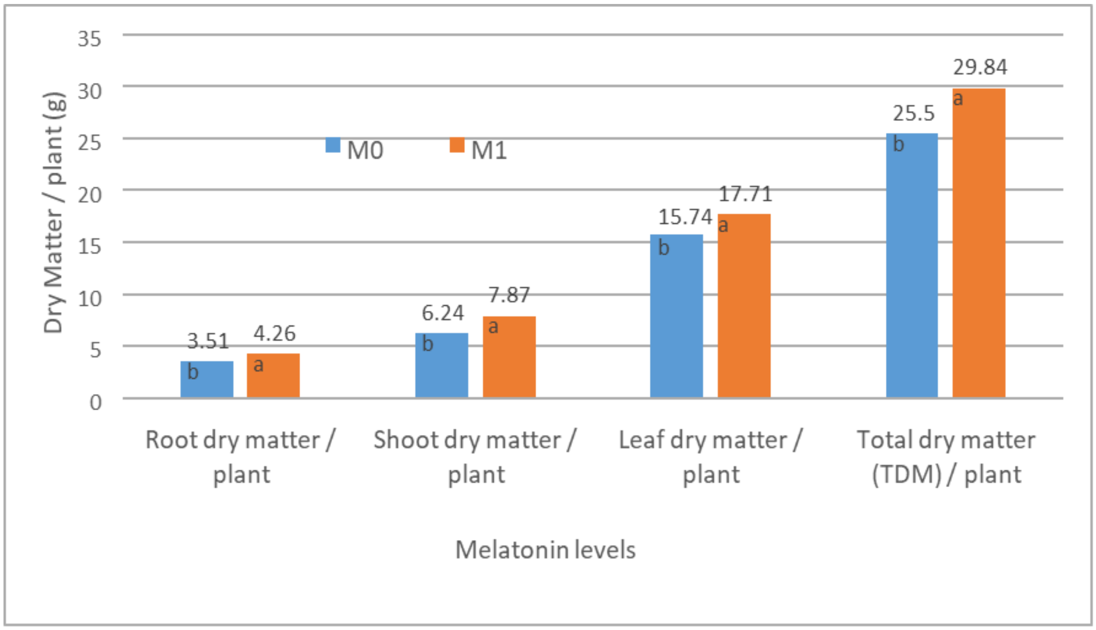

Figure 16. Effect of different melatonin levels on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice (LSD $_{(0.05)}$ = 0.10, 0.43, 1.15 and 1.48 for dry weights of root, shoot, leaf and total dry matter (TDM), respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM)

Drought stress decreased the deposit of dry materials. Reduction in total dry matter production under the dought condition and the positive effect of melatonin on total dry matter production are presented in Table 8 and Appendix XIII. The use of melatonin under drought stress revealed a significant ($P \leq 0.01$) enhancement in the root, shoot, leaf dry weights, and TDM. It was revealed from statistically significant Drought × Melatonin interaction (Table 8). M_1 supplementation during D_1 drought stress increased root dry weight plant^{-1} by 22.65% ; shoot dry weight plant^{-1} by 28.38% ; leaf dry weight plant^{-1} by 16.01% and total dry matter (TDM) plant^{-1} by 19.97% (compared to drought-affected plants without melatonin application). Root, shoot, leaf dry weights and TDM increased by 30.74%, 40.85%, 22.66% and 27.88% respectively after M_1 supplementation of drought-stressed (D_2) plants (compared to drought treatment alone). However, M_1 supplementation was noticed as the most effective dose for the increment of plant root, shoot, leaf dry weights, and TDM under both levels of drought. Furthermore, the parameters of drought-stressed plants receiving supplementary melatonin were still lower than the values of non-drought stress treatment.

Table 8. Interaction effect of different drought periods and melatonin levels on the dry weights of root, shoot, leaf and total dry matter (TDM) plant^{-1} of rice
LSD.

Treatments	**Root dry weight plant^{-1} (g)**	**Shoot dry weight plant^{-1} (g)**	**Leaf dry weight plant^{-1} (g)**	**Total dry matter (TDM) plant^{-1} (g)**
$D_0 M_0$	4.43 b	8.24 b	19.63 a	32.31 b
$D_0 M_1$	5.07 a	9.61 a	20.27 a	34.95 a
D_1M_0	3.40 d	5.99 c	14.80 c	24.19 d
D_1M_1	4.17 c	7.69 b	17.17 b	29.02 c
D_2M_0	2.70 e	4.48 d	12.80 d	19.98 e
D_2M_1	3.53 d	6.31 c	15.70 bc	25.55 d
LSD $_{(0.05)}$	**0.17**	**0.74**	**1.99**	**2.56**
CV (%)	**2.40**	**5.80**	**6.52**	**5.09**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying

Drought stress reduced rice plant growth significantly, as evidenced by decreases in shoot and root length, shoot and root fresh weight, and shoot and root dry weight and exogenous melatonin reversed the adverse effect. Similar result was demonstrated by Silalert and Pattanagul (2021) and Li et al. (2022). Melatonin is a plant growth regulator and plays a vital role in regulating plant growth and development (Arnao and HernándezRuiz, 2015). One of the most intriguing aspects of research on melatonin in plants is the postulated similarity between its function and that of IAA (indole 3-acetic acid). IAA is an auxin involved in various physiological processes, the most notable of which is its role as a growth promoter (Arnao and Hernández-Ruiz, 2006). It is believed that melatonin and IAA can have a co-regulatory impact on plant growth by working in a combined or similar fashion to promote root morphogenesis (Murch et al., 2001). Moreover, improved antioxidant defense system, osmoregulation, water status, photosynthetic pigment levels, and various other physiological attributes were reported in melatonin added plants under different abiotic stress including drought stress which ultimately improved the growth (Ahmad et al., 2019; Alharbi et al. 2021; Silalert and Pattanagul 2021).

4.1.9 Panicle Length (cm)

As higher panicle length could provide a higher number of grains, so panicle length is regarded as an important yield contributing character. Results revealed that the panicle length of rice was also significantly ($P \leq 0.01$) affected by various levels of drought (Figure 17 and Appendix XIV). Here, drought stress caused about 20.80% and 31.60% downfall in the length of panicle with D_1 and D_2 treatments respectively when compared to control plants without drought stress.

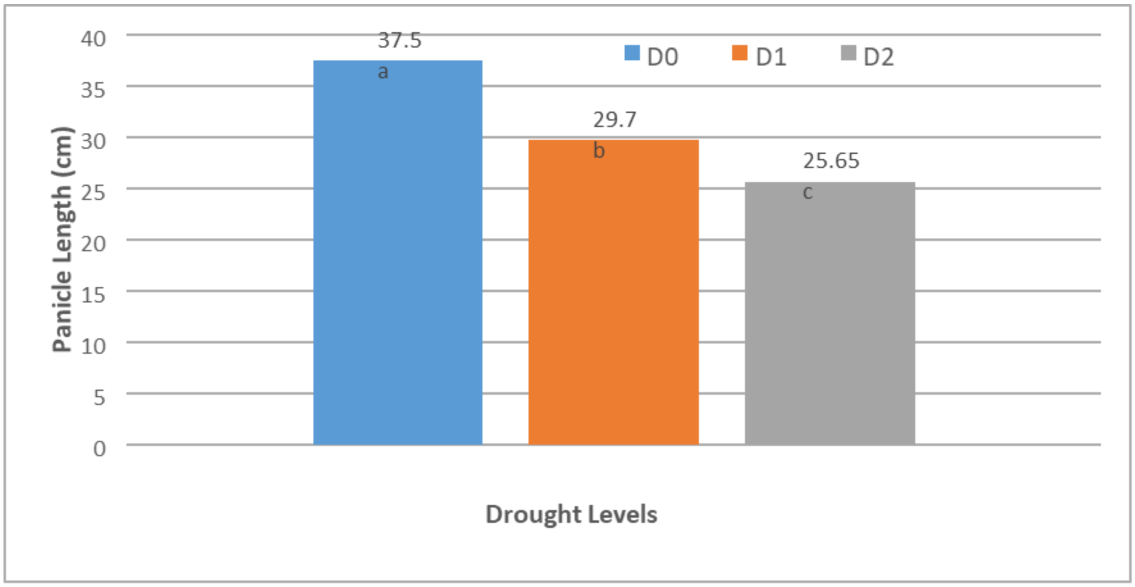

Figure 17. Effect of different drought periods on the panicle length of rice (LSD $_{(0.05)}$ = 1.65 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

Panicle length increased significantly ($P \leq 0.01$) after the supplementation of melatonin (Figure 18 and Appendix XIV). Panicle length of melatonin treated plants increased by 8.35% with M_1 treatments compared to control plants that did not receive any melatonin.

The application of M_1 increased the panicle length.

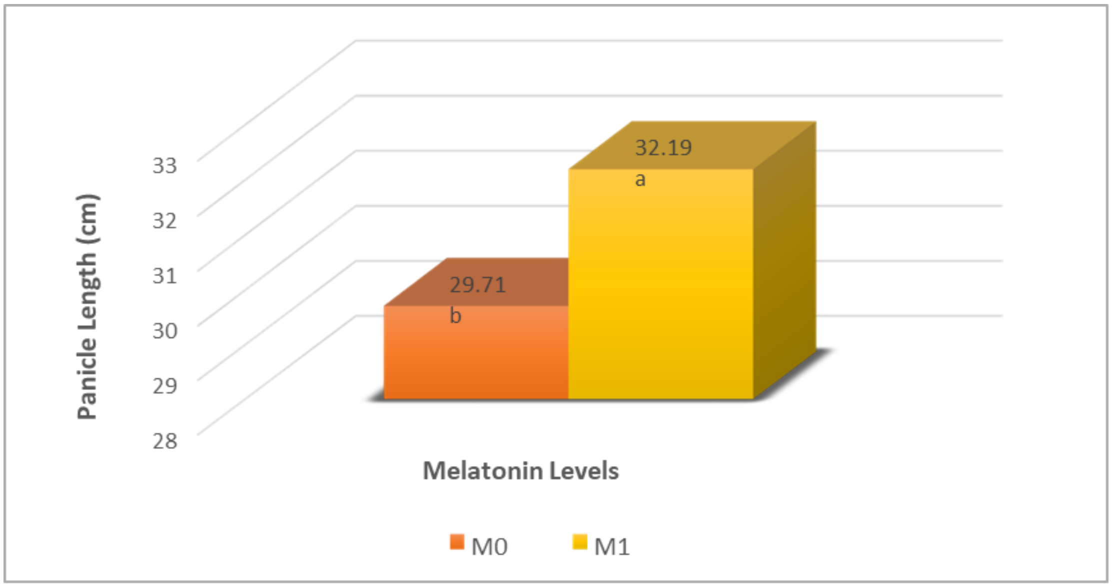

Figure 18. Effect of different melatonin levels on the panicle length of rice (LSD $_{(0.05)}$ = 1.35 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin, M_1= 100 µM Melatonin)

A marked decrease in panicle length by drought stress (D_1 and D_2) was found in rice plants but the application of melatonin (M_1) improved the panicle length at both levels of drought which was evident from Drought × Melatonin interaction (Table 9 and Appendix XIV). Percent increase of panicle length in the treatment D_1M_1 was 11.61% , indicating a remarkable difference with D_1M_0 where no melatonin was applied. Whereas, it was 14.02% increase with D_2M_1 when compared with D_2M_0 with drought treatment only.

Table 9. Interaction effect of different drought periods and melatonin levels on panicle length of rice

Treatments	**Panicle Length (cm)**
D_0M_0	37.10 a
D_0M_1	37.90 a
D_1M_0	28.07 c
D_1M_1	31.33 b
D_2M_0	23.97 d
D_2M_1	27.33 c
LSD(0.05)	**2.33**

CV(%)	**4.15**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.10 No. of effective and non-efective Tillers Plant^{-1}

Tiller formation in rice is a very important agronomic trait for grain production and number of tillers provide valuable information about the stress profile of a plant under abiotic stress

(Suzuki *et al*., 2005). The number of tillers plant^{-1} were significantly $(P \leq 0.01)$ reduced by increasing level of drought (Figure 19 and Appendix XV). D_1 and D_2 treatment reduced tiller number by 23.22% and 51.51% respectively for effective tillers /plant and increased tiller number by 73.45% and 102.26% respectively for non-effective tillers /plant when compared to control. In respect of drought effect, the result showed that the number of effective tillers plant^{-1} were greatly affected even at D_1 treatment but the maximum reduction in number of tillers plant^{-1} was found at D_2 treatment.

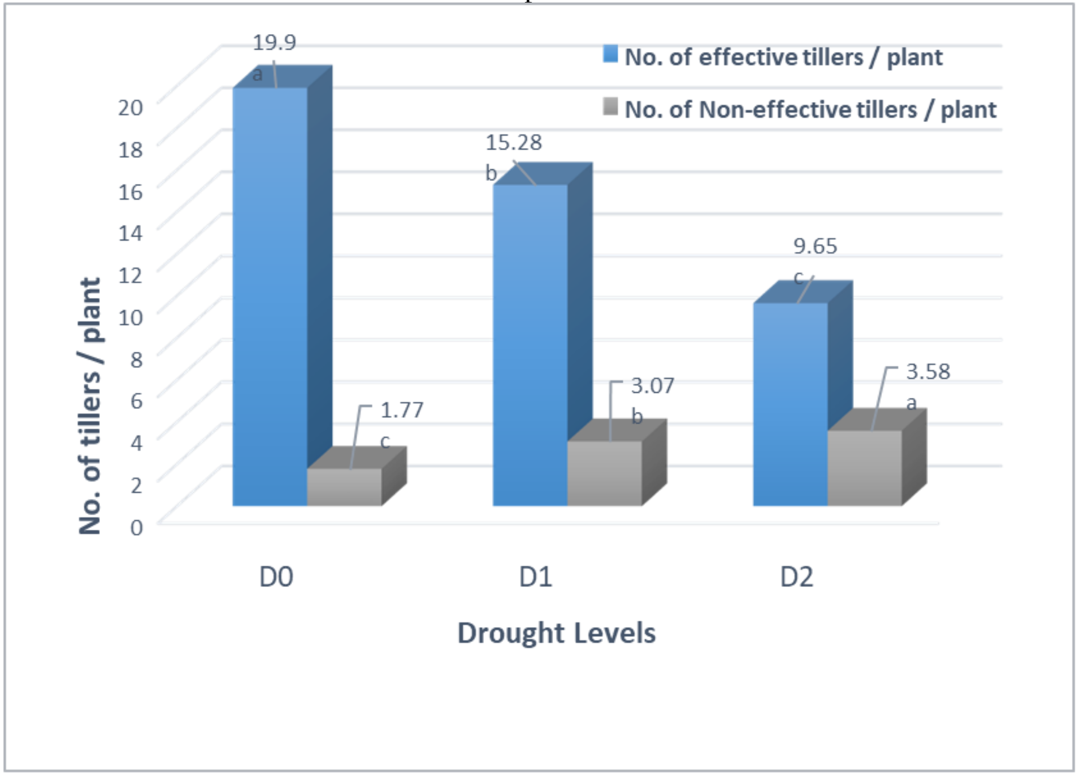

Figure 19. Effect of different drought periods on number of tillers plant^{-1} of rice after transplanting (LSD $_{(0.05)}$ = 1.06 and 0.19 for effective and non-effective tillers respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

By exogenous application of melatonin, the number of tillers plant^{-1} was increased significantly $(P \leq 0.01)$ at different days after transplanting (Figure 20 and Appendix XV). The number of tillers plant^{-1} enhancement over control was 17.70% and 20.19% by effective and non-effective tillers respectively. So, the parameters of plants not receiving supplementary melatonin (control) were still lower than the values of melatonin treatment.

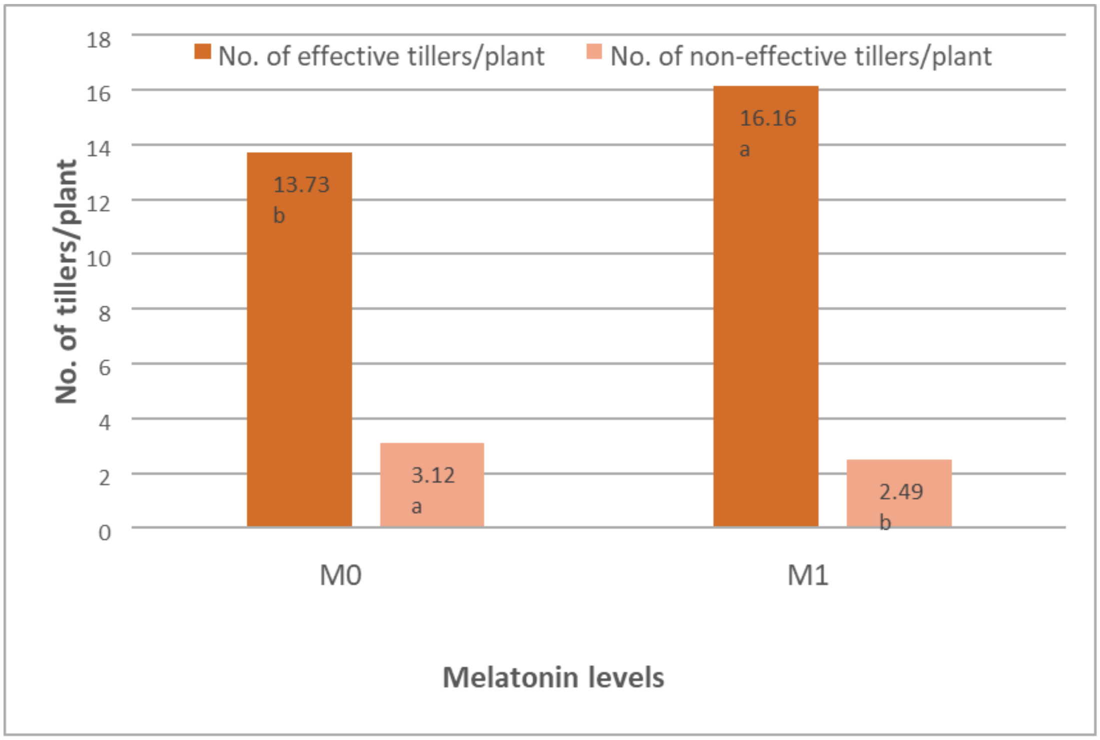

Figure 20. Effect of different melatonin levels on number of tillers plant^{-1} of rice after transplanting (LSD $_{(0.05)}$ = 0.87 and 0.15, for effective and non-effective tillers respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

Drought stress greatly affected the development and viability of tillers plant^{-1}. It was evident from statistically significant Drought × Melatonin interaction (Table 10 and Appendix XV). Under D_1 drought, the no. of effective tillers plant^{-1} increment at M_1 (D_1 M_1) was 21.52% when compared to drought treatment (D_1 M_0) alone. On the other hand, under D_2 drought, the no. of effective tillers plant^{-1} increased by 32.53% at M_1 treatment (D_2 M_1) when compared to drought treatment (D_2 M_0) alone.

The no. of non-effective tillers plant^{-1} increased with both levels of drought but the application of melatonin decreased this parameter which was evident from statistically significant Drought × Melatonin interaction (Table 10 and Appendix XV). The no. of noneffective tillers plant^{-1} decreased by 28.01% after M_1 supplementation of droughtstressed (D_1M_1) plants when compared to drought treatment (D_1M_0) alone. Whereas, M_1 supplementation during drought stress (D_2) decreased by 23.83% when compared to drought treatment (D_2M_0) alone.

Table 10. Interaction effect of different drought periods and melatonin levels on the number of effective and non-effective tillers plant^{-1} of rice

Treatments	**No. of Effective Tillers Plant^{-1}**	**No. of Non-Effective Tillers Plant^{-1}**
D_0M_0	19.10 b	1.73 e
D_0M_1	20.70 a	1.80 e
D_1M_0	13.80 d	3.57 b
D_1M_1	16.77 c	2.57 d
D_2M_0	8.30 f	4.07 a
D_2M_1	11.00 e	3.10 c
LSD(0.05)	**1.50**	**0.27**
CV(%)	**5.53**	**5.23**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

In the present study drought stress decreased the tiller number. Number of effective tillers decreased but number of non-effective tillers increased in rice plant under drought stress as observed by Pant et al. (2019). Singh et al. (2018) also reported a reduction in tiller number plant^{-1} under drought stress. The number of tillers reduces due to reduced growth and photosynthesis processes of plant (Quampah et al., 2011). However, melatonin addition with drought treatment increased the tiller number, compared to drought treatment alone. Melatonin increased productive tillers, grains per panicle, and 1000grain weights and yield of rice under that combined stress of cadmium-lead (Jiang et al.
2022).

4.1.11 No. of Spikelets Panicle^{-1}

Rice grain yield is closely related to the number of filled grains panicle^{-1}. No. of filled grains panicle^{-1} decreased significantly ($P \leq 0.01$) with the increase of drought (Figure 21 and Appendix XVI). The highest number of filled grain panicle^{-1} was counted at control condition and the lowest number of filled grain per panicle was recorded at the D_2 level of drought. A decrease of 31.39% and 43.98% number of filled grains panicle^{-1} was observed with D_1 and D_2 level of drought respectively compared to control (no drought). An increase of 76.38% and 163.09% number of non-filled grains panicle^{-1}

was observed with D_1 and D_2 level of drought respectively compared to control (no drought).

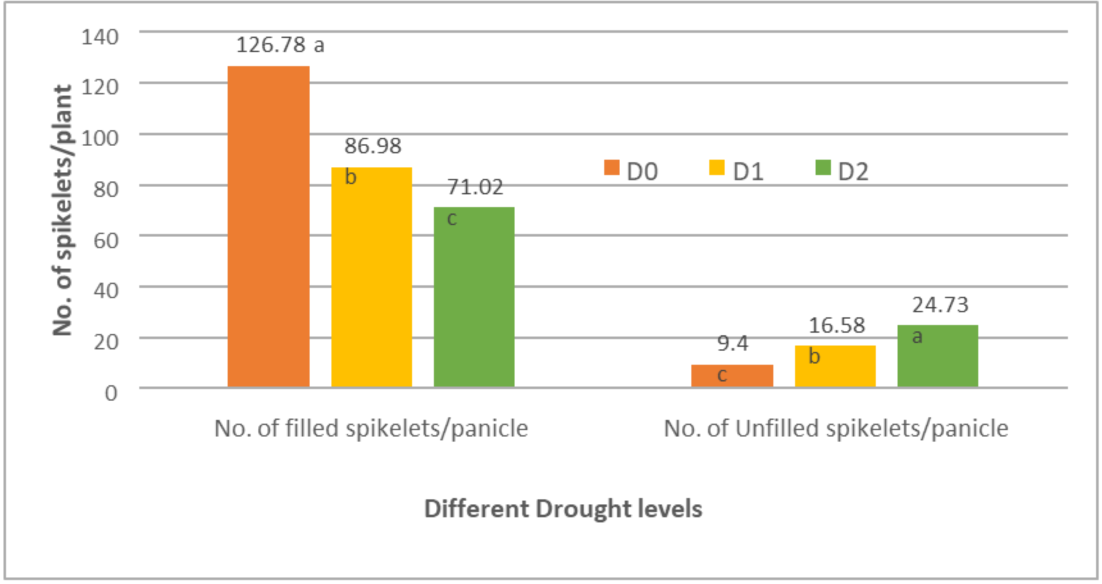

Figure 21. Effect of different drought periods on the number of filled and unfilled spikelets panicle^{-1} of rice (LSD $_{(0.05)}$ = 2.62 and 0.79 for number of filled and unfilled spikelets, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

Significant ($P \leq 0.01$) influence of melatonin on filled grains panicle^{-1} was observed. A gradual increase in the number of filled grains panicle^{-1} was observed with the increase of melatonin level (Figure 22 and Appendix XVI). No. of filled grains panicle^{-1} increased by 14.67% after M_1 supplementation when compared to control (without melatonin). No. of non-filled grains panicle^{-1} decreased by 23.35% after M_1 supplementation when compared to control (without melatonin).

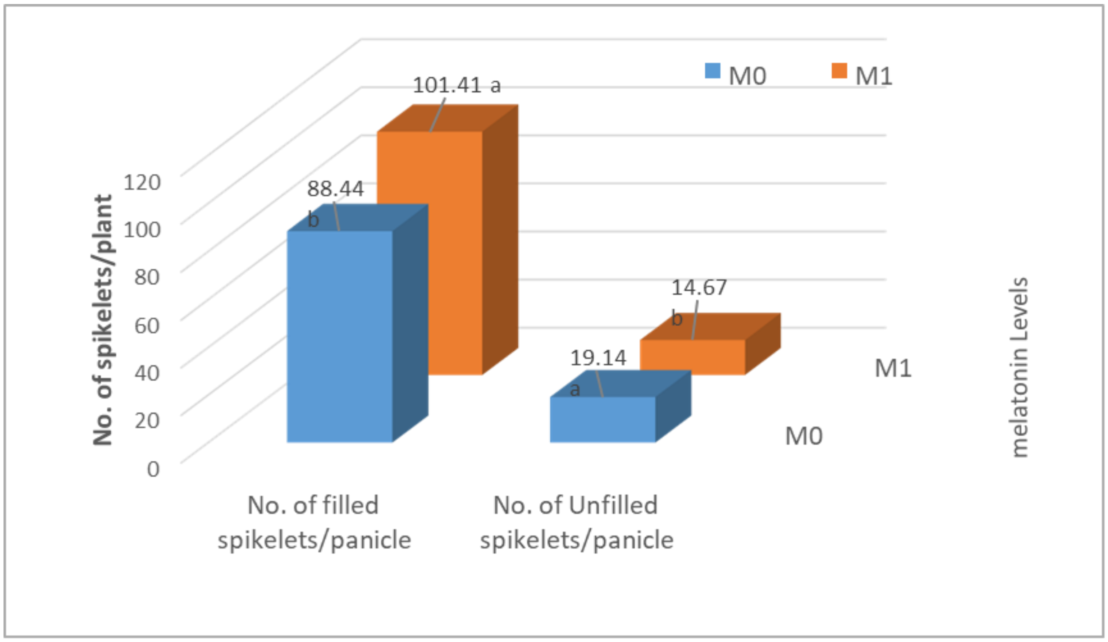

Figure 22. Effect of different melatonin levels on the number of filled and unfilled spikelets panicle^{-1} of rice (LSD $_{(0.05)}$ = 2.14 and 0.64 for number of filled and unfilled spikelets, respectively and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

The interaction effect of Drought × Melatonin treatment had a significant ($P \leq 0.01$) effect on filled grains panicle^{-1} which was evident from statistically significant Drought × Melatonin interactive effect (Table 11 and Appendix XVI). Melatonin supplementation at the rate of M_1 was sufficient to improve the number of filled grains panicle^{-1} significantly, by 22.27% and 16.51% at D_1 and D_2 level of drought respectively when compared to drought treatment alone.

It was revealed that drought increased the number of unfilled spikelets panicle^{-1} but melatonin application decreased the number of unfilled spikelets panicle^{-1} under both D_1 and D_2 level of drought condition. It was shown by statistically significant ($P \leq 0.01$) Drought × Melatonin interaction (Table 11 and Appendix XVI). Under D_1 droughtstressed condition, the number of unfilled spikelets panicle^{1} decreased by 33.32% at M_1 (D_1 M_1) in comparison with D_1 drought treatment alone (D_1 M_0) . Whereas, under D_2 treated condition, a decrease of 22.91% in the number of unfilled spikelets panicle^{-1} was observed at M_1 treatment (D_2 M_1) compared to D_2 M_0 treatment where no melatonin was added.

Table 11. Interaction effect of different drought periods and melatonin levels on the number of filled and unfilled spikelets panicle^{-1} of rice

Treatments	**No. of Filled Spikelets Panicle^{-1}**	**No. of Unfilled Spikelets Panicle^{-1}**
D_0M_0	121.47 b	9.60 e
D_0M_1	132.10 a	9.20 e
D_1M_0	78.27 d	19.90 c
D_1M_1	95.70 c	13.27 d
D_2M_0	65.60 e	27.93 a
D_2M_1	76.43 d	21.53 b
LSD(0.05)	**3.70**	**1.11**
CV (%)	**2.14**	**3.62**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

4.1.12 1000 Grain Weight (g)

To study the effect of drought stress on rice yield, the average weight of 1000 grains grown under control and, two different drought levels were measured. 1000 grain weight significantly ($P \leq 0.01$) decreased with the increase of drought levels (Figure 23 and Appendix XVII). A gradual decrease in 1000 grain weight was observed with the increase in drought concentration and it was 34.26% and 47.90% with the treatment D_1 and D_2 respectively compared to non-drought stress control.

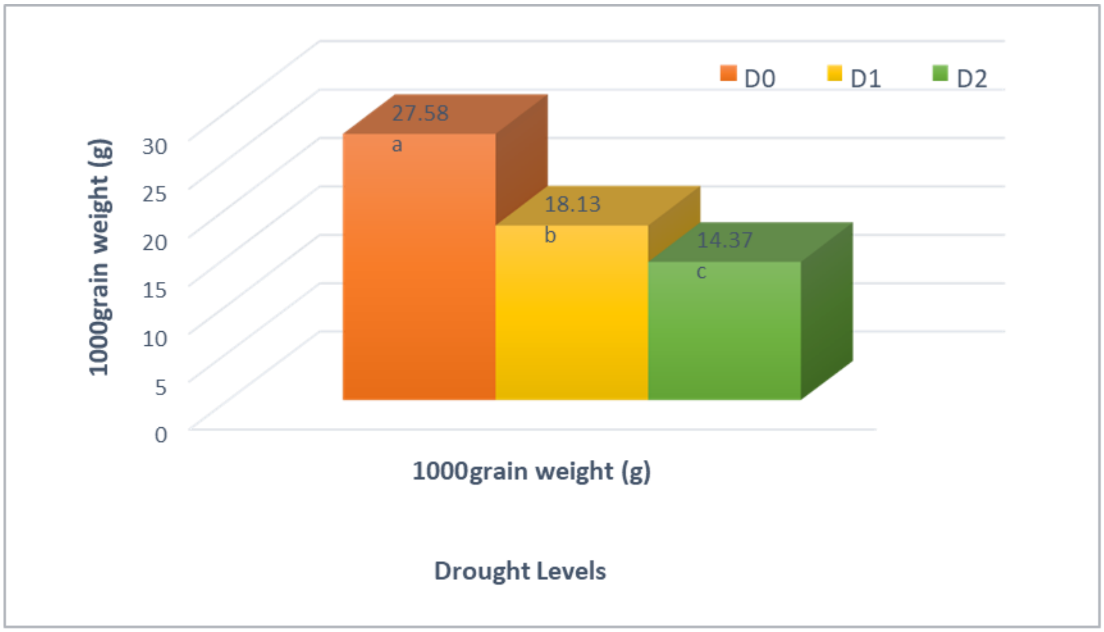

Figure 23. Effect of different drought periods on the 1000 grain weight of rice (LSD (0.05) = 1.18 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

Regarding the effect of melatonin supplementation, it was obvious that melatonin supplementation improved 1000 grain weight significantly ($P \leq 0.01$) and this increase was 18.97% for M_1 compared to control (Figure 24 and Appendix XVII).

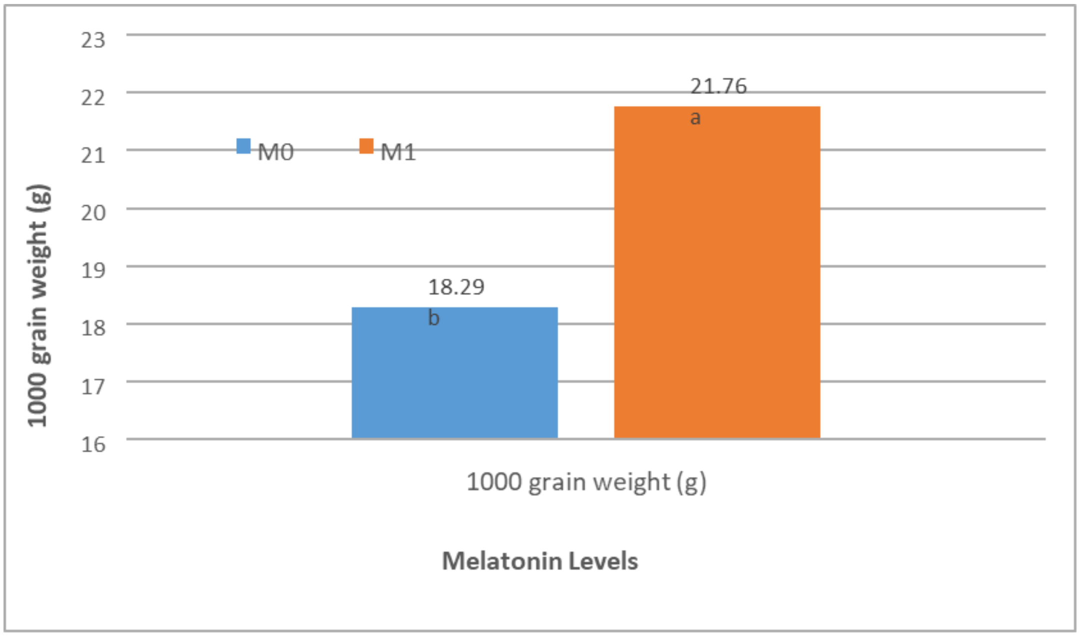

Figure 24. Effect of different melatonin levels on the 1000 grain weight of rice (LSD $_{(0.05)}$ = 0.97 and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin , M_1= 100 µM Melatonin)

1000 grain weight significantly ($P \leq 0.05$) responded to melatonin levels under drought stress to increase the above-mentioned trait. A statistically significant Drought × Melatonin interaction supported this finding (Table 12 and Appendix XVII). 1000 grain weight was increased by 26.77% after M_1 supplementation of drought-stressed (D_1M_1) plants when compared to drought treatment (D_1M_0) alone. Whereas, M_1 supplementation during drought stress (D_2) increased the 1000 grain weight by 29.29% when compared to drought treatment (D_2M_0) alone.

4.1.13 Grain Yield Plant^{-1}

The ultimate desirable product of yield components of rice is grain yield. Drought stress led to significant ($P \leq 0.01$) reduction in the grain yield plant^{-1} with the most drastic reduction being observed at D_2 treatment (Figure 25 and Appendix XVII). The loss of grain yield due to D_1 and D_2 level of drought was 26.47% and 39.90% respectively over non-drought stressed control.

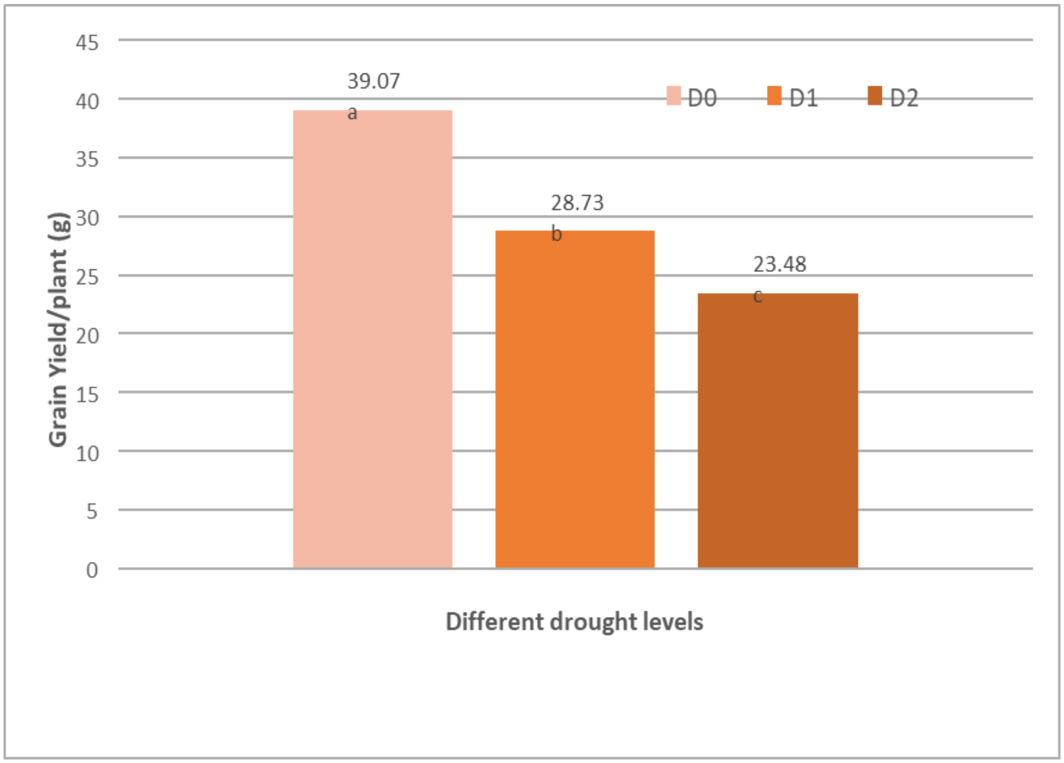

Figure 25. Effect of different drought periods on the grain yield plant^{-1} of rice (LSD $_{(0.05)}$ = 0.72 for grain yield and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (D_0=No drought, D_1= withholding of water for 10 days , D_2= withholding of water for 20 days)

The impact of melatonin application on rice grain yield revealed that melatonin was found to be more fruitful in improving grain yield of rice under drought stress. The melatonin levels had significant ($P \leq 0.01$) effect on rice grain yield (Figure 26 and Appendix XVII). The grain yield increased by 19.08% at M_1 level of melatonin compared to control.

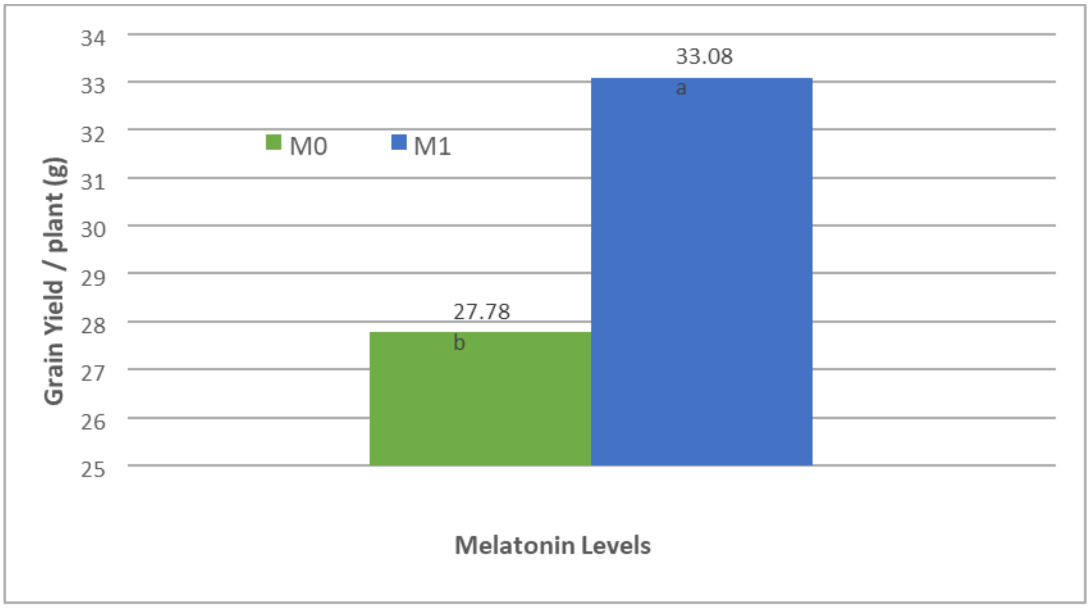

Figure 26. Effect of different melatonin levels on the grain yield plant^{-1} of rice (LSD $_{(0.05)}$ = 0.59 for grain yield and bars with different letters are significantly different at $p \leq 0.05$ applying LSD) (M_0=0 µM Melatonin, M_1= 100 µM Melatonin)

Drought reduced the grain yield of rice. By exogenous application of melatonin, the production of grains was increased under drought condition (Plate XVII). It was shown by statistically significant Drought × Melatonin interaction (Table 12 and Appendix XVII). Percent increase of grain yield in the treatment D_1M_1 was 29.79%, indicating a remarkable difference with D_1M_0 where no melatonin was applied. Whereas, it was 28.07% increase with D_2M_1 when compared with D_2M_0 with drought treatment only.

Table 12. Interaction effect of different drought periods and melatonin levels on the 1000 grain weight, grain yield plant^{-1} of rice

Treatments	**1000 grain weight (g)**	**Grain Yield Plant^{-1} (g)**
D_0M_0	26.33 b	37.73 b
D_0M_1	28.82 a	40.41 a
D_1M_0	15.99 d	25.01 e
D_1M_1	20.27 c	32.46 c
D_2M_0	12.53 e	20.59 f
D_2M_1	16.20 d	26.37 d

LSD(0.05)	**1.68**	**1.02**
CV(%)	**4.60**	**1.84**

Values in a column with different letters are significantly different at $p \leq 0.05$ applying LSD.

In rice plants, drought increased non-effective tiller plant^{-1} and non-filled grain panicle^{-1} in rice plant. Panicle length, effective tiller plant^{-1}, filled grain panicle^{-1}, 1000-grain weight, grain yield plant^{-1} decreased under drought stress. Positive effect was observed in rice plant underin case of different yield contributing characters and yield. In another study, aromatic rice was examined for its tolerance to combined lead-cadmium stress. Productive tillers, grains per panicle, and 1000-grain weights and yield were decreased by that stress. Different doses of melatonin were applied either spraying or mixing with irrigation water. Melatonin increased productive tillers, grains per panicle, and 1000grain weights and yield of rice under that combined stress (Jiang et al. 2022). Melatonin biosynthesis-related genes have various biological functions. Roles of a caffeic acid Omethyltransferase (OsCOMT) gene in mediating rice grain yield through dual regulation of leaf senescence and vascular development. Transgenic assays suggested that OsCOMT significantly delays leaf senescence at the grain filling stage by inhibiting degradation of chlorophyll and chloroplast, which, in turn, improves photosynthesis efficiency. All of these improved physiological attributes and finally the yield (Jiang et al.
2022).

Drought stress reduces rice yield. In this experiment, the effects of foliar application of melatonin increased photosynthesis, stress resistance, carbon and nitrogen metabolism and yield of rice during seed filling stage under drought stress. The application exogenous melatonin increased the antioxidant enzyme activity, inhibited the production of reactive oxygen species, decreased cell membrane damage under drought stress, alleviated the inhibition of photosynthetic capacity by drought stress, improved the carbon and nitrogen assimilation ability, and alleviated the yield loss caused by drought stress. Compared with drought stress, the treatment of melatonin increased the number of pods per plant, the grain number per plant and the hundred grain weight by 2.9%, 0.8%, and 17.2% on average of two years, respectively, and the yield (grain weight per plant) increased by 14.7% (Huangfu et al. 2022).

The application exogenous melatonin increased the antioxidant enzyme activity, inhibited the production of reactive oxygen species, decreased cell membrane damage under drought stress, alleviated the inhibition of photosynthetic capacity by drought stress, improved the carbon and nitrogen assimilation ability, and alleviated the yield loss caused by drought stress. In rice, Compared with drought stress, the treatment of melatonin increased the number of panicle per plant, the grain number per plant and the hundred grain weight by 2.9%, 0.8%, and 17.2% on average of two years, respectively, and the yield (grain weight per plant) increased by 14.7% (ZOU et al. 2020).

CHAPTER V

Summary and Conclusion

To evaluate the role of melatonin under drought stress, an experiment was carried out at the net house of the Department of Agricultural Botany and Plant Physiology Laboratory of Shere-Bangla Agricultural University, Dhaka, Bangladesh in *Boro* season during the period from December 2020 to May 2021.

The experiment was carried out to assess the role of melatonin in improving morphological, physiological and yield performance of rice plant (*Oryza sativa* L. cv. BRRI dhan29) under drought stress. The experiment was laid out in Randomized Complete Block Design (RCBD). Rice was grown with 0 and 100 µM melatonin alone or in combination with drought. The control plant was watered as per requirement while the others were subjected to drought stress by withholding water. Treatments were applied 15 days after transplanting. The treatments were: Control (C), 100 µM melatonin (M), withholding of water for 10 days (D1), D1+M, withholding of water for 20 days (D2) and D2+M.

Then data was recorded on plant height, no. of tillers plant^{-1}, leaf area, leaf membrane stability index (MSI%), relative water content (RWC%), chlorophyll content (SPAD value), proline content, dry weights of root, shoot, leaf and total dry matter (TDM) plant^{1}, panicle length, no. of spikelets panicle^{-1}, 1000 grain weight, grain yield plant^{-1}. The collected data were statistically analyzed for evaluation of the treatment effect and a significant variation among the treatments was found while different drought levels and melatonin levels were applied in different combinations.

Because of water deficit the RWC of rice plants decreased in plants. The proline accumulation was noticed to combat the internal water shortage to some extent. Melatonin application increased the proline further and the RWC was increased. Drought decreased the SPAD value of rice plant indicating the destruction of chlorophyll. The phenotypic appearance also proved the reduction of greenness and yellowing of rice leaves under drought stress. Observing the growth parameters, it is clear drought stress exerted adverse effects on growth parameters during different growth stages. As a result of altered physiological and biochemical processes under drought stress the yield attributes of rice plants showed reduction and the yield also decreased noticeably under drought stress. Drought stress caused about 20.80% and 31.60% decrease in the length of panicle, No. of Filled Spikelets Panicle^{-1} decreased by 36% and 46%, 1000 grain weight decreased by 39 and 52%, Grain Yield Plant^{-1} decreased by 33 and 45%, compared to drought stress alone. When melatonin was supplemented with drought treatments length of panicle, No. of Filled Spikelets Panicle^{-1}, 1000 grain weight and Grain Yield Plant^{-1} increased, compared to drought stress alone.

The result of the experiment revealed that almost all the morphological, physiological and yield contributing characters were decreased significantly except no. of noneffective tillers, no. of unfilled spikelets panicle^{-1} and proline content due to imposition of drought. Plants grown in control condition (without drought) performed best in recording the morphological, physiological and yield contributing characters of rice. In fact, there was a gradual decrease of all the parameters with the increase of drought stress.

Further studies are required to understand the metabolic and biochemical role of melatonin in improving morphological, physiological and yield performance of rice under drought stress. Different other combinations of drought and melatonin can be considered for further study. Targeting different growth stage of rice plant the different treatment combinations can be applied to know the melatonin-induced response of rice plant under drought stress condition.

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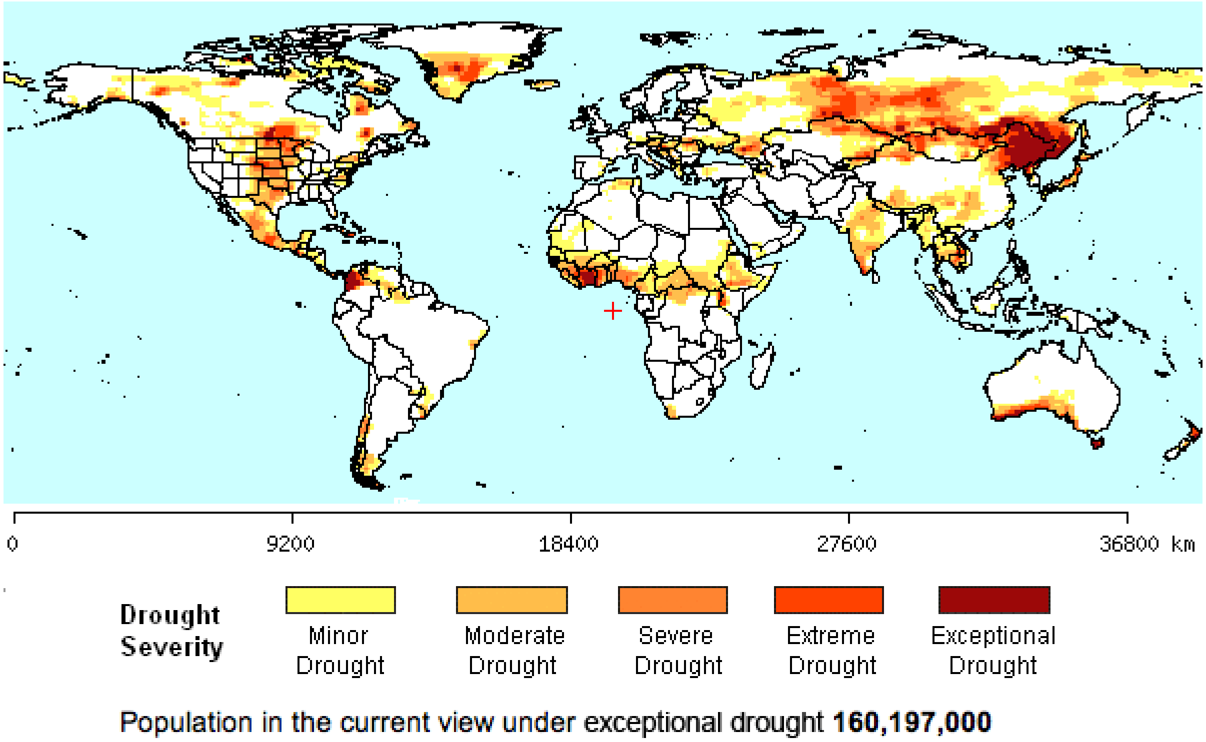
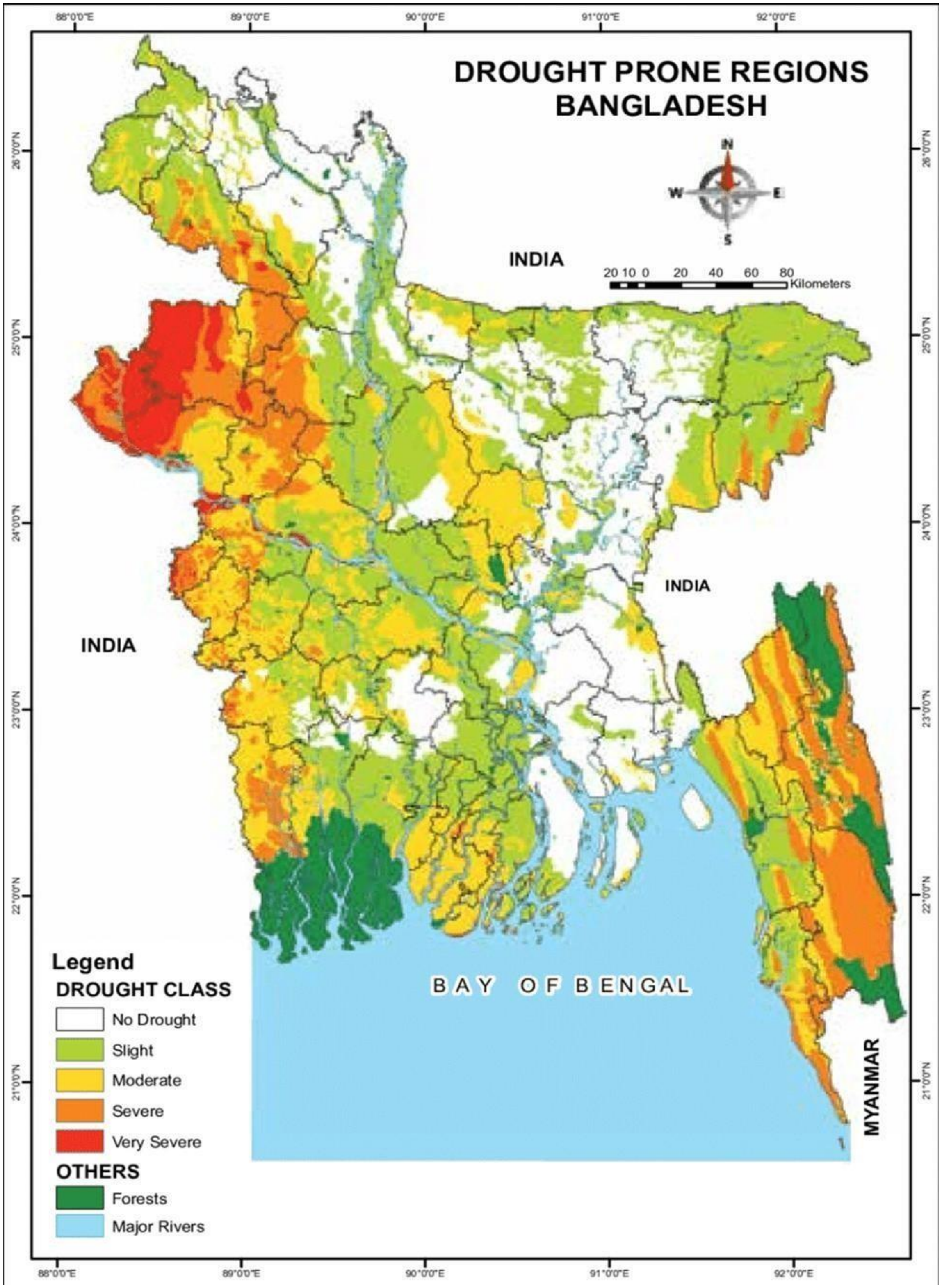
APPENDICES

Appendix I. Global extents and distributions of drought affected soils



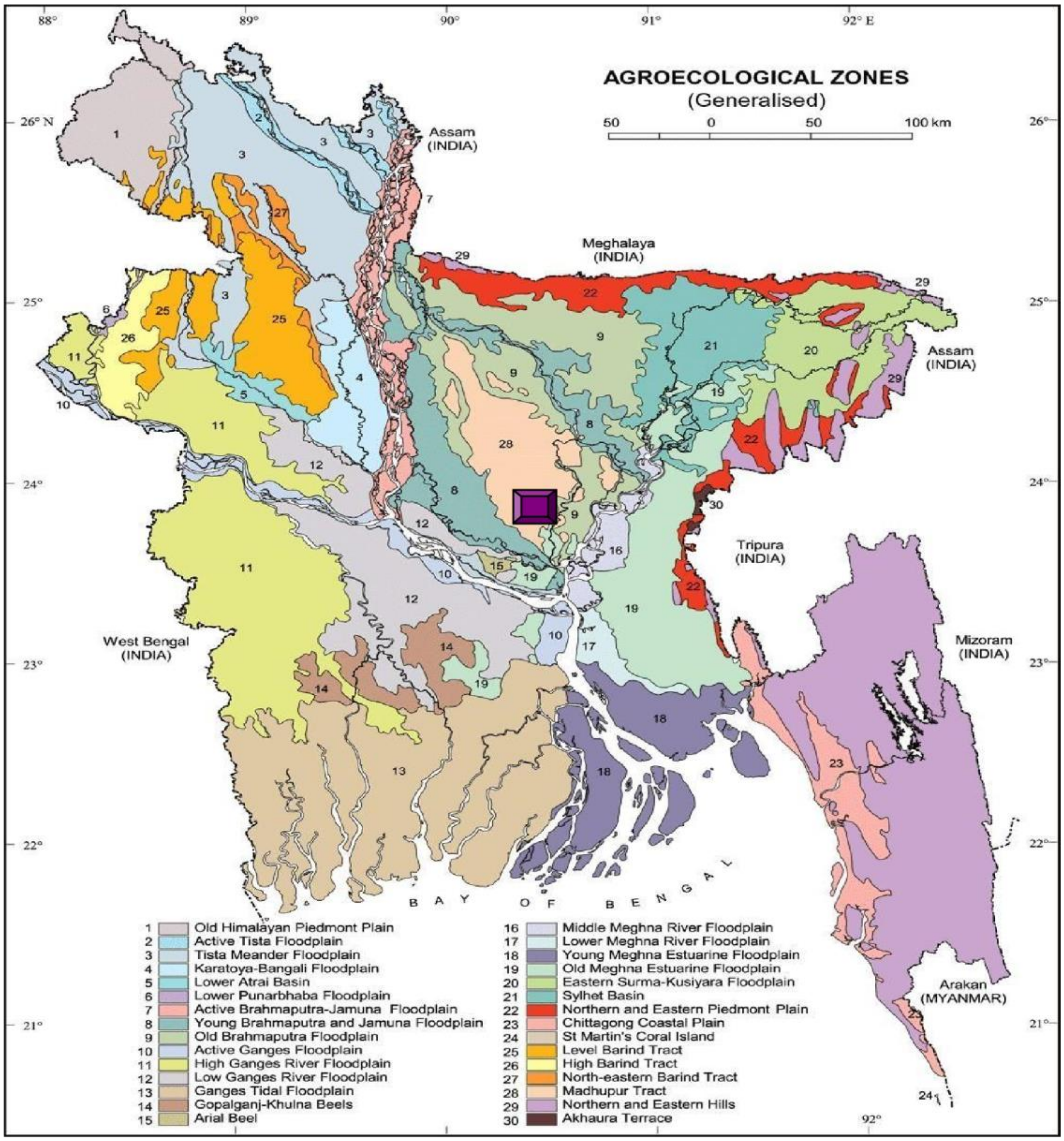
Source: Wicke *et al*., (2011)

Appendix II. Drought prone areas of Bangladesh map, 2006



(Source : BARC ,2006)

Appendix III. Experimental location on the map of Agro-Ecological Zones of Bangladesh



🟪 The experimental site under study

Appendix IV. Morphological Characteristics of the Experimental Field

Morphology	**Characteristics**
Location	SAU Farm, Dhaka
Agroecological zone	Madhupur Tract (AEZ- 28)
General Soil Type	Deep Red Brown Terrace Soil
Parent material	Madhupur clay
Topography	Fairly level
Drainage	Well drained
Flood level	Above flood level
Soil series	Tejgaon

(SAU Farm, Dhaka)

Appendix V. Physical and Chemical properties of the initial soil sample

Characteristics	**Value**
Particle size analysis	
% Sand (2.0-0.02 mm)	22.53
% Silt (0.02-0.002 mm)	56.72
% Clay (<0.002 mm)	20.75
Textural class	Silt Loam
pH (1: 2.5 soil- water)	5.6
Bulk Density (g/cc)	1.45
Particle Density (g/cc)	2.52
Organic carbon (%)	0.47
Organic matter (%)	0.81
Total N (%)	0.05
Available P (ppm)	18.1
Available K (meq/100g soil)	0.10
Available S (ppm)	2.006

(SAU Farm, Dhaka)

Appendix VI. Maximum and minimum monthly temperature (^{o}C), relative humidity and rainfall during December, 2020 to May, 2021 at the farm of SAU

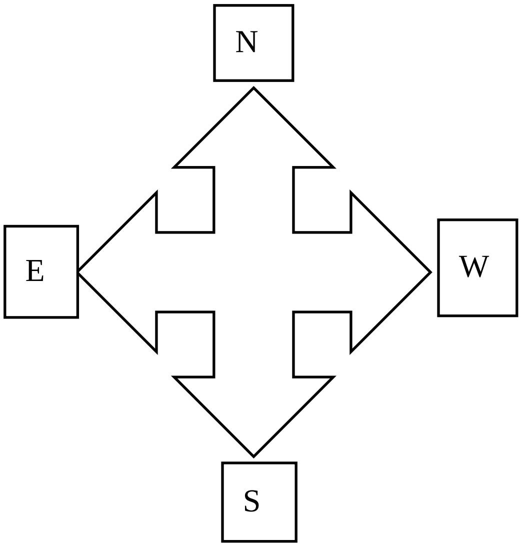
Name of the Months	**Average air temperature (^{O}C)**		**Relative Humidity (%)**	**Rainfall (mm)**
	Maximum	**Minimum**		
December, 2020	31	18	63	1.9
January, 2021	28	16	61	3.5
February, 2021	27	13	57	12.3
March, 2021	34	15	57	8.1
April, 2021	34	16	57	73.4
May , 2021	35	20	66	178.5

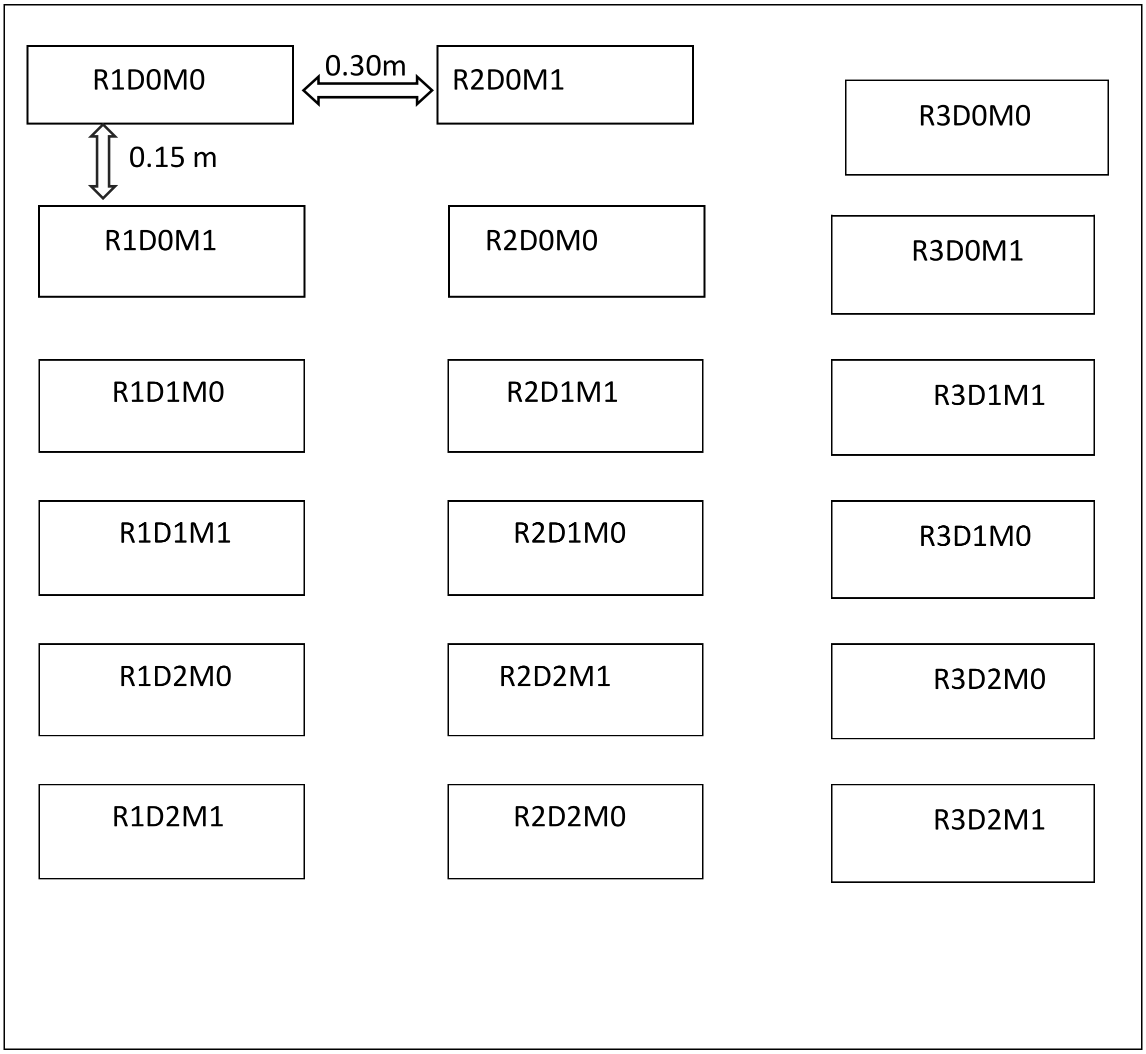
(Weather station, Sher-e-Bangla Agricultural University, Dhaka-1207)

Appendix VII . Layout of the experiment

Pot Size : 30 cm × 35 cm

Pot to pot distance : 0.15 m
Block to block distance : 0.30 m





Appendix VIII. Analysis of variance of the data on plant height as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	df	Mean square of plant height at different days after transplanting (DAT)			
		30	60	90	At Harvest
Replication	2	115.72	45.22	60.05	46.82
Drought periods	2	452.29^{**}	544.06^{**}	609.52^{**}	555.29^{**}
Melatonin Levels	1	116.03^{**}	111.01^{**}	121.68^{**}	168.67^{**}
Drought periods X Melatonin Levels	2	3.58 NS	3.42^{*}	6.80^{NS}	4.22 NS
Error	10	0.94	0.73	3.13	8.85

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix IX Analysis of variance of the data on number of tillers plant^{-1} at harvest as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	df	Mean square of number of
		Tillers Plant^{-1} at harvest
Replication	2	28.23
Drought periods	2	23.61^{**}
Melatonin Levels	1	12.50^{**}
Drought periods X Melatonin Levels	2	1.27 NS

Error	10	0.32

**, indicates significant at 1% level of probability
*, indicates significant at 5% level of probability ^{NS}, indicates Non significant

Appendix X. Analysis of variance of the data on leaf area, membrane stability index (MSI%) and relative water content (RWC%) as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	**df**	**Mean square of**		
		Leaf Area	**Membrane stability index (MSI%)**	**Relative water content (RWC%)**
Replication	2	270.67	56.20	9.78
Drought periods	2	1273.22**	706.23**	442.88**
Melatonin Levels	1	196.96**	148.92**	82.79**
Drought periods X Melatonin Levels	2	1.50 NS	12.85**	7.73 NS
Error	10	3.90	7.43	4.72

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XI. Analysis of variance of the data on chlorophyll content (SPAD value) as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	**df**	**Mean square of chlorophyll content (SPAD value) at different days after transplanting (DAT)**	
		60	**90**
Replication	2	53.38	6.00
Drought periods	2	166.47**	118.76**
Melatonin Levels	1	45.76**	52.02**
Drought periods X Melatonin Levels	2	2.39 NS	4.31 NS
Error	10	1.34	3.95

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XII. Analysis of variance of the data on proline content as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	**df**	**Mean square of proline content**
Replication	2	0.54
Drought periods	2	15.28**
Melatonin Levels	1	3.42**
Drought periods X Melatonin Levels	2	0.42 ^{NS}
Error	10	0.23

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XIII. Analysis of variance of the data on dry weights of root, shoot, leaf and total dry matter (TDM) as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	df	Mean square of dry weights of			
		Root	Shoot	Leaf	TDM
Replication	2	2.96	1.35	17.99	24.38
Drought periods	2	4.05**	18.90**	51.23**	182.06**
Melatonin Levels	1	2.49**	11.99**	17.41**	84.89**
Drought periods X Melatonin Levels	2	0.02 NS	0.08 NS	2.11 NS	3.48 NS
Error	10	0.01	0.17	1.19	1.98

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XIV. Analysis of variance of the data on panicle length as influenced by combined effect

Source of Variation	df	Mean square of Panicle Length
Replication	2	82.82
Drought periods	2	217.67**
Melatonin Levels	1	27.63**
Drought periods X Melatonin Levels	2	3.17 ^{NS}
Error	10	1.65

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XV. Analysis of variance of the data on number of effective and noneffective

tillers plant^{-1} as influenced by combined effect of drought periods and melatonin levels of

rice

Source of Variation	df	Mean square of number of	
		Effective Tillers Plant^{-1}	**Non-Effective Tillers Plant^{-1}**
Replication	2	21.89	1.98
Drought periods	2	158.11**	5.26**
Melatonin Levels	1	26.40**	1.81**
Drought periods X Melatonin Levels	2	0.79 NS	0.55**
Error	10	0.68	0.02

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XVI. Analysis of variance of the data on no. of filled and unfilled spikelets panicle^{-1} as influenced by combined effect of drought periods and melatonin levels of rice

Source of Variation	df	Mean square of number of	
		Filled Spikelets Panicle^{-1}	**Unfilled Spikelets Panicle^{-1}**
Replication	2	299.67	79.83
Drought periods	2	4948.90**	353.13**
Melatonin Levels	1	756.60**	90.23**
Drought periods X Melatonin Levels	2	22.46*	18.73**

Error	10	4.14	0.37

**, indicates significant at 1% level of probability

*, indicates significant at 5% level of probability

^{NS}, indicates Non significant

Appendix XVII. Analysis of variance of the data on 1000 grain weight, grain yield plant^{1} as influenced by combined effect drought levels and melatonin levels of rice

Source of Variation	**df**	**Mean Square of**	
		1000 Grain Weight	**Grain Yield/Plant**
Replication	2	7.24	28.89
Drought periods	2	277.96**	377.56**
Melatonin Levels	1	54.32**	126.46**
Drought periods X Melatonin Levels	2	1.24 ^{NS}	8.82**
Error	10	0.85	0.31

, *, indicates significant at 5% level of probability

^{NS}, indicates Non significant

** , indicate s significant at 1% level of probability

PLATES

Plate I . Seedbed Preparation

Plate II . Seed Sowing

Plate III . Netting On Seedbed

Plate IV . Uprooting of seedling from seedbed

Plate Ⅴ. Transplanting of seedling in the experimental pot

Plate VI . Different Treatment Effects

Plate VII . Leaf Collection

Plate VIII. Boiling of the plant samples in water bath for MSI% measurement