DIFFERENTIAL AND DYNAMIC RESPONSE OF RICE VARIETY UMA UNDER VARYING DROUGHT STRESS REGIMES

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By

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THESIS

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KERALA, INDIA
2021

DECLARATION

I hereby declare that the thesis entitled **"Differential and dynamic response of rice variety Uma under varying drought stress regimes"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: 13/12/2021

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CERTIFICATE

Certified that the thesis entitled **"Differential and dynamic response of rice variety Uma under varying drought stress regimes"** is a bonafide record of research work done independently by **Ms. Akshaya Ramakrishnan (2019-11-250)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree,diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Akshaya Ramakrishnan (2019-11-250)**, a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Physiology**, agree that the thesis entitled **"Differential and dynamic response of rice variety Uma under varying drought stress regimes"** may be submitted by **Ms. Akshaya Ramakrishnan** in partial fulfillment of the requirement for the degree.

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Akshaya Ramakrishnan

DEDICATED
TO
MY FAMILY
MY ADVISOR
TEACHERS
FRIENDS
FARMERS

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per cent	Per cent
CD	Critical difference
Cm	Centimeter
et al.	Co-authors/Co-workers
Fig.	Figure
Mm	Milli Molar
M	Molar
G	Gram
H	Hour
Fr. Wt	Fresh weight
i.e.	That is
μ	Micro
CRD	Completely randomized design

INTRODUCTION

1. INTRODUCTION

Rice, one of the world's most important crops, has the evolutionary advantage of being semi-aquatic. As a result, it has minimal adaptations to water scarcity and is particularly vulnerable to drought stress. Nowadays, rice production is being threatened by climate change. Rice production is reduced as air temperature rises as a result of climate change, according to recent studies. Rice output is reduced as the rainfall pattern changes when drought frequency and intensity increases. Drought stress in South and Southeast Asia can result in a 40% reduction in yearly productivity of the crop (Li *et al.,* 2015). India and surrounding regions of Nepal are one of the world's greatest drought-prone locations among the different rainfed rice-growing ecologies. In India, there are 20.4 million hectares of rainfed rice growing areas. Droughts in India in 2002, 2003, 2009, and 2010 resulted in severe reductions in rice production, especially in eastern Indian states. Similarly, the severe drought that ravaged areas of eastern Uttar Pradesh and Bihar in 2015 wreaked havoc on the rice harvest (Dar *et al.,* 2020).

The main staple crop cultivated in the fields of Kerala is rice, with about 600 varieties adopted across the paddy fields of the state. Uma (MO-16) developed by the Rice Research Station, Moncompu of Kerala Agricultural University, is one of the most popular rice variety in Kerala. More than 60% of the state's rice land is presently devoted to this variety. It is a non-lodging cultivar with a medium growing season (115-120 days) and yields around 8 to 9 tonnes of paddy per hectare. Uma is suitable for Kole, Kuttanad and Kari soils and also suitable for all three seasons, demonstrating its adaptability to a broad range of environmental circumstances. Environmental factors, on the other hand, restrict the actual yield of Uma. Besides direct drought conditions in the field, huge floods can also influence the water availability in the field during the summer, due to soil piping mechanism effected by torrential rain water flow through enlarged micro or macro soil pipes which can lead to an uncontrolled subsurface water flow into streams or rivers. As a result, rice tracts that would probably face a rainless period followed by the flood become drought prone and this could fetch the state to the threat of severe drought. This highlights the need towards the development of varieties having the capability to withstand varying intensities and duration of drought stress conditions. The requirement to study the drought response of variety Uma is due to its wider adoption across the state. With this outlook, the proposed study focuses to identify the potential traits of Uma to combat drought stress conditions and also exemplify the traits that are missing in Uma, for varietal improvement. The novelty of the study is based on the fact that it envisages to unravel the differential response of variety Uma to a series of stress induction patterns across generations. The morphological, physiological and biochemical responses, metabolite profiling and seed parameters envisaged in the study will help to assess the stress damage as well as the tolerance mechanisms.

In this context, the present study on differential and dynamic response of Uma variety under drought stress at vegetative stage and its impact on stress responses in subsequent generation are attempted with the main objective of identifying potential drought stress specific biomarkers representing a trend in metabolite dynamics across generations. This approach is aimed to demonstrate in a simple way that a natural drought stress exposure can influence stress responses of rice variety, Uma in the present as well as future generations. This can ultimately lead to the identification and selection of potential traits for inclusion in crop improvement programmes.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice is one of the most profitable crops with a long history of cultivation, and it is today regarded an important food product as well. Rice seed and products account for over 40% of the food consumed by half of the world's population. Rice plantation is Asia's oldest kind of agriculture, providing more than 80% of the calories and 75% of the protein consumed by people (Sabetfar *et al.,* 2013).

Drought reduces worldwide rice output by 18 million tonnes each year on average. As a result, this abiotic stress is a key limitation in rice production in water-stressed areas. Drought-prone rice fields are projected to cover 23 million hectares in Asia alone. Drought is a major production restriction in eastern India, which has more than 10 million hectares of drought-prone highland and lowland fields. Drought-related yield losses are estimated to cost an average of $250 million per year (Bernier *et al.,* 2008). Drought stress, which is linked to phenological processes, decreases yield potential not only because of a shortage of water, but also because of the timing and length of drought stress (Bernier *et al.,* 2008). Such specific impacts of drought stress in the morphological, physiological, biochemical, yield attributes and seed parameters of rice plants as well as the glimpses of stress memory responses are discussed under this chapter.

2.1 Effect of drought stress on morphological and physiological responses in rice

Drought stress is defined as moderate loss of water that causes stomatal closure and gas exchange limitations. Desiccation is a much larger loss of water that can cause major disruptions in metabolism and cell structure, as well as the eventual cessation of enzyme-catalyzed reactions which leads to the physiological as well as morphological reactions in plants (Jaleel *et al.,* 2007).

2.1.1 Plant height

According to Singh *et al.* (2018) regardless of the rice genotype, drought stress lowered plant height. Due to a shortage of water, drought stress decreased metabolic activity. As a result of the reduced turgor pressure, the plant's cell division and cell elongation activities were affected, and the plant height was reduced. As reported by

Farooq *et al.* (2010), changes in leaf development and hydration status were examined among the indica rice cultivar IR64 and four near-isogenic lines that were distinct for various characteristics. Two soil water regimes were used on the plants: well-watered and progressive soil drying as indicated by the fraction of transpirable soil water (FTSW). Drought significantly decreased plant height in stressed treatments compared to well-watered control. Plant height rose steadily for IR64 until day 6 when drought was imposed, however it continued to rise under well-watered circumstances.

A greenhouse experiment was conducted to evaluate performance of 15 rice cultivars grown in Mississippi, USA. Studies on twenty morpho-physiological parameters of rice seedlings subjected to soil moisture stress showed that the rate of cell expansion determined the rice cultivars' shoot growth (plant height and leaf area). Plants at 66 per cent field capacity and 33 per cent field capacity were shown to be substantially shorter than plants at 100 per cent field capacity in this study. Ultimately, results showed that the total drought response index (TDRI) could be used as an index for easy screening of drought tolerant varieties (Singh *et al.,* 2017).

2.1.2 Number of tillers

Bunnag and Pongthai (2013) stated that water is the most essential compound for plant development and growth; they have studied drought effects on several drought-tolerant rice cultivars in the vegetative stage from the field circumstances. Results showed that drought stress had a direct effect on rice plant growth, reducing plant height and the number of tillers per plant because plants were unable to absorb soil water when it became scarce, resulting in a reduction in the vital nutrients accessible to the plants.

2.1.3 Relative water content

Water use efficiency (WUE) is a crucial component determining plant productivity under drought conditions, and it is crucial to increase crop performance under water limited settings. WUE has substantial positive associations with agronomic measures like relative water content (RWC) and stomatal conductance.

Thus, the focus of the study conducted by Pandey and Shukla (2015) was mostly on new information about the impacts of drought on rice, rice responses and drought stress adaptation mechanisms by assessing RWC. Hence the ability to maintain these measures for an extended amount of time during a drought encounter is a desirable trait. Thripathy *et al.* (2000) demonstrated that visual grading of leaf wilting and measuring leaf RWC can be used to determine the severity of stress on a daily basis. Leaf samples were taken from both control (well-watered) and stressed plants (at 60–65 per cent RWC), and the results were such that stressed plants maintained lesser RWC compared to well-watered plants.

Studies conducted at KAU indicated that out of the 42 varieties screened, Swarnaprabha and Kattamodan had high recovery from drought stress as well as higher relative water content (RWC), membrane stability index (MSI), osmolyte accumulation and higher antioxidant properties. The infliction of stress resulted in a steady decrease in RWC. The tolerant check Sahbhagi Dhan had the highest RWC in the vegetative stage under drought stress, followed by Neeraja, Swarnaprabha, Kattamodan, and Onam. Under drought stress, cultivars with a higher RWC are more likely to maintain protoplast hydration for a longer period of time, ensuring productivity (Swapna and Shylaraj, 2017).

2.1.4 Membrane thermostability

Thripathy *et al.* (2000) stated that the most important selection criteria for drought resistance in cereals were cell-membrane stability (CMS). The genomic region responsible for cell membrane stability was identified by examining 104 rice (*Oryza sativa* L.*)* doubled haploid (DH) lines generated from a cross between CT9993–5-10–1-M and IR62266-42–6-2 in a greenhouse, with a progressively developing drought stress environment by withholding water in 50-day-old plants. Later, they identified that cell membrane stability was mainly dependent on the genotypic nature of the plant. The discovery of QTLs (quantitative trait locus) for this essential characteristic will aid in the development of drought tolerant rice varieties.

2.1.5 Chlorophyll content

In the study conducted by Akram *et al.* (2013), panicle initiation stage was discovered to be the most important factor in influencing many physiological characteristics. The metabolic imbalance formed as the result of water stress at the time of panicle onset, dramatically lowered physiological processes leading to impaired enzyme activity and stomatal conductivity. Chlorophyll pigments have deteriorated, resulting in a significant reduction in PAR, photosynthetic rate, transpiration rate, and other parameters like relative water content. According to Pandey and Shukla (2015), chlorophyll is a key pigment in the photosynthetic machinery that absorbs light and delivers it to the photosystem's reaction centre. Soil dryness is a problem for reduction in chlorophyll a and b. Carotenoids, on the other hand, play additional roles in aid chloroplast photosystem structure in processes like light harvesting and photoprotection. Stress-induced disablement in pigment synthesis pathways or pigment degradation, cause loss of the chloroplast membrane and enhanced lipid peroxidation which may all contribute to the decrease in chlorophyll content.

To screen and analyse the physiological foundation of heat and drought resistance in three rice genotypes viz. ADT 43, TKM 9 and N22, Kumar *et al.* (2015) conducted a study on the effects of combined heat and drought stresses on chlorophyll stability index, membrane thermal stability, and abscisic acid (ABA) levels. Stressed applied during the panicle initiation (PI) and anthesis stages of crop growth showed that genotype N22 was determined to be the tolerant genotype, followed by TKM 9, and ADT 43 was found to be the sensitive genotype. Stresses in anthesis stage resulted in a build up of osmolyte, ABA contents and alterations in membrane stability, regardless of genotype. This indicates that these biochemical characteristics can be used to screen rice genotypes for combined stresses and to learn more about the stress tolerance mechanisms.

2.2 Effect of drought stress on biochemical responses in rice

The acclimation and adaptation potential of any plant to unfavourable environmental encounters are determined by metabolic readjustments brought about by activation/deactivation of many biochemical pathways. They are involved in redox homeostasis, osmotic adjustment, protein homeostasis, and so on.

2.2.1 Proline

Plants accumulate several forms of organic and inorganic solutes in the cytosol in response to water deficits in order to reduce osmotic potential and preserve cell turgor. Osmotic adjustment is a biochemical mechanism that is highly dependent on the pace of plant water stress. The buildup of osmolytes in the cytoplasm causes osmotic adjustment, which improves water absorption from drying soil.

In plants that are subjected to a variety of stresses, proline plays an important role on plant stress adaptation. Kemble and Mac-Pherson (1954) presented the first report on free proline buildup in rye grasses as a result of water stress. Proline functions as an osmolyte, and its buildup aids performance and drought resistance. Rice that has been subjected to drought stress has shown changes in proline content. Proline serves as a metal chelator, an antioxidative defence molecule, and a signalling molecule during stress, in addition to being a good osmolyte. As a result, proline content can be utilised as a screening factor for drought-tolerant rice varieties (Pandey and Shukla, 2015). Chutia and Borah (2012) conducted a study on genotypes of the rice from Assam wherein seven varieties of Assam were evaluated. Many of them spontaneously accumulated proline as a significant organic osmolyte. In stressed plants, proline is considered to have an adaptive function in regulating osmotic adjustment and safeguarding subcellular structures.

2.2.2 Malondialdehyde (MDA)

Drought stress promotes the production of malondialdehyde (MDA), which is an indicator of oxidative stress and serves as an index of lipid peroxidation. Damage to the plasma membrane by peroxide causes release of contents, rapid desiccation, and cell death (Shukla *et al.,* 2012). Under drought stress, the photosynthetic light curve, malondialdehyde (MDA) concentration, and reactive oxygen metabolism of two autotetraploid rice lines and their equivalent diploid rice lines revealed that net photosynthetic rate fell substantially, especially under severe drought stress and strong photosynthetic active radiation, but it decreased less in autotetraploid lines under the same conditions. When compared to their diploid counterparts, the autotetraploid rice had a reduced MDA concentration and a lower rate of superoxide anion generation, indicating that cell membranes were less peroxidized (Yang *et al.,* 2014)

In vitro drought stress of -0.5 and -2.0 MPa was imposed on 10 to 20 day old rice seedlings for 24 h and it was observed that activities of total superoxide dismutases (SODs) as well as ascorbate peroxidase (APX) showed consistent increase with increasing levels of drought stress; however catalase activity declined. The results suggested that drought stress induced oxidative stress in rice plants function as an important component of antioxidative defense system (Sharma and Dubey, 2005).

2.2.3 Protein

A study conducted by Muthurajan *et al.* (2010) revealed the role of proteins during the panicle formation stage of rice. The peduncle (uppermost internode) elongation driven panicle exsertion is an important physiological mechanism for high grain output in rice. The peduncle elongation to its full potential was restricted even after re-watering, following a drought stress exposure at heading/panicle emergence. Drought caused delayed heading and entrapment of spikelets lower down the panicle within the flag-leaf sheath, increasing sterility in the lower un-emerged spikelets as well as the upper superior spikelets whose exsertion was delayed. Intermittent drought stress reduced relative water content (RWC) and increased abscisic acid (ABA) levels in the peduncles, but both recovered to normal levels after re-watering. Examination of proteins from peduncles collected under well-watered, drought-stressed, and re-watered plants revealed at least two-fold differential expressions of 31 proteins in response to drought, with a majority of these alterations being fully reversed by re-watering.

In another study, a pot experiment was done to see if foliar sprayed proline might help rice to cope with the simultaneous impacts of heat and drought stress. Total soluble protein (TSP), glycine betaine (GB), and leaf proline levels were all reduced under a stressful setting, whereas malondialdehyde (MDA) levels increased. In comparison to control, simultaneous heat and drought stressors resulted in the greatest decrease in osmoprotectants and an increase in MDA levels. The use of proline significantly increased TSP levels (Hanif *et al.,* 2021)

According to Sharma and Dubey (2005), 10 to 20 days old rice seedlings subjected to *in vitro* drought stress, exhibited an increasing trend in concentration of super oxide ions and lipid peroxidation products in stressed samples compared to control. Whereas, the protein and thiol concentrations were less in stressed tissues compared to control. Observations indicated that drought stress caused oxidative stress in plant parts and antioxidant defense mechanism has a crucial role in free radical scavenging mechanism, which aids in limiting the stress damage.

2.2.4 Metabolite profiling using GC-MS (Gas Chromatography–Mass Spectrometry)

In the study conducted by Shu *et al.* (2011) the response of rice seedlings to drought induced stress was studied using proteome analysis. Several proteins were involved in glucose metabolism, lipid metabolism *etc*., each with different expression patterns. Differentially expressed metabolites were discovered using a cDNA microarray and GC-MS analysis. They were able to deduce the metabolic pathway influenced by drought induced stress, after combining these data with the proteomic analyses. These findings showed that during drought, energy consumption from storage compounds increased. Furthermore, higher expression of anabolic pathway enzymes was associated with an increase in the amount of six amino acids. Energy conversion from carbohydrates or fatty acids to amino acids was thought to have increased. They were able to figure out how rice plants adapt to drought by looking at fundamental metabolic networks.

Melandri *et al.* (2020) investigated central metabolism and oxidative stress status in the flag leaf of 292 indica rice (*Oryza sativa*) accessions using a mix of metabolomics and high-throughput colorimetric tests. Plants were cultivated in the field and subjected to either well-watered or drought conditions during the reproductive stage to determine the metabolic pathways linked to drought-induced grain yield reduction. To examine the variance in polar metabolites under well-watered and drought circumstances, leaf samples from the 292 accessions were analysed using untargeted GC-MS-based metabolite profiling. A total of 88 metabolites were discovered, the majority of which were primary metabolites such as amino acids, sugars, and organic acids. The quantification of each of the three most common sugars (sucrose, fructose, and glucose) was also measured using spectrophotometry.

Jasmonic acid (JA) and salicylic acid (SA) are important signalling molecules that play a role in plant development, growth, and defence. When a plant is subjected to biotic and abiotic stressors, their quantitative levels vary. Quantifying the amounts of JA and SA-mediated signalling and metabolic pathways in plants, tissues, and organs could help to get a better understanding their probable roles. Their extraction and quantification, on the other hand, are not simple and remain technically hard. GC-MS was used to extract and quantify them from rice seedling leaves in a study by Cho *et al.* (2013).

2.3 Effect of drought stress on yield attributes of rice

2.3.1 Spikelet fertility

During flowering, five rice genotypes were subjected to high temperature, water stress, and a combination of high temperature and water stress to see how they responded with respect to spikelet fertility. Microscopic examinations revealed that treatment and genotype variations in anther dehiscence had a strong association with the amount of pollen grains on the stigma. In all five genotypes, high-temperature stress resulted in decreased spikelet sterility that ultimately led to the sterility of the grains (Rang *et al.,* 2011).

2.3.2 Grain yield

Panicle initiation stage was discovered to be the most important growth stage influencing many physiological characteristics, because of the metabolic imbalance formed as the result of water stress at the time of panicle onset (Akram *et al.*, 2013). The most commonly employed parameters are paddy yield, 1000-grain weight (test weight), viable grains per panicle, and grain sterility per panicle for rice breeding programmes to detect water deficit tolerance. Furthermore, it was discovered that water stress at panicle initiation had more influence on plant growth and paddy yield; hence water stress throughout this development period may be prohibited.

Crop yield stability necessitates a reduction in yield losses induced by environmental stressors like drought. An investigation on central metabolism and oxidative stress status in the flag leaf of 292 indica rice (*Oryza sativa*) accessions were done using a mix of metabolomics and high-throughput colorimetric tests. Plants were cultivated in the field and subjected to either well-watered or drought conditions during the reproductive stage to determine the metabolic pathways linked to drought-induced grain yield reduction. The major mechanisms implicated in drought-induced leaf metabolic reprogramming were photorespiration, protein breakdown, and nitrogen recycling. Ultimately, they could find that malondialdehyde, on the other hand, was a reliable biomarker for grain yield reduction, suggesting that under drought stress, lipid peroxidation is the most significant restriction (Melandri *et al.*,2020).

2.3.3 Test weight

Sing *et al.* (2018) conducted an experiment wherein a seven-day drought therapy was administered to different rice genotypes Swarna sub1, Nagina 22, NDR 102, NDR 97 and Shusk Samrat in a pot experiment, at the reproductive stage. The response of rice genotypes revealed substantial genetic variability in test weight of Swarna Sub1 (46%), NDR 102 (20.47%), NDR 97 (17.08%), and modest decrease in Nagina 22 (12.44%). Under water stress, grain size, shape and eventually weight decrease in grains were noted. Water stress decreased the amount of water in the plant, which inhibited the reproductive development and grain growth.

2.4 Effect of drought stress on seed parameters of plants

2.4.1 Speed of germination

The goal of the study of Chao *et al.* (2021) was to assess genotypic variations in germination speed and to discover the factors that influence germination speed. Germination speed was strongly linked with grain moisture content at early stages of soaking in both paddy rice and dehulled brown rice. Germination speed of grains from crops exposed to water shortage in the field was somewhat slower than those with higher water availability.

2.4.2 Seedling vigour index

The primary goal of the study conducted by Batool *et al.* (2014) was to compare the responses of two maize varieties (Islamabad gold and Sawan) to water stress, wherein seedlings were split into two halves after germination, with the first half serving as a control and the second half being exposed to drought in airtight desiccators with a 20 per cent $MgSO_4$ solution. The germination index, seedling vigour index, fresh and dried weights of seedlings all showed a substantial reduction after the drought exposure. In a study conducted by Salam *et al.* (2017), rice varieties/genotypes were screened for drought tolerance and shortlisted based on seedling vigour index, and characterised for drought tolerance using microsatellite markers. Uma was identified as one among the rice varieties maintaining optimum seedling vigour index even under stressed condition.

2.5 Stress memory responses

The stress memory responses have been recently reviewed extensively (Yaish, 2017), wherein it was briefly described that when plants are stressed, they flower earlier than usual to avoid the unfavourable conditions and generate seeds as quickly as possible in order to save the species. The resulting seeds may pass on the stressed plants' accumulated epigenetic information to their progenies. Epigenetics, in its current understanding, refers to heritable changes in gene expression that can result in phenotypic diversity without a change in DNA sequence. As a result, adaptive evolution may emerge as a result of this process. Epigenetic transgenerational memory refers to the transmission of epigenetic information across generations. Heritable epialleles, as well as nucleosome recycling during cell division, could cause such transgenerational epigenetic alterations.

The primed condition in Arabidopsis (*Arabidopsis thaliana*) remained functional in the next generation without any extra treatment, according to the study conducted by Slaughter *et al.* (2012). They compared the reactions of Arabidopsis plants that had been primed with either b-aminobutyric acid (BABA) or a specific bacterial isolate. The progeny of primed plants had a faster and higher accumulation of defense-related gene transcripts in the salicylic acid signalling pathway, as well as improved disease resistance. In comparison to the descendants of unprimed plants, the primed state of plants was passed down to their progeny and gave enhanced protection from pathogen attack.

According to Ling *et al.* (2018), crop productivity is hampered by abiotic and biotic stressors. Plants can withstand subsequent and otherwise lethal conditions after being exposed to a non-lethal stress, known as priming; the priming effect lasts even after a stress-free time. In *Arabidopsis thaliana*, the molecular basis of heat-shock memory and the effect of priming was examined, which showed that priming plants with a non-lethal heat stress led to splicing de-repression after a second heat stress exposure. Non-primed plants, on the other hand, displayed considerable splicing suppression. The ability of plants to endure successive and potentially lethal heat stress was linked to 'splicing memory,' according to these findings.

Plants are constantly subjected to a variety of abiotic and biotic stimuli that affect their ability to grow and reproduce. As a result, they require a variety of sophisticated adaptations to cope with harsh environmental conditions without jeopardising their healthy development, reproductive success or final survival. This necessitates a complex network of transcriptional and post-transcriptional regulators, as well as epigenetic switches, to control gene expression. Plants exposed to various abiotic stressors have shown changes in chromatin modifications such as DNA and histone methylation (Pandey *et al.,* 2016).

Xiao *et al*., (2017) reviewed by citing evidences that exposure to one type of abiotic stress could induce cross tolerance to other types of stresses. However, the underlying physiological and molecular mechanisms of such priming-induced enhancement of stress tolerance in crop plants are yet to be deciphered

Studies conducted in ornamental plant *Silene dioica,* by employing reiterated (i.e, repeated cycles) drought revealed the effects on pigment composition of leaves. The results also highlighted the importance of structural changes in the photosynthetic apparatus in response to reiterated drought; which left a stress imprint that directed plant responses to subsequent stress episodes. The foreseeable effect of the first drought was found to improve the physiological response to reiterated drought (Soriano and Bosch, 2016).

It has been recently demonstrated that stress memory can be developed in rice under water deficit stress. Long noncoding RNA (lnRNA), DNA methylation and endogenous phytohormones (especially abscisic acid) participate in short-term drought memory, possibly acting as memory factors to activate drought-related memory transcripts in pathways such as photosynthesis and proline biosynthesis, so as to respond to the subsequent stresses (Li *et al*., 2019).

Cross generational memory response due to the prior exposure of stress in the parent generation and its influence over the progeny in the seedling stage could be assessed by exposure to multiple abiotic stresses. In this context, the response of rice seedlings to PEG induced osmotic stress and NaCl induced salinity stress were reviewed.

In a study conducted by Swapna and Shylaraj (2017), a total of 42 high-yielding rice varieties were collected from Kerala Agricultural University's various research stations. The experimental setting included hydroponic treatments at various osmotic potentials, which were artificially produced by varying strengths of polyethylene glycol (PEG 6000), followed by pot planted studies in the rain-out zone. Antioxidant enzyme activities, relative water content, cell membrane stability, photosynthetic pigments, proline concentration, and plant growth indices of drought-tolerant cultivars were assessed. Furthermore, the standard scores of these rice types were evaluated under stress and recovery settings using the Standard Evaluation System for rice scoring scale. Two rice varieties, Swarnaprabha and Kattamodan, having higher drought tolerance were discovered based on the assessed parameters.

The transmission of the primed condition from one generation to the next is known as transgenerational priming (Luna and Ton, 2012). It was observed that the offspring of drought primed barley plants displayed relative longer roots than the progeny of non-primed plants after the parental plants were subjected to drought stress during reproductive stages (Nosalewicz *et al.* 2016). Drought primed plants' offspring have also been found to have a greater tolerance to heat stress during grain filling, with increased photosynthetic capacity, less cell membrane damage and larger grain yields (Zhang *et al.,* 2016). Thermo-tolerance was induced in the progeny of the primed plants under high temperature stress through heritable epigenetic alternation and signalling transduction, both of which triggered prompt modifications of defence related responses in antioxidation, transcription, energy production, and protein destination and storage (Wang *et al*., 2016). Abiotic stress priming may even promote cross-tolerance to additional abiotic stimuli that occur later. Drought priming, for example, was found to enhance cross-tolerance to heat stress during grain filling in wheat, resulting in much lower grain yield loss (Wang *et al.* 2015).

With an aim to test the effects of varying salt levels on the germination, growth, and yield of four irrigated rice (*Oryza sativa L.*) cultivars, an experiment was carried out with six different NaCl concentrations: 0, 30, 60, 90, 120, and 150 mM. Different salt levels were shown to have a detrimental impact on seed germination, plant height, tiller number, and leaf area index in all rice cultivars. With increased salt stress, all yield components, such as the number of panicles, panicle length, spikelets per panicle, filled grain and grain weight, fell dramatically. The grain production of all four rice types was reduced by 36-50 per cent when the NaCl content was increased to 150 mM. BRRI Dhan41 performed better in salinity stress up to a point (Hasanuzzaman *et al*., 2009)

Salt tolerance of plants can be improved using a variety of agrobiotechnology-supported programmes. Seed priming, often known as "pre-treatment," is the most biosafety and socioeconomically acceptable of the options. Seed priming causes the dormant seed to experience abiotic stress. It partially reprogrammes the seed metabolome so that it is exposed to such poor conditions and can adjust to salt stress more effectively. The seed's endosperm was reported to be weakened by partial hydration during priming, which channelled energy reserves, prepared the seed for radicle protrusion (germination) and recharged the antioxidant machinery (Banerjee *et al*., 2018).

A recent research initiative intended to examine the reactions of two rice genotypes to recurrent and non-recurrent stressors by examining their drought memory responses at several levels including physiological, biochemical, and metabolomic scales, as well as in global PCA. In order to gather a high number of observations, seventy variables were measured on given scales. The memory responses were evident in almost all. Under drought conditions, the lowland genotype, particularly plants not subjected to recurrent water shortages, displayed greater damage to the photosynthetic system, although having a more obvious memory response effect after rehydration. The upland genotype, on the other hand, appeared to be more drought resistant, as evidenced by lesser biochemical damage. Each genotype exhibited distinct biochemical and metabolomic characteristics, as well as similar physiological activity (Auler *et al.,* 2021).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study conducted on "Differential and dynamic response of rice variety Uma under varying drought stress regimes" was carried out as two experiments at College of Agriculture, Vellanikkara. This chapter includes the materials and methods used in carrying out the experiments of the study.

3.1 Experiment 1- Net house study

3.1.1 Location

Pot culture experiment was done in net house of Plant Physiology department of College of Agriculture, Vellanikkara

3.1.2 Season

The experiment was conducted during the period of March to August

3.1.3 Variety

Uma (MO-16), one of the most popularly cultivated variety in Kerala, developed by the Rice Research Station, Moncompu of Kerala Agricultural University was used in this study. The variety is currently occupying more than 60 percentage of the rice cultivated area of the state. It is a medium duration (115-120 days) non-lodging variety which yields about 8 to 9 tonnes of paddy per hectare. Uma is recommended for Kole, Kuttanad, and Kari soils and suited for all three seasons which indicates its adaptability to a wide range of environmental conditions.

3.1.4 Details of experiment

3.1.4.1 Plant material

Pre-germinated 10 day old seedlings of var. Uma were transplanted from trays to nine inch tall pots at the rate of two seedlings per pot. The pots were filled with farm yard manure (FYM) and soil from the paddy field at Kottepadam, Madakkathara in 1:2 ratio. Basal dose of fertilizer was also given prior to the transplanting and the pots were housed in nethouse

3.1.4.2 Drought stress imposition

During vegetative stage (40 days after transplanting), rice plants were exposed to a drought stress for a week by controlled irrigation to maintain a field capacity (FC) of 60 % gravimetric method (Parvathi *et al.*, 2013). The stressed plants were rewatered to 100% FC after a week of stress exposure. Stress imposed pots were placed in modified customized rain out shelter (ROS)-like structures. Uniformly irrigated non-stressed control plants were also simultaneously maintained. The experiment was established under completely randomized design (CRD) with two treatments and 10 replications

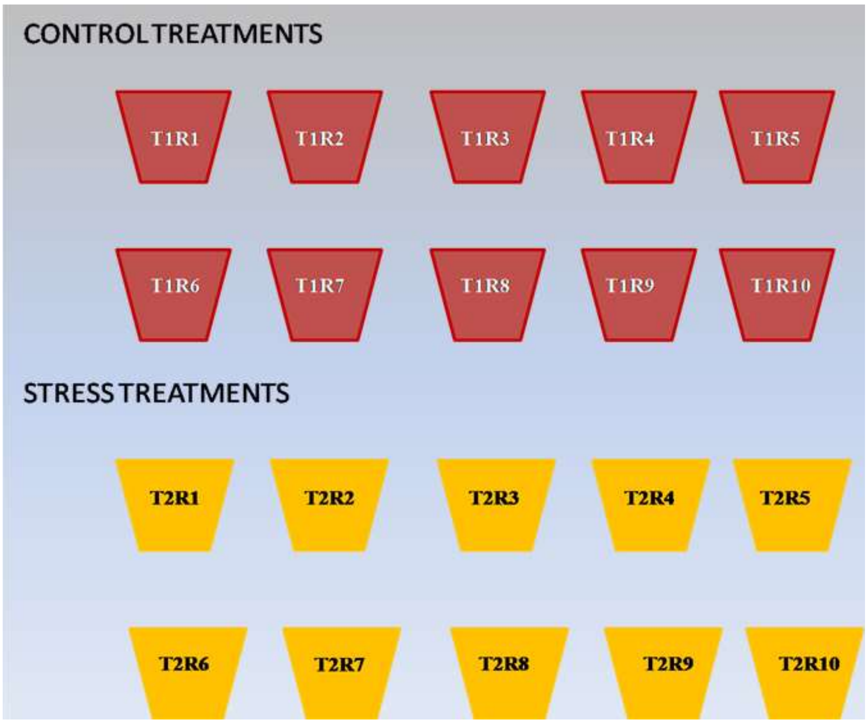


Plate 1: Experiment 1 layout (T1 – Control, **T2** – Stressed plant, **R-**replications**)**

Plate 2: Pots were placed under modified customized rain out shelter (ROS)-like structures and were weighed for gravimetric stress imposition

3.1.5 Observations recorded

Plant parameters such as number of tillers and plant height were recorded in the vegetative stage prior to stress induction. To undertake stress imposition by gravimetric method (Parvathi *et al.*, 2013), continuous monitoring of pot weight were done during the stress induction to maintain 60% field capacity. As part of assessing stress damage post stress induction, physiological and biochemical parameters were also recorded.

3.1.5.1 Morphological observations

a) Plant height

Plant height was recorded for plants maintained as two treatments (non-stressed and stressed plants) 40 days after transplanting. The measurement of plant height was made by recording the length from base to the longest leaf and was expressed in cm.

b) Number of tillers

Number of tillers formed was recorded during vegetative stage at 40 days after transplanting for plants maintained as two treatments (non-stressed and stressed plants).

3.1.5.2 Physiological observations

a) Relative water content

Turner (1981) described a method for measuring relative water content. In this method, the relative water content was measured for physiologically active third opened leaf from top. The fresh weights of the sample leaves were recorded, and they were placed in petri dishes with distilled water, then leaves were removed after 2 hours and the turgid weight was recorded till two consecutive readings recorded the same. Later, dry weight of the sample was estimated by drying them in an oven at 60-80°C for 2 days.

Formula for calculating relative water content (RWC)

RWC (%) = [(FW – DW) / (TW – DW)] *100

b) Membrane thermostability

This assay measures electrolyte leakage from leaf tissue after exposure to high temperature, based on the increased electrolyte diffusion resulting from heat induced cell membrane permeability (Sullivan, 1972). Membrane thermostability is expressed in terms of electrical conductivity (EC).

Membrane injury index = EC of control / EC of treated

Membrane thermostability (%) = 100 – Membrane injury index

c) Chlorophyll content

Total chlorophyll content was estimated by non-destructive method using Chorophyll Content Meter MC-100 (Apogee Inc., Ltd., USA) and expressed as µmol m^{-2} leaf area

3.1.5.3 Biochemical observations

a) Proline estimation

Bates *et al.* (1973) standardized a technique to assess proline concentration in leaves from non-stressed and drought-stressed plants. Leaves (100 mg) from non-stressed and drought-stressed plants were crushed with a mortar and pestle in 10 mL of 3 percent sulphosalicylic acid, centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected for proline measurement. Ninhydrin (1.25 g) was dissolved in 30 mL glacial acetic acid, followed by 20 mL 6 M phosphoric acid, which was maintained at 40°C for 24 hours. 2 mL plant extract, 2 mL acid Ninhydrin, and 2 mL glacial acetic acid were combined and placed in a water bath and heated for 1 hour at 100°C. Optical density (OD) values were measured at 520 nm using toluene as the blank after 4 mL of toluene was added to the contents and forcefully stirred for a few seconds. Proline content (µmoles/g fresh wt.) was determined independently from the OD measurements using a standard curve

b) Malondialdehyde (MDA) estimation

The MDA content was determined using the Hodges *et al.* (1999) method. For each sample, one gram of frozen leaf was crushed in 8 ml of ice-cold phosphate buffer (0.05 mol/L, pH 7.8). At 4°C, the homogenate was centrifuged for 30 minutes at 15000 rpm. The filtrate was then diluted five times before being tested for MDA concentration. The 1.0 mL dilution was then added to a 2.5 mL, 0.5 percent thiobarbituric acid solution (dissolved in 20% trichloroacetic acid) and heated for 15 minutes. The MDA concentration (nmol/ml) of the filtrate was measured using a spectrophotometer at 532 and 600 nm after rapid cooling and filtering.

c) Protein estimation

The total soluble protein was calculated using Lowry *et al.* (1951) technique and represented as mg g^{-1} of fresh weight.

3.1.5.4 Yield attributes

a) Spikelet fertility

It is estimated as the ratio of number of filled spikelet to the total number of spikelet and expressed in percentage (%) (Prasanth *et al.*, 2016)

Spikelet fertility (%) = (Number of filled spikelet / Total number of spikelet) *100

b) Grain yield

Grain yield was recorded after harvest from individual plants, using an electronic balance and expressed as grain yield per plant in grams.

c) Test weight

Randomly selected 100 grains from each replication was weighed using an electronic balance and expressed as 100 (test) grain weights in gram.

3.2 Experiment 2- Laboratory petriplate experiment

3.2.1 Location

Plant Physiology laboratory in college of agriculture, Vellanikkara

3.2.2 Details of experiment

The assessment of stress memory response at seedling stage in the subsequent generation was attempted as a comparative study using seeds collected from non-stressed and drought- stressed plants of Experiment 1 as well as a fresh set of seeds of rice variety Uma.

3.2.2.1 Standardisation of stress level for different abiotic stresses

Effective stress levels of NaCl-induced salinity and PEG-induced osmotic stresses were standardized using variety Uma. Germinated seeds were subjected to various concentration of NaCl as well as PEG (polyethylene glycol) and control treatments.

Treatments

1. Control, 100 mM, 200 mM, 300 mM - NaCl.
2. Control, -2, -4, -6 bar - PEG 6000

3.2.2.1.1 Observations recorded

Plant parameters such as root length, shoot length and root to shoot ratio were recorded. Accordingly; percentage reduction in growth over control was estimated for both salt stress as well as osmotic stress treatments, which was used as the basis to fix the stress level to be adopted in 3.2.1.2.

3.2.2.2 Stress imposition using standardized stress levels of NaCl and PEG

On the basis of the standardised stress levels from 3.2.1.1, wherein fifty percent reduction in growth over control was attained, NaCl-induced salinity and PEG-induced osmotic stresses were imposed to the subsequent generation seeds along with the control treatments as detailed in 3.2.1.

3.2.2.2.1 Observations recorded

a) Growth parameters

(i) Speed of germination

From the day of seed spread until the seventh day, seeds with radicle protrusion were counted every day. A total of 400 seeds were utilised in four repetitions. The speed of germination was estimated using a formula, and the results were presented in numbers. (Sreepriya *et al.*, 2019)

Speed of germination = $X1/Y1 + (X2-X1)/Y2+\ldots..+ (Xn - (Xn-1))/ Yn$
X1- Number of seeds germinated at first seed count
X2- Number of seeds germinated at second seed count
Xn- Number of seeds germinated at n^{th} day
Y1- Number of days from sowing to first seed count
Y2- Number of days from sowing to second seed count
Yn- Number of days from sowing to n^{th} seed count

(ii) Seedling vigor index

On the 14th day of the germination test, five seedlings were randomly picked from each petri-dish and measured the lengths (cm) of roots and shoots with a 30 cm steel-make scale. The seedling lengths were then calculated by summing each seedling's root and shoot lengths. The following formula was used to compute the seedling vigour index. (Ghosh *et al.*, 2021

Seedling vigour index = ((Germination (%) × Seedling length (cm) *100)

Based on the observations on speed of germination and seedling vigour index, the seed materials from the stressed plants of Experiment 1, were classified into high vigour seedlings and moderate vigour seedlings and maintained separately for the subsequent experiments.

(iii) Plant parameters such as root length, shoot length were recorded and expressed in cm.

b) Biochemical parameters

(i) Malondialdehyde content was also estimated over the different treatments (Ref. 3.1.5.3 Hodges *et al.* (1999)).

(ii) Metabolite profiling using GC-MS/MS(Gas Chromatography–Mass Spectrometry)

Sample preparation for GC- MS/MS

Shoot tissues from rice seedlings were collected from the treatments and ground in liquid nitrogen and stored at -80°C. Fifty milligram of ground frozen tissue was mixed with 3.5 mL of water:methanol:chloroform in 1:3:1 ratio and sonicated for 40 minutes. The extracted solution was centrifuged at 2000 rpm for 5 minutes. The supernatant was freeze dried using lyophilizer (model-Operon FDU 7003). The samples were reconstituted with 750µL of acetonitrile and placed in a vortex mixer to dissolve the compounds in acetonitrile. For each sample, 2mL of reconstituted sample was taken with disposable syringes, filtered through syringe filters, and then transferred to GC vials and later used for estimating the volatile metabolites by GC-MS/MS.

Gas Chromatography-Mass Spectrophotometry (GC-MS/MS) Analysis

A TSQ 8000 MS/MS (Make: Thermo fisher scientific) with a triple quadrupole mass spectrometer detector was used to analyse rice extracts in chloroform:methanol:water (1:3:1) mixture. In the GC-MS system, the Agilent DB – 5MS +DG column (30m length, 0.250mm diameter, and 0.25m film filter) was utilised. With a flow rate of 1mL/min, helium was used as the carrier gas. The temperature was initially set at 110°C (with a hold for 2 minutes), then ramped at 15°C/min to 150°C (with a wait of 1 minute), then escalated at 10°C/min to 250°C (with a hold for 5 minutes), for a total experiment run time of 21 minutes. A 2-liter sample was injected. Both the injector and the detector were maintained at 250°C. Mass spectra having a spectral range of m/z 50-700 amu were acquired using electron ionisation at 70 eV (Shu et al., 2011).

3.3 Statistical Analysis

Statistical analysis was carried out using GRAPES software for both experiments (Gopinath *et al.*, 2021).

RESULTS

4. RESULTS

The major results obtained from the study on "Differential and dynamic response of rice variety Uma under varying drought stress regimes" are included in this chapter.

4.1 EXPERIMENT 1- NET HOUSE STUDY

A controlled customized pot culture experiment was carried out in the net house facility of Department of Plant Physiology, College of Agriculture, Vellanikkara using rice variety Uma, wherein gravimetric drought stress imposition at vegetative stage to a moderate stress level of 60% field capacity was accomplished by gradual dry down technique (Plate 3). Subsequently, assessment of stress damage and monitoring of stress specific candidate biomarkers were attempted, so as to characterize the drought induction response of variety, Uma.

Plate 3: Representative phenotype of drought stressed (60%FC) and non-stressed (100% FC) plants of rice variety, Uma at vegetative stage

4.1.1 Morphological observations

Prior to stress imposition, the plant height and number of established tillers per plant of all the plants used in the study across different treatments were assessed to ensure that uniformly established plants were subjected to different treatments. The mean data on plant height and number of tillers are given in Table 1. Plant height was estimated after 40 days of transplanting, prior to the stress imposition and it was found that there was no significant difference between the two treatments. The mean number of tillers established per plant was also recorded during vegetative stage after 40 days of transplanting for plants earmarked for non-stress and stress treatments, which were also on par.

Table 1: Plant height and number of tillers of plants earmarked for stress and non-stress treatments 40 days after transplanting prior to stress imposition

Treatments	Number of tillers/plant	Plant height (cm)
Control	14.6	64.14
Stress	14.8	62.57
CD (0.05)	NS	NS

4.1.2 Physiological observations for assessment of stress damage post stress imposition

Relative water content in the leaf tissues after drought stress exposure is an indication of stress undergone by the plant. Treatment means of both control and stressed plants are shown in Table 2. Relative water content of both treatments differed significantly with stressed plants having relatively lower water content compared to non-stressed control plants. Membrane thermostability of the leaf tissues after drought stress exposure is the indirect demonstration of membrane leakage as well as the tolerance of cell membrane against stress in non-stressed and stressed plants. Stressed plants possessed significantly lower membrane thermostability compared to non-stressed control plants (Table 2). Chlorophyll content in the leaf tissues of stressed and non-stressed tissues estimated by non-destructive sampling after drought stress exposure indicated that non-stressed tissues contained more amount of chlorophyll (452.03 µmol/m^2) than stressed tissues (288.49 µmol/m^2), reiterating adequately imposed stress conditions (Table 2).

Table 2: Relative water content (%), membrane thermostability and chlorophyll content (µmol/m^2) of both non-stressed control and stressed plants (one week stress exposed) post drought stress imposition at vegetative stage in rice variety, Uma

Treatments	Membrane thermostability (%)	Chlorophyll content (µmol/m^2)	Relative water content (%)
Control	63.91 ^{a}	452.03^{a}	89.58^{a}
Stress	13.73 ^{b}	288.49^{b}	68.75^{b}
CD (0.05)	7.99	47.46	2.11

4.1.3 Biochemical observations on candidate drought stress specific biomarkers across different phenophases

4.1.3.1 Proline content

Amino acid proline is produced in the stressed leaves when there is a sudden stress exposure, which has a significant role in the osmolyte regulation with special context to drought stress. Proline estimation in leaf tissues one week post stress exposure at vegetative stage showed that stressed tissues accumulated significantly more amount of proline (4.09 µmol/g) compared to that of the non-stressed control tissues (3.58 µmol/g).When proline contents were estimated at panicle initiation stage, there were indications that there was no significant difference between proline levels in the control and stressed plants which were exposed to drought stress at vegetative stage, although there was a drop in the levels compared to that observed at vegetative stage.

However, at grain filling stage, there was a trend such that control treatment plants accumulated considerably higher amounts of proline (0.307 µmol/g) than the stressed plants (0.015 µmol/g) (Table 3). The results indicated a differential regulation of the metabolite, proline across different phenophases in response to a stress exposure.

Table 3: Proline contents in rice leaf tissue one week post drought stress imposition and across different phenophases in variety, Uma

Treatments	**Proline content (µmol/g)**		
	Vegetative stage	**Panicle initation stage**	**Grain filling stage**
Control	3.58^b	0.005	0.307^a
Stress	4.09^a	0.633	0.015^b
CD (0.05)	0.48	NS	0.246

4.1.3.2 Malondialdehyde (MDA) estimation

Malondialdehyde (MDA) is a remarkable indicator of lipid peroxidation process taking place inside plant cell on the advent of a stress and in particular in drought stress, it is a potential biomarker for yield stability. So it's estimation after stress exposure has vital importance.

MDA estimation at vegetative stage one week post stress exposure, revealed that its contents varied significantly in the stressed (4.48 nmol/ml) and non-stressed control (2.62 nmol/ml) tissues (Table 4). However, there were differential regulations in MDA levels in subsequent phenophases. In panicle initiation stage, although the MDA levels dropped drastically, tissues from stressed plants showed a higher MDA content (0.998 nmol/ml) than the control one (0.583 nmol/ml); whereas at grain filling stage, there was reduction in the MDA levels in the same stressed plants with a lower MDA content (2.41 nmol/ml) compared to control plants (3.22 nmol/ml).

Table 4: Malondialdehyde contents in rice leaf tissue one week post drought stress imposition and across different phenophases in variety, Uma

Treatments	MDA content (nmol/ml)		
	Vegetative stage	Panicle initation stage	Grain filling stage
Control	2.62^b	0.583^b	3.22^a
Stress	4.48^a	0.998^a	2.41^b
CD (0.05)	0.91	0.267	0.04

4.1.3.3 Protein estimation

Rapid decrease in the total soluble protein content is an indication of the influence of stress. Due to the sudden differential regulation in proline and MDA levels at panicle initiation stage, after a prior stress exposure at vegetative stage, the protein levels were assessed. Total soluble protein estimated at the panicle initiation stage of the crop in both well-watered and stress affected plants, was significantly higher in control plants (52.33 mg/g) than stressed plants (21.35 mg/g) (Table 5).

Table 5: Protein content in rice leaf tissue at panicle initiation stage in control and stressed plants in variety, Uma

Treatments	Protein content (mg/g)
Control	52.33^a
Stress	21.35^b
CD (0.05)	18.09

4.1.4 Yield attributes

4.1.4.1 Spikelet fertility

Spikelet fertility was estimated for the control and stressed plants, wherein the drought stressed plants exhibited a lower spikelet fertility percentage (44.41%) compared to that of control (66.36%) (Table 6). The mean data of the grain yield of plants are given in Table 6. There was a significant difference between the grain yields of stressed and control plants. Control plants possessed a higher yield (4.26 g/plant) compared to that of the stressed plants (2.05 g/plant). The mean data of the test weight of plants are given in Table 6. There was no significant difference between the test weight of the stressed and control plants and were found to be on par.

Table 6: Yield attributes of control and stressed plants of rice variety, Uma

Treatments	**Grain yield (g/plant)**	**Spikelet fertility (%)**	**Test weight (g/plant)**
Control	4.26^a	66.36^a	1.85
Stress	2.05^b	44.41^b	1.79
CD (0.05)	0.93	8.40	NS

Correlation analysis was performed to observe any interesting trends in the relation between the candidate biomarkers monitored in the study and the yield attributes. It was interesting to note that there was a positive correlation between the MDA contents at grain filling stage and yield attributes like spikelet fertility and grain yield (Table 7).

Table 7: Correlation analysis between the post-stress characteristics of the stressed and non-stressed control plants

	Chl	**RWC**	**MTS**	**P-V**	**P-P**	**P-G**	**M-V**	**M-PI**	**M-G**	**PT-PI**	**SF**	**GY**
Chl	1	0.951**	0.915*	-0.841*	-0.656	0.746	-0.903*	-0.731*	0.94**	0.793*	0.793***	0.768**
RWC	0.951**	1	0.988***	-0.825*	-0.758	0.864*	-0.918**	-0.901*	0.996***	0.948**	0.914*	0.923**
MTS	0.915*	0.988***	1	-0.836*	-0.845*	0.876*	-0.941**	-0.897*	0.997***	0.955**	0.899*	0.909*
P-V	-0.841*	-0.825*	-0.836*	1	0.66	-0.824*	0.755	0.93**	-0.829*	-0.953**	-0.779	-0.621
P-P	-0.656	-0.758	-0.845*	0.66	1	-0.684	0.895*	0.631	-0.812*	-0.764	-0.684	-0.747
P-G	0.746	0.864*	0.876*	-0.824*	-0.684	1	-0.692	-0.948**	0.865*	0.92**	0.627	0.622
M-V	-0.903*	-0.918**	-0.941**	0.755	0.895*	-0.692	1	0.727	-0.941**	-0.863*	-0.905*	-0.942**
M-PI	-0.731*	-0.901*	-0.897*	0.93**	0.631	-0.948**	0.727	1	-0.892*	-0.949***	-0.763*	-0.571
M-G	0.94**	0.996***	0.997***	-0.829*	-0.812*	0.865*	-0.941**	-0.892*	1	0.952**	0.91*	0.925**
PT-PI	0.793*	0.948**	0.955**	-0.953**	-0.764	0.92**	-0.863*	-0.949***	0.952**	1	0.842**	0.737*
SF	0.793***	0.914*	0.899*	-0.779	-0.684	0.627	-0.905*	-0.763*	0.91*	0.842**	1	0.847***
GY	0.768**	0.923**	0.909*	-0.621	-0.747	0.622	-0.942**	-0.571	0.925**	0.737*	0.847***	1

*** Correlation is significant at 0.001 level (two tailed) , ## ** Correlation is significant at 0.01 level (two tailed), ## * Correlation is significant at 0.05 level (two tailed)**(Chl**- Chlorophyll content， **RWC**-Relative water content， **MTS**-Membrane thermostability， **P-V**- Proline content at vegetative stage (post stress)， **P-P-**Proline content at panicle initation stage， **P-G**-Proline content at grain filling stage， **M-V**-Malondialdehyde (MDA) content at vegetative stage (post stress)， **M-PI**-Malondialdehyde (MDA) content at panicle initation stage， **M-G**-Malondialdehyde (MDA) content at grain filling stage， **Pt-PI**-Protein content at panicle initation stage， **SF**-Spikelet fertility， **GY**-Grain yield)

4.2 EXPERIMENT 2- LABORATORY EXPERIMENT

The seeds collected from non-stressed and drought-stressed plants of Experiment 1 as well as a fresh set of seeds of rice variety Uma were used in a comparison study to examine stress memory response at the seedling stage in the future generation. In order to impose PEG induced osmotic stress and NaCl induced salinity stress as a stress exposure in the next generation at seedling stage, an experiment was carried out to standardise the sublethal stress levels to be employed to seedlings. Subsequently, the sublethal stress level selected was employed to uniformly germinated seedlings to systematically assess the comparative growth responses and monitor the metabolite dynamics.

4.2.1 Standardisation of sublethal stress levels for stress imposition using NaCl induced salinity stress and PEG induced osmotic stress

The effective stress levels of NaCl-induced salinity and PEG-induced osmotic stresses were standardised. Uniformly germinated seedlings of rice variety, Uma were subjected to different concentrations of NaCl as well as PEG 6000 (Polyethylene Glycol 6000) and control treatments for the standardisation. The phenotype of the seedlings under different treatments is as shown in Plate. 2 and Plate. 3.

Plate.4 Phenotype of the seedlings of rice variety, Uma under various concentration of PEG (polyethylene glycol) induced osmotic stress (Concentration of PEG expressed as bars)

Plate.5 Phenotype of the seedlings of rice variety, Uma under various concentration of NaCl induced salt stress (Concentration of NaCl expressed as millimolar; mM)

Plant parameters such as root length, shoot length and root to shoot ratio were recorded post stress imposition. Treatment means of these seedling parameters across different NaCl concentrations of 100, 200 and 300 mM after three days of stress exposure are given in the Table 8. The root length (8.07 cm) as well as shoot length (4.05 cm) recorded the highest in control treatment which reduced drastically under NaCl stress. It was observed that as the concentration of NaCl increased root growth as well as shoot growth exhibited a significantly decreasing trend.

The seedlings exposed to control treatment also possessed significantly higher root to shoot ratio (3.54) compared to the stressed treatments. Moreover, as NaCl concentration increased the root to shoot ratio decreased significantly (Table 8).

Table 8: Effect of different concentrations of NaCl on seedling parameters such as root length, shoot length and root to shoot ratio in rice variety Uma

Treatments	Root length (cm)	Shoot length (cm)	Root to Shoot ratio
Control	8.07^a	4.05^a	3.55^a
100 mM	5.60^b	3.27^b	2.73^b
200 mM	2.61^c	1.90^c	1.92^c
300 mM	1.07^d	0.72^d	1.65^d
CD (0.05)	0.77	0.54	0.22

In response to PEG induced osmotic stress, root length, shoot length and root to shoot ratio were measured after three days of incubation under different levels of PEG viz. -2, -4 and -6 bars. Table 9 shows the treatment means of various parameters assessed during the standardization of rice variety, Uma under PEG induced osmotic stress.

The root length, shoot length and root to shoot ratio of the control seedlings were significantly higher (6.22, 3.80 and 4.067 cm respectively) than under PEG induced osmotic stress. However, the seedling response to stress exposure at -6 bars of PEG was on par with -4 bars PEG levels in case of all the parameters assessed (Table 9).

Table 9: Effect of different concentrations of PEG 6000 on seedling parameters such as root length, shoot length and root to shoot ratio in rice variety Uma

Treatments	Root length (cm)	Shoot length (cm)	Root to Shoot ratio
Control	6.22^{a}	3.80^{a}	4.07^{a}
-2 bar	1.69^{b}	1.43^{b}	1.89^{b}
-4 bar	1.29^{bc}	0.66^{c}	1.52^{c}
-6 bar	0.77^{c}	0.36^{c}	1.44^{c}
CD (0.05)	0.79	0.52	0.27

The percent reduction in growth over control was computed to ascertain the sublethal stress level in case of both the stresses. Based on reduction in growth to a tune of 25-50%, 100 mM NaCl was selected as sublethal stress level for the subsequent salinity stress assays. However, in case of osmotic stress, since there was a drastic reduction across -2 to -6 bars, -4 bars PEG was chosen as the stress level (Table 10 and 11).

Table 10: Percent reduction in growth over control of rice variety Uma under salinity stress induced by different NaCl concentrations

Treatments NaCl concentration (mM)	Percent reduction in root growth over control	Percent reduction in shoot growth over control	Percent reduction in total length over control
100	30.57^{c}	19.13^{c}	26.72^{c}
200	67.42^{b}	53.23^{b}	62.67^{b}
300	86.67^{a}	82.34^{a}	85.24^{a}
CD (0.05)	10.45	15.55	9.52

Table 11: Percent reduction in growth over control of rice variety Uma under osmotic stress induced by different PEG concentrations

Treatments PEG concentration (bars)	Percent reduction in root growth over control	Percent reduction in shoot growth over control	Percent reduction in total length over control
-2	72.37^{b}	62.48^{c}	68.62^{c}
-4	78.99^{b}	82.16^{b}	80.25^{b}
-6	87.79^{a}	90.58^{a}	88.85^{a}
CD (0.05)	8.19	7.59	5.28

4.2.2 Stress imposition using standardised stress levels of NaCl and PEG

4.2.2.1 Analysis of growth parameters of the seed materials selected for the study

(i) Speed of germination

Treatment means of the speed of germination of control and stressed seeds from previous generation and a new set of fresh seeds of variety, Uma were examined. Surprisingly, there were two categories of seeds among those collected from the first generation stress exposed plants (Experiment 1), based on the speed and vigour of the germinated seeds, which were significantly different. One set possessing both higher speed of germination and seedling vigour index was termed as high vigour seedlings and another set exhibiting lowest speed of germination and seedling vigour index was termed as moderate vigour seedlings. The fresh set and control set of seeds, collected from previous generation experiment, exhibited similar speed of germination which were on par. The high vigor seedlings in stress exposed seeds showed comparatively high speed of germination (96.49) (Table 12).

Table 12: Speed of germination of control, stress-high vigor, stress-moderate vigour and fresh set of seeds used in the study

Treatments	Speed of germination
Control	67.22^{b}
Stress-high vigour	96.49^{a}
Stress-moderate vigour	42.79^{c}
Fresh set	65.69^{b}
CD (0.05)	9.31

(ii) Seedling vigour index

The seedling vigour index of control and stressed seeds from previous generation and a new set of fresh seeds of Uma was also examined.

Seedling vigour index of the two classes of progenies of the first generation stressed plants was significantly different. High vigour seedlings showed a higher seedling vigour index of 1066.57 and the lowest was recorded for the moderate vigor seedlings (782.76) (Table 13).

Table 13: Seedling vigour index of control, stress-high vigor, stress-moderate vigour and fresh set of seeds used in the study

Treatments	Seedling vigour index
Control	861.13^{b}
Stress-high vigour	1066.57^{a}
Stress-moderate vigour	782.76^{b}
Fresh set	1001.19^{a}
CD (0.05)	98.79

4.2.2.2 Assessment of stress response in the next generation

(i) Growth characterization

Plant parameters such as root length and shoot length of the seedlings across different treatments in the stress experiment were recorded and expressed in cm.

(a) Growth response of control set of seeds from first generation

In case of control treatment, the seedlings showed significantly higher growth (6.46 cm root length and 3.35 cm shoot length) while comparing treatment means. But the treatment means of growth response of seedlings under PEG (-4 bar) and NaCl (100 mM) induced stresses was on par with each other with respect to both shoot length as well as root length (Table 14).

Table 14: Shoot length and root length of control set of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation

Treatments	Root length (cm)	Shoot length (cm)
Control	6.46^a	3.35^a
Osmotic stress (PEG -4 bar)	4.29^b	1.18^b
Salt stress (NaCl 100 mM)	4.48^b	1.18^b
CD (0.05)	0.83	0.39

Plate 6: Representative phenotype of control set of seedlings exposed to different abiotic stresses in the next generation

(b) Growth response of high vigour seeds from the stressed set of plants from first generation

The non-stressed high vigour seedlings exhibited significantly higher root length as well as shoot length than PEG (-4 bar) and NaCl (100 mM) treatments, which were on par in terms of root growth. However, in case of shoot length, salt stressed as well as the control treatments was on par with each other (Table 15).

Table 15: Shoot length and root length of stressed set of seedlings of high vigour exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation

Treatments	Root length (cm)	Shoot length (cm)
Control	6.25^{a}	3.15^{a}
Osmotic stress (PEG -4 bar)	3.31^{b}	1.56^{b}
Salt stress (NaCl 100 mM)	4.58^{b}	2.85^{a}
CD (0.05)	1.43	0.57

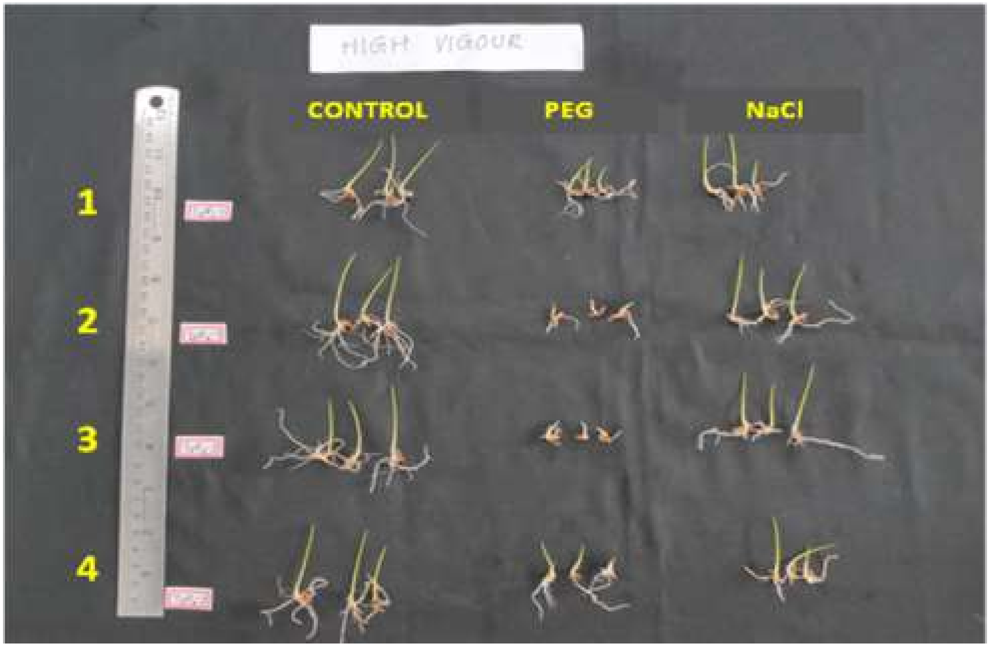


Plate 7: Representative phenotype of high vigour set of seedlings exposed to different abiotic stresses in the next generation

(c) Growth response of moderate vigour seeds from the stressed set of plants from first generation

In this case, both root and shoot parameters (5.34 cm root length and 2.55 cm shoot length) were significantly higher in control treatments than stressed seedlings, although the growth under PEG (-4 bar) and NaCl (100 mM) induced stresses was on par (Table 16).

Table 16: Shoot length and root length of stressed set of seedlings of moderate vigour exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation

Treatments	Root length (cm)	Shoot length (cm)
Control	5.34^a	2.55^a
Osmotic stress (PEG -4 bar)	2.68^b	0.95^b
Salt stress (NaCl 100 mM)	2.38^b	1.37^b
CD (0.05)	1.59	1.05

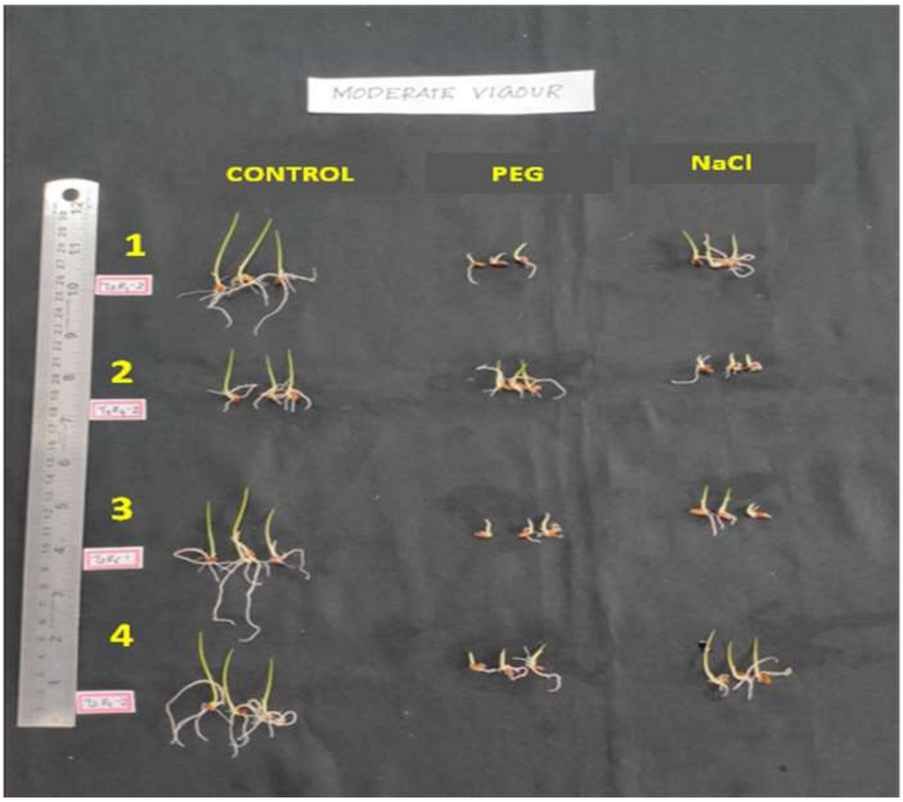


Plate 8: Representative phenotype of moderate vigour set of seedlings exposed to different abiotic stresses in the next generation

(d) Growth response of fresh set of seeds

Root length and shoot length of seedlings belonging to the fresh set were significantly higher in control treatment than under stress; with each treatment being significantly different from each other in case of both these parameters (Table 17).

Table 17: Shoot length and root length of fresh set of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation

Treatments	Root length (cm)	Shoot length (cm)
Control	6.42^{a}	3.71^{a}
Osmotic stress (PEG -4 bar)	3.93^{b}	1.47^{c}
Salt stress (NaCl 100 mM)	4.60^{c}	2.31^{b}
CD (0.05)	0.63	0.26

Plate 9: Representative phenotype of seedlings of fresh set of seedlings exposed to different abiotic stresses in the next generation

(ii) Stress recovery growth characterisation

Recovery in growth after sublethal stress was accounted for parameters such as root length and shoot length in each treatment.

(a) Response of control set of seeds from first generation after recovery growth

Root length as well as shoot length was significantly different from each other in the three treatments after recovery growth, with the non-stressed control showing higher recovery growth compared to other treatments. Even then, the recovery growth of root length and shoot length of salt stressed and osmotic stress plants are on par with each other (Table 18).

Table 18: Shoot length and root length of control set of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation after recovery growth

Treatments	Root length (cm)	Shoot length (cm)
Control	7.11a	6.37a
Osmotic stress (PEG -4 bar)	5.67b	5.16b
Salt stress (NaCl 100 mM)	5.95b	4.71b
CD (0.05)	1.02	1.21

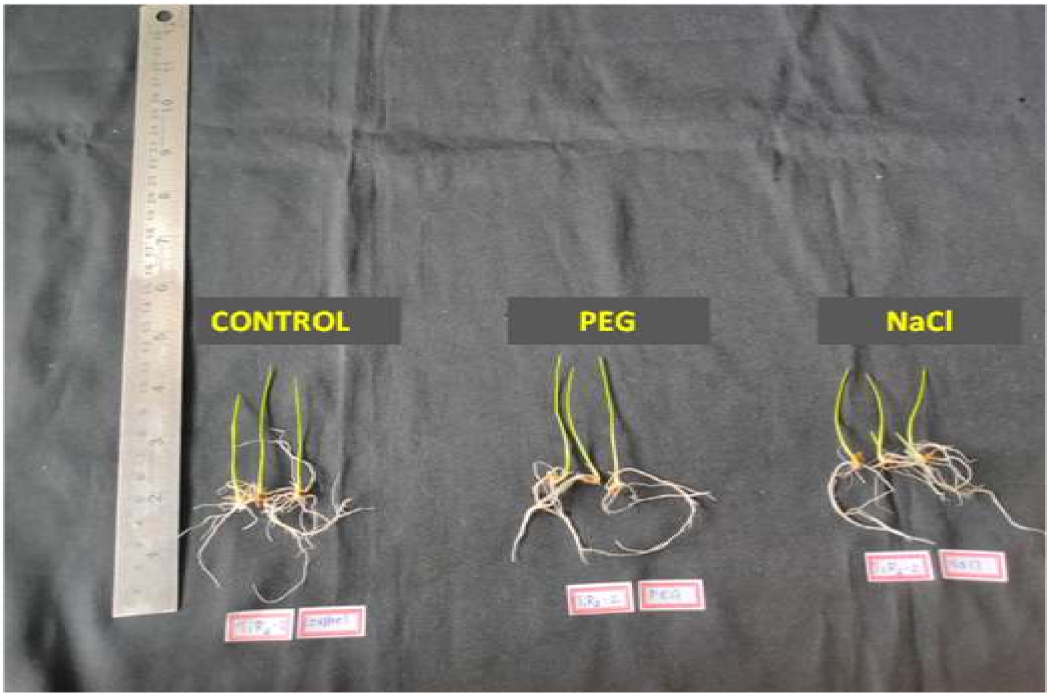


Plate 10: Representative recovery phenotype of seedlings of control set of seedlings exposed to different abiotic stresses in the next generation

(b) Response of high vigour set of seeds from first generation after recovery growth

Root length as well as shoot length was significantly different from each other in three treatments after recovery growth. Non-stressed seedlings showed higher recovery growth compared to other treatments. Recovery growth of root length of salt stressed and osmotic stress plants were on par with each other, whereas recovery growth of shoot length in control as well as salt stress were on par with each other (Table 19).

Table 19: Shoot length and root length of high vigour set of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation after recovery growth

Treatments	Root length (cm)	Shoot length (cm)
Control	7.11^{a}	5.09^{a}
Osmotic stress (PEG -4 bar)	3.92^{b}	3.64^{b}
Salt stress (NaCl 100 mM)	5.04^{b}	4.99^{a}
CD (0.05)	1.16	1.01

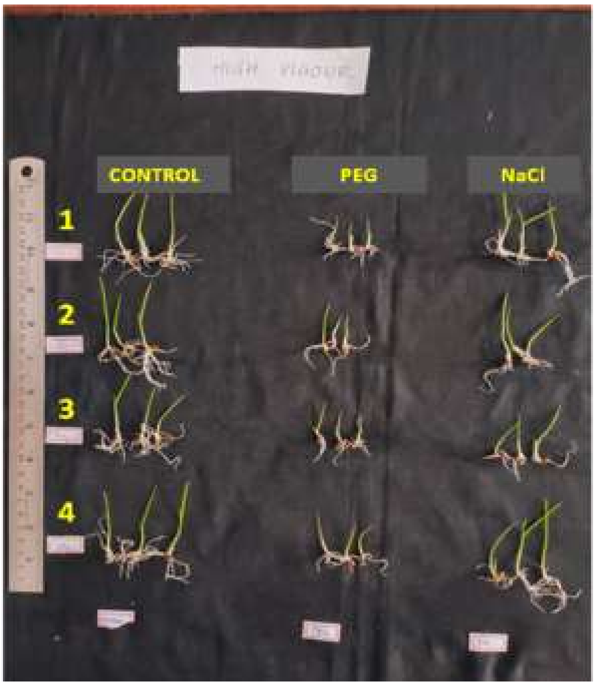


Plate 11: Representative recovery phenotype of seedlings of high vigour set of seedlings exposed to different abiotic stresses in the next generation

(c) Response of moderate vigour set of seeds from first generation after recovery growth

Root length as well as shoot length was significantly different from each other in the three treatments after recovery growth. Similarly, the non-stressed seedlings showed higher recovery growth compared to other treatments, although the recovery growth of root length and shoot length of salt stressed and osmotic stress plants are on par with each other (Table 20).

Table 20: Shoot length and root length of moderate vigour set of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation after recovery growth

Treatments	Root length (cm)	Shoot length (cm)
Control	6.43^{a}	5.24^{a}
Osmotic stress (PEG -4 bar)	3.66^{b}	2.49^{b}
Salt stress (NaCl 100 mM)	3.55^{b}	3.35^{b}
CD (0.05)	1.39	1.41

Plate 12: Representative recovery phenotype of seedlings of moderate vigour set of seedlings exposed to different abiotic stresses in the next generation

(d) Response of fresh set of seeds after recovery growth

Root length was not significantly different from each other in three treatments after recovery growth. Control seedlings exhibited showed higher recovery growth compared to other treatments. Recovery growth in shoot length of all the treatments was significantly different with each other. Whereas recovery growth of shoot length in osmotic stress was on par with control as well as salt stress treatments (Table 21).

Table 21: Shoot length and root length of moderate vigour set of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the fresh set of seedlings after recovery growth

Treatments	Root length (cm)	Shoot length (cm)
Control	6.37	6.72^{a}
Osmotic stress (PEG -4 bar)	6.11	5.10^{ab}
Salt stress (NaCl 100 mM)	5.95	4.33^{b}
CD (0.05)	NS	1.79

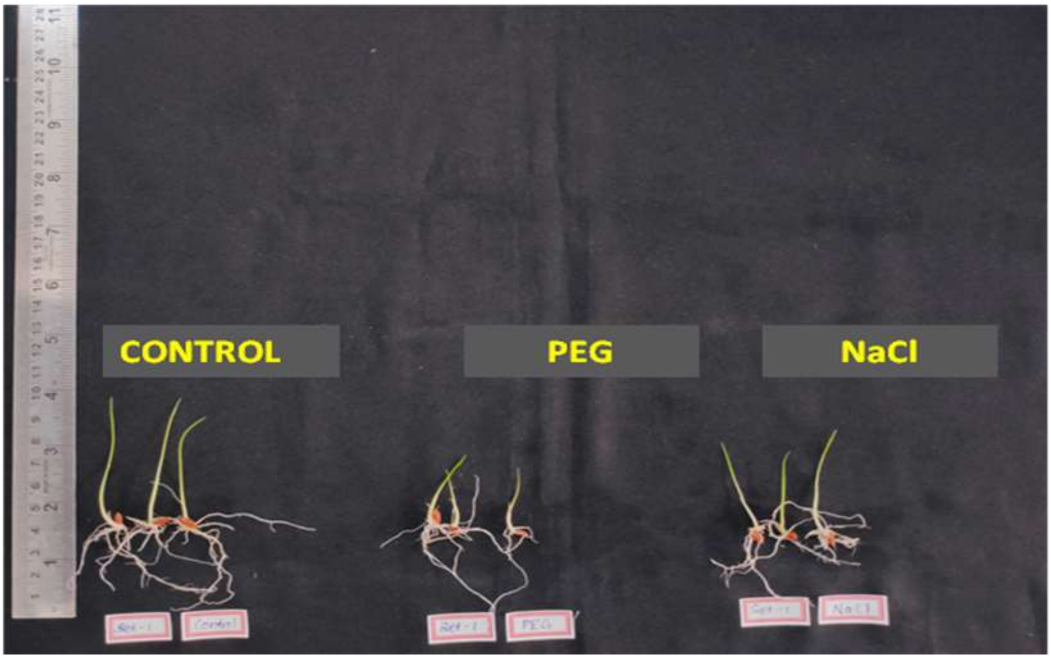


Plate 13: Representative recovery phenotype of seedlings of fresh set of seedlings exposed to different abiotic stresses in the next generation

(iii) Biochemical characterisation

(a) Malondialdehyde content

Malondialdehyde content was estimated over the different treatments and expressed as nmol/ml. The contents were significantly different between each treatment in both moderate vigour seedlings and fresh set of seedlings. But in case of high vigour seedlings, MDA content in control and salt stress treatments were on par with each other. There was no significance difference between stress and non-stress treatments of the control set of seedlings, although the accumulation levels were significantly different under both the stresses (Table 22).

Table 22: Malondialdehyde content (nmol/ml) of seedlings exposed to PEG induced osmotic and NaCl induced salinity stresses in the next generation across different

Treatments	MDA content (nmol/ml)			
	Control	Stress-High Vigour	Stress-Moderate Vigour	Fresh
Control	3.58^{ab}	0.74^{b}	1.72^{b}	0.96^{b}
Osmotic stress (PEG -4 bar)	4.01^{a}	4.37^{a}	3.50^{a}	3.00^{a}
Salt stress (NaCl 100 mM)	3.16^{b}	0.60^{b}	0.61^{c}	0.71^{b}
CD (0.05)	0.65	0.19	0.59	0.49

(b) Metabolite profiling using GC-MS/MS (Gas Chromatography–Mass Spectrometry)

In order to assess the metabolite dynamics due to stress exposures in the next generation, Gas Chromatography-Mass Spectrometry (GC-MS/MS) analysis was done across the seeds collected from control and stressed plants from first generation as well as a fresh set of seeds of rice variety Uma. The metabolites analyzed will be limited to the volatiles and to a narrower spectrum subject to the limited derivatization steps included in the sample extraction. GC-MS/MS spectra of the different treatments were obtained as the ouput of GC-MS/MS analysis as given in Plates 4 -7. Peaks in the graph indicate the relative abundance of the volatile compound over the time period.

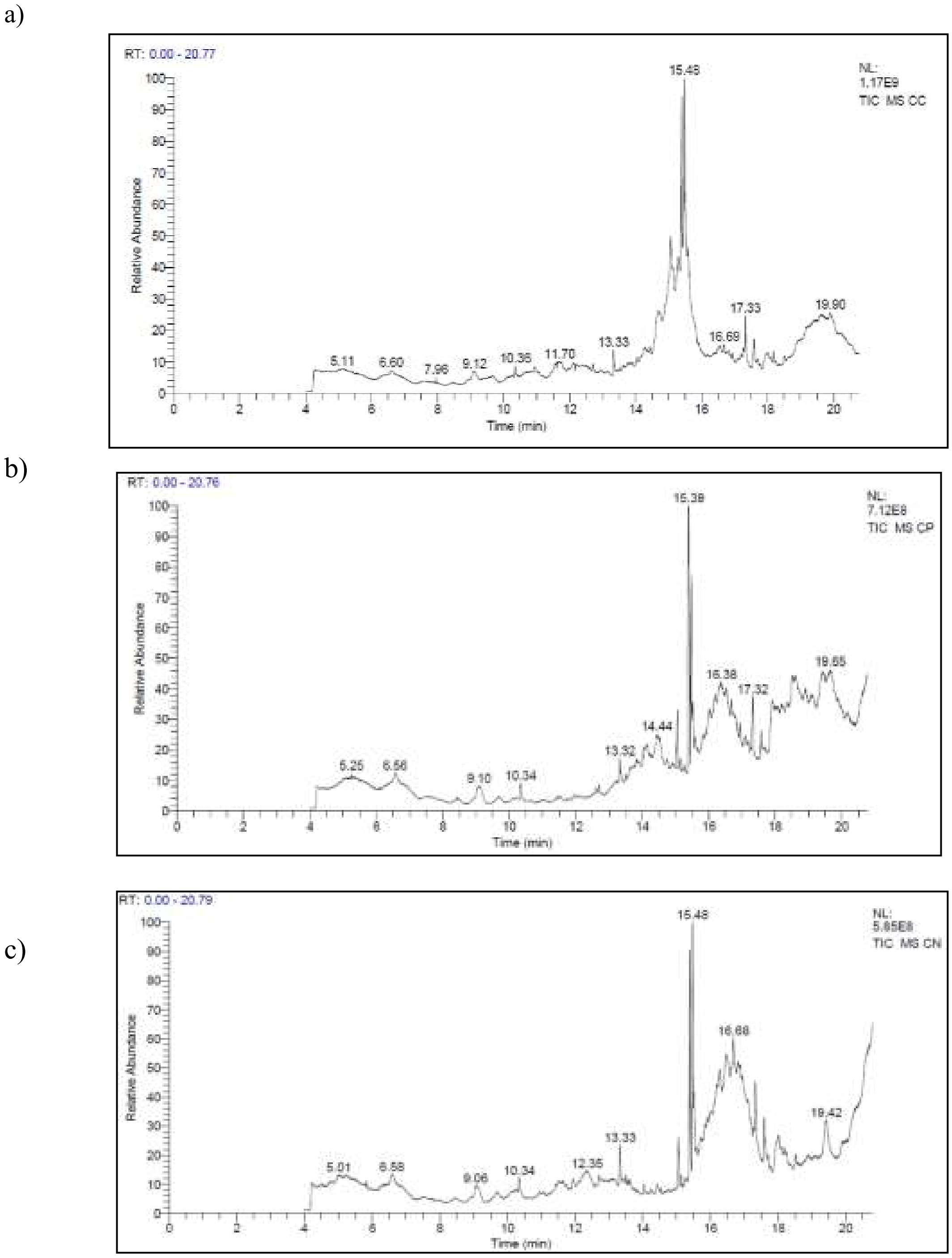


Plate 14: GC-MS/MS spectra of shoot tissues of seedlings from control set of seeds of rice variety, Uma (from first generation) under different treatments: a) Control b) PEG (-4 bar) c) NaCl (100 mM)

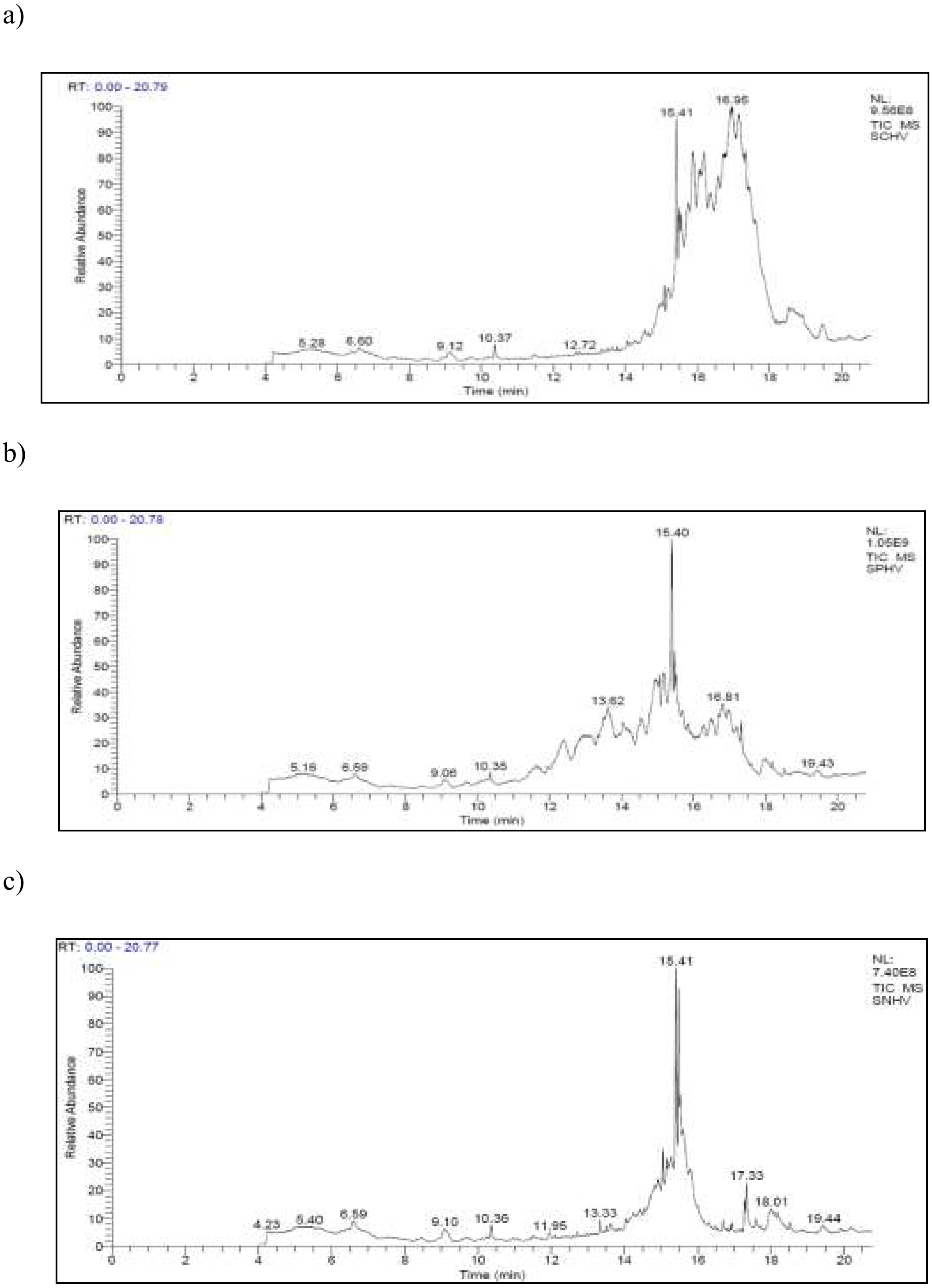
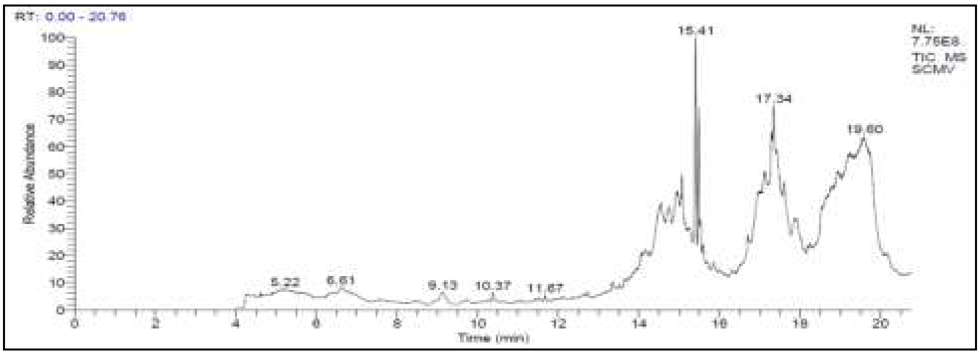
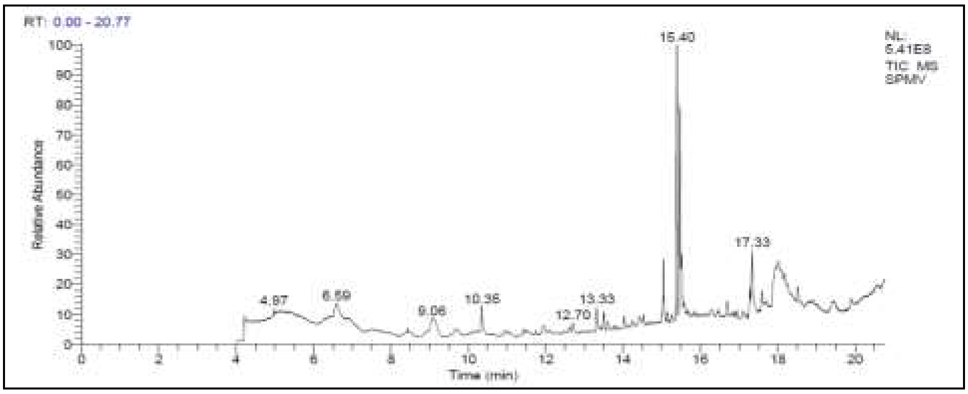


Plate 15: GC-MS/MS spectra of shoot tissues of seedlings from stressed set of seeds of high vigour (from first generation) under different treatments: a) Control b) PEG (-4 bar) c) NaCl (100 mM)

a)



b)



c)

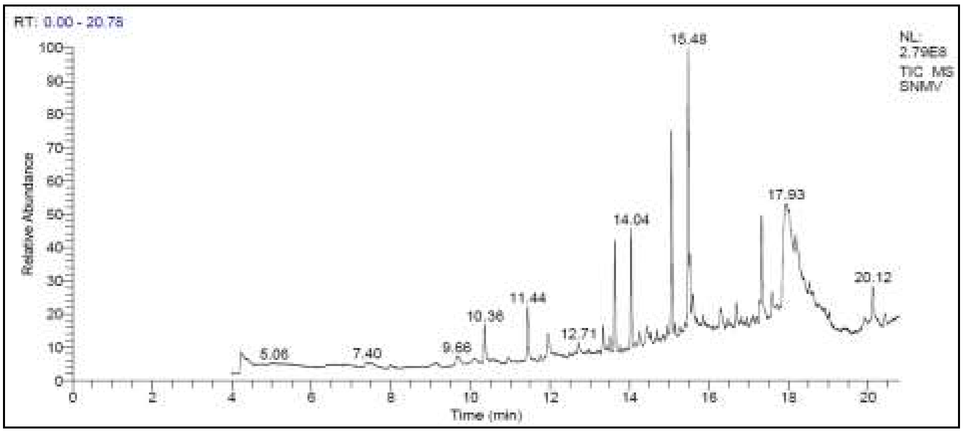


Plate 16: GC-MS/MS spectra of shoot tissues of seedlings from stressed set of seeds of moderate vigour (from first generation) under different treatments: a) Control b) PEG (-4 bar) c) NaCl (100 mM)

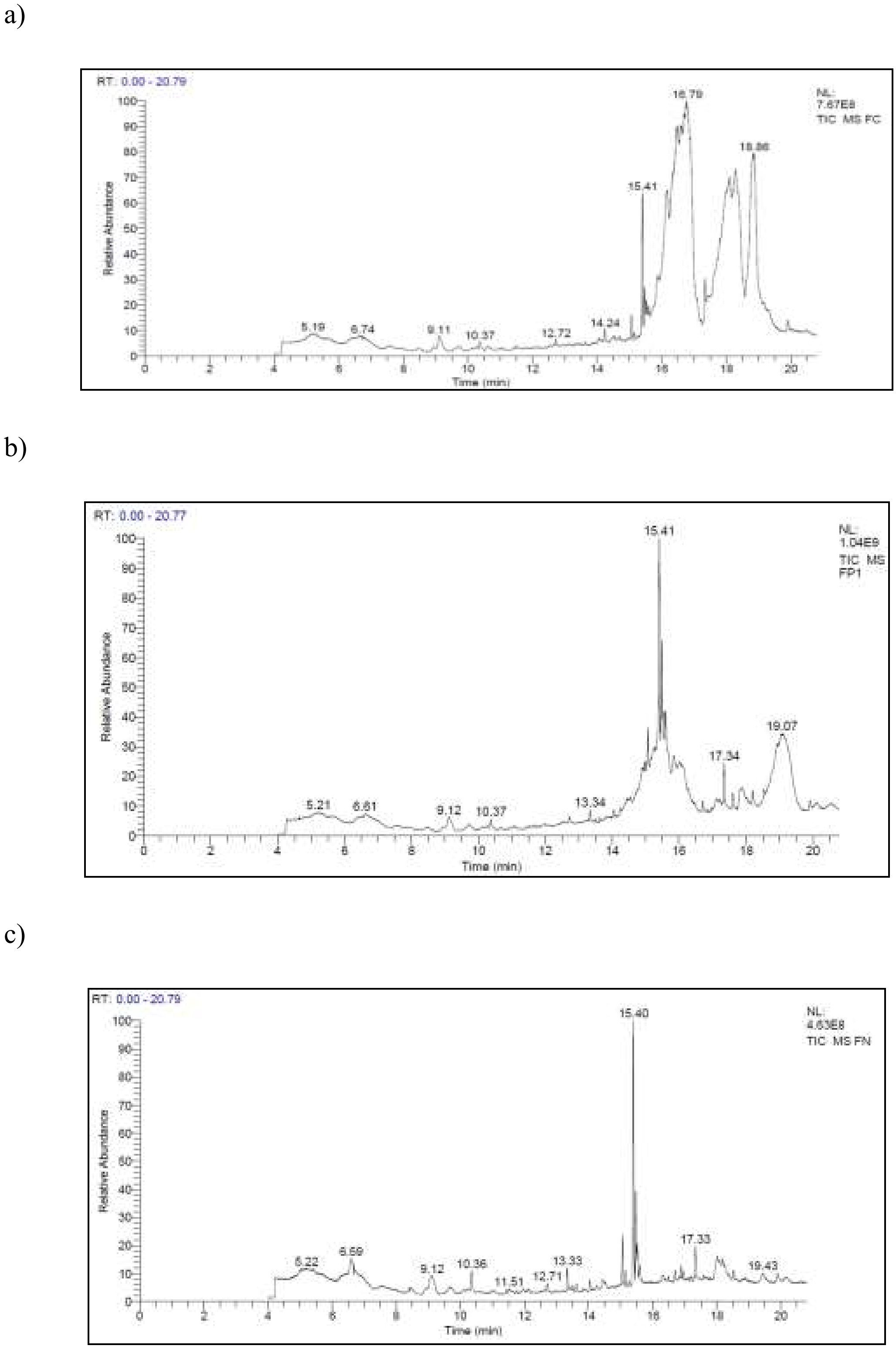


Plate 17: GC-MS/MS spectra of shoot tissues of seedlings from fresh set of seeds of rice variety, Uma under different treatments: a) Control b) PEG (-4 bar) c) NaCl (100 mM)

From the GC-MS/MS spectra, the interpretations were made based on the relative abundance or area percentage and probability of the compound over the time period. Highest peak (H) shows the compound which shows relatively higher area percentage over time, whereas unique peak (U) indicates those peaks which are unique to the treatment. The data compiled from the GC-MS/MS profiling is represented in Tables 23-26.

Table 23: Metabolite variables in shoot tissues of rice seedlings from control set of seeds (from first generation) subjected to different abiotic stresses

Trt.	Compound Name	RT	Prob.	Area	Tp.
CC	9,19Cyclolanost24en3ol	15.48	54.98	19.48	H
	Diphenyl sulfone	15.4	76.69	11.94	
	9,19Cyclolanost24en3ol	15.59	60.43	5.83	
	2,8,9Trioxa5aza1silabicyclo(3.3.3)undecane	17.33	32.6	5.21	
	9,19Cyclolanost24en3ol	14.68	69.12	4.95	
	Pregna6,16diene11,20diol	18	13.99	3.33	
	9,19Cyclolanost24en3ol	15.28	47.45	2.92	
	Octadecanoic acid	17.59	56.74	2.35	
	9,19Cyclolanostan3ol,24methylene,(3á)	19.9	10.66	2.07	
	Tetradecanoic acid	13.33	63.91	1.62	
	Dodecanal	9.1	32.55	1.58	
	Lupeol	12.11	51.01	1.27	
	Lupeol	12.39	49.02	1.18	
CP	Diphenyl sulfone	15.39	97.82	13.32	H
	n-Hexadecanoicacid	15.47	66.45	11.08	U
	Ç-Sitosterol	17.92	49.04	8.37	U
	Ç-Sitosterol	18.62	74.54	4.6	
	Friedelan3one	16.39	31.68	4.18	U
	9,19Cyclolanost7en3ol	14.44	25.61	3.74	
	2,8,9Trioxa5aza1silabicyclo(3.3.3)undecane	17.32	37.1	3.13	
	9,19Cyclolanostan3ol,24methylene,(3á)	19.65	65.22	3.11	

Trt.	Compound Name	RT	Prob.	Area	Tp.
	7,9Ditertbutyl1oxaspiro(4,5)deca6,9diene2,8dione	15.06	92.1	2.99	
	Dodecanal	9.1	32.52	2.38	
	7,8Epoxylanostan11ol,3acetoxy	17.58	31.29	2.18	
	4,4,6a,6b,8a,11,11,14bOctamethyl1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a	16.81	37.2	1.74	
	Tetradecanoic acid	13.32	44.97	1.56	
	Obtusifoliol	14.04	57.44	1.29	U
	Phthalic acid	14.54	3.31	1.19	
	4H1,4Epoxy4a,7methanonaphthalene, 1,5,6,7,8,8ahexahydro	6.56	8.61	1.11	
CN	n-Hexadecanoic acid	15.48	64.4	12.56	H
	2Propenenitrile	15.4	0.68	9.59	
	Diphenyl sulfone	15.4	96.73	9.59	
	9,19Cycloergost24(28)en3ol	16.68	50.12	5.6	
	Decanedioic acid, bis(2ethylhexyl)ester	19.42	69.25	4.13	
	Sebacic acid, 2ethylhexylundecyl ester	19.42	2.19	4.13	
	2,8,9Trioxa5aza1silabicyclo(3.3.3) undecane 1methoxy	17.33	24	3.66	
	Ethanol	18	51.92	2.69	U
	Lup20(29)en3ol,acetate, (3á)	20.57	60.83	2.44	
	7,9Ditertbutyl1oxaspiro(4,5)deca6,9diene2, 8dionePropanoic acid	15.06	82.58	2.3	
	Tetradecanoic acid	13.33	67.64	2.09	
	Phenol, 2,4bis(1,1dimethylethyl)	10.34	31.32	1.42	
	Phenol, 2,4bis(1,1dimethylethyl)	10.34	31.32	1.42	
	Dodecanal	9.12	29.64	1.03	

Trt.- Treatments, RT – Retention time, Prob. – Probability of the compound, Area- Area in percentage , Tp – Type of peak, H – Highest peak, U- Unique peak; CC- control control, CP- control PEG, CN- control NaCl

Table 24: Metabolite variables in shoot tissues of rice seedlings from stressed set of seeds of high vigour (from first generation) subjected to different abiotic stresses

Trt.	Compound Name	RT	Prob.	Area	Tp.
SCHV	Diphenyl sulfone	15.40	90.67	12.9	H
	Diphenyl sulfone	15.41	97.16	11.27	
	Lup20(29)en3ol,acetate, (3á)	16.95	33.67	7.25	
	9,19Cyclolanost24en3ol,acetate, (3á)	17.16	42.5	7.16	
	Lupeol	15.48	53.87	3.55	
	Lup20(29)en3ol,acetate, (3á)	16.56	50.35	2.56	
	10Acetoxy2hydroxy1,2,6a,6b,9,9,12 aheptamethyl1,3,4,5,6,6a	19.49	12.23	2.55	
	9,19Cyclolanost24en3ol	17.65	26.11	2.28	
	Dodecanal	9.13	32.44	1.24	
	Ç-Sitosterol	18.53	43.25	1.17	
SPHV	Lupeol	14.94	81.68	11.05	H
	Lup20(29)en3ol,acetate, (3á)Lupeol	13.62	56.85	9.43	
	Lup20(29)en3ol,acetate, (3á)	13.62	56.85	9.43	
	Lupeol	15.17	72.72	4.48	
	2,8,9Trioxa5aza1silabicyclo(3.3.3) undecane 1methoxy	17.33	44.34	1.88	
	9,19Cyclolanost24en3ol,acetate (3á)	17.18	46.15	1.65	
	Pregna6,16diene11,20diol	17.95	28.56	1.5	
SNHV	Estra1,3,5(10)trien17áol	15.48	23.43	21.46	H
	Diphenyl sulfone	15.41	84.78	16.76	
	Lup20(29)en3ol,acetate (3á)	15.59	76.03	9.21	
	3,4Oxazolidinedicarboxylicacid	17.33	22.74	4.96	
	Ethanol, 2,2'(dodecylimino) bisThiourea	18	66.78	4.22	U
	Dodecanal	9.1	53.97	2.68	
	9-Octadecynoic acid	17.28	7.39	2.45	

Trt.	Compound Name	RT	Prob.	Area	Tp.
	Ethanol, 2phenoxyCyclobutane carbonitrile	6.59	41.98	1.52	U
	Phenol, 2,4 bis(1,1dimethylethyl)	10.36	45.51	1.42	
	Tetradecanoic acid	13.33	38.29	1.2	
	Decanedioic acid	19.43	56.45	1.17	
	lProline,Nbutoxycarbonyl, isohexyl ester	11.95	4.26	0.75	U
	Cinnamic acid	12.11	28.66	0.18	

Trt.- Treatments, RT – Retention time, Prob. – Probability of the compound, Area- Area in percentage, Tp – Type of peak, H – Highest peak, U- Unique peak; SCHV- stress control high vigour, SPHV- stress PEG high vigour, SNHV- stress NaCl high vigour

Table 25: Metabolite variables in shoot tissues of rice seedlings from stressed set of seeds of moderate vigour (from first generation) subjected to different abiotic stresses

Trt.	Compound Name	RT	Prob.	Area	Tp.
SCMV	9,19Cycloergost24(28)en3ol	17.35	53.27	12.88	H
	Diphenyl sulfone	15.41	96.96	9.86	
	Friedelan3one	19.74	36.97	9.73	
	n-Hexadecanoic acid	15.49	52.6	7.36	
	Friedelan3one	19.64	41.68	5.71	
	4,8,13Cyclotetradecatriene1,3diol,1,5,9 trimethyl12(1methylethyl)	19.64	4.91	5.71	
	Obtusifoliol	15.07	10.79	4.54	
	Obtusifoliol	14.95	60.09	4.51	
	Ethyl isoallocholate	17.93	11.25	4.42	
	Obtusifoliol	14.51	55.47	2.99	
	Obtusifoliol	14.55	55.47	2.44	
	ÇSitosterol	18.54	36.89	2.3	
	Obtusifoliol	14.17	18.86	1.42	
	Dodecanal	9.13	31.52	1.29	
	9,19Cyclolanost24en3ol,acetate, (3á)	16.7	14.38	1.05	

Trt.	Compound Name	RT	Prob.	Area	Tp.
SPMV	Diphenyl sulfone	15.4	96.84	18.35	H
	n-Hexadecanoic acid	15.48	49.32	16.28	
	3,4Oxazolidinedicarboxylicacid, 2(1methylethyl),3methylester, (2Rcis)	17.33	23.5	6.78	
	Ethanol, 2,2'(dodecylimino)bisThiourea,	18.01	34.64	4.68	
	9Ditertbutyl1oxaspiro(4,5)deca6,9diene2,8dionePropanoic acid	15.06	91.95	4.62	
	Ethanol, 2,2'(dodecylimino)bisThiourea,	17.91	56.28	4.07	
	Dodecanal	9.09	34.12	3.73	
	Ethanol, 2phenoxyCyclobutane carbonitrile	6.59	53.24	2.55	
	10Acetoxy2hydroxy1,2,6a,6b,9,9,12aheptamethyl1,3,4,5,6,6a,6b,7,8,8a,9,10	19.42	19.57	2.35	
	Phenol, 2,4bis(1,1dimethylethyl)	10.35	52.45	2.35	
	Octadecanoic acid	17.59	60.62	1.95	
	9,12Octadecadienoicacid, ethyl ester	17.28	6.22	1.81	
	Tetradecanoic acid	13.33	64.79	1.77	
	Ethanol, 2,2'(dodecylimino)bisThiourea,	17.95	54.56	1.74	
	Glycine	14.43	23.72	1.24	U
	3,5ditertButyl4hydroxybenzaldehyde	13.51	62.92	1.19	
	Glycine,	18.52	13.61	1.16	U
	lAlanine,Nethoxycarbonyl,octyl ester	16.68	10.06	1.12	U
	9,12,15Octadecatrienoicacid	18.91	17.75	1.02	
SNMV	Ethanol, 2,2'(dodecylimino)bisThioureaiPropyl	17.93	30.06	17.64	H
	n-Hexadecanoic acid	15.48	50.27	15.36	
	7,9Ditertbutyl1oxaspiro(4,5)deca6,9die	15.06	96.4	9.94	

Trt.	Compound Name	RT	Prob.	Area	Tp.
	ne2,8dione,Propanoic acid				
	2,8,9Trioxa5aza1silabicyclo(3.3.3)undecane	17.33	23.58	7.95	
	1Cyclohexyldimethylsilyloxy3phenylpropane	14.04	15.97	5.66	
	1Cyclohexyldimethylsilyloxy3phenylpropane	13.63	24.91	5.36	
	1Cyclohexyldimethylsilyloxy3,5dimethylbenzene	20.12	38.25	3.53	
	Diethyl Phthalate	11.44	68.57	3.08	
	Benzothiazole, 2(2hydroxyethylthio)Acethydrazide	15.59	75.03	2.31	
	Phenol, 2,4bis(1,1dimethylethyl)Phenol	10.36	57.52	2.27	
	12methyltetradecanoateOxiranedodecanoic acid	18.19	18.08	2.17	
	Diisooctyl phthalate	16.31	13.26	2.09	
	lProline,Nbutoxycarbonyl,isohexyl ester	11.95	3.84	2.01	U
	Octadecanoic acid	17.59	20.27	1.65	
	Tetradecanoic acid	13.33	49.58	1.57	
	Ethyl isoallocholate	14.44	30.26	1.54	
	lAlanine,Nethoxycarbonyl,octyl ester	16.68	10.08	1.3	U

Trt.- Treatments, RT – Retention time, Prob. – Probability of the compound, Area- Area in percentage, Tp – Type of peak, H – Highest peak, U- Unique peak; SCMV- stress control moderate vigour, SPMV- stress PEG moderate vigour, SNMV- stress NaCl moderate vigour

Table 26: Metabolite variables in shoot tissues of rice seedlings from fresh set of seeds subjected to different abiotic stresses

Trt.	Compound Name	RT	Prob.	Area	Tp.
FC	2H1Benzopyran6ol,3,4dihydro2,8dimethyl 2(4,8,12trimethyltridecyl),[2R[2R* (4R*,8R*)]	18.86	92.22	22.52	H
	Lupeol	16.83	72.64	12.88	
	9,19Cyclolanost24en3ol,acetate, (3á)	18.37	47.92	6.53	
	Diphenyl sulfone	15.41	95.97	5.45	
	1Heptatriacotanol	17.33	21.1	1.59	
	Dodecanal	9.11	35.46	1.31	
	NHexadecanoicacid	15.48	62.15	1.3	
	Dibutyl phthalate	15.54	8.38	0.91	
FP	Diphenyl sulfone	15.41	72.14	16.07	H
	Ç-Sitosterol	19.11	73.93	14.64	
	Á-Sitosterol	18.94	5.56	4.02	
	9,10Secocholesta5,7,10(19)triene1,3diol,25 [(trimethylsilyl)oxy]	17.6	18.91	1.06	
	Lupeol	16.14	68.65	2.81	
	Obtusifoliol	15.6	6.71	6.46	
	Estra1,3,5(10)trien17áol	15.49	12.21	10.63	
	9,19Cyclolanostane3,7diol	14.45	9.29	1.28	
	Dodecanal	9.12	40.77	1.76	
FN	Diphenyl sulfone	15.4	97.46	24.58	H
	NHexadecanoicacid	15.48	58.09	11.47	
	Dodecanal	9.12	31.88	5.07	
	7,9Ditertbutyl1oxaspiro(4,5)deca6,9diene2,	15.07	90.77	4.97	

Trt.	Compound Name	RT	Prob.	Area	Tp.
	8dione				
	Ethanol, 2,2'(dodecylimino)bis	18.01	39.93	4.85	
	Ethanol, 2phenoxy Cyclobutane carbonitrile	6.59	50.52	4.46	
	2,8,9Trioxa5aza1silabicyclo(3.3.3)undecane, 1methoxy	17.33	39.26	3.33	
	2Oxazolamine	5.18	13.19	2.5	
	Tetradecanoic acid	13.33	55.51	2.46	
	Benzothiazole, 2(2hydroxyethylthio)	15.6	91.39	2.42	
	Phenol, 2,4bis(1,1dimethylethyl)	10.36	54.65	2.42	
	Decanedioic acid, bis(2ethylhexyl)ester	19.43	41.8	1.95	
	Glycine	14.44	19.27	1.79	U
	10Heptadecen8ynoicacid, methyl ester, (E)	5.03	16.24	1.39	
	Hexadecanoic acid, methyl ester	15.15	66.74	1.24	
	2Propenoicacid, 3(4methoxyphenyl),2ethylhexyl ester	19.9	50.21	1.2	
	Methyl 9cis,11transoctadecadienoate	16.89	9.66	1.18	
	Anthracene, 9,10dihydro9methyl10propyl1	4.04	4.7	1.06	
	Glutarimide, N(2octyl)	18.52	34.44	1.02	

Trt.- Treatments, RT – Retention time, Prob. – Probability of the compound, Area- Area in percentage , Tp – Type of peak, Hp – Highest peak, U- Unique peak; FC- Fresh control, FP- Fresh PEG, FN- Fresh NaCl

Based on the highest peak area percentage, two compounds from each treatment was sorted out for further comparative analysis (Table 27).

Table 27: Comparative analysis of the top metabolite candidates across the four different types of seeds used as plant material for next generation stress characterisation

Treatments		Area (%)	Compound name
CONTROL	CONTROL	19.48	9,19Cyclolanost24en3ol
		11.94	Diphenyl sulfone
	PEG	13.32	Diphenyl sulfone
		11.08	n-Hexadecanoic acid
	NaCl	12.56	n-Hexadecanoic acid
		9.59	2-Propenenitrile
		9.59	Diphenyl sulfone
FRESH	CONTROL	22.52	2H1Benzopyran6ol
		12.88	Lupeol
	PEG	16.07	Diphenyl sulfone
		14.64	Ç -Sitosterol
	NaCl	24.58	Diphenyl sulfone
		11.47	n-Hexadecanoic acid
STRESS-HIGH VIGOUR	CONTROL	11.27	Diphenyl sulfone
		7.25	Lup20(29)en3ol,acetate, (3á)
	PEG	12.9	Diphenyl sulfone
		11.05	Lupeol
	NaCl	21.46	Estra1,3,5(10)trien17áol
		16.76	Diphenyl sulfone
STRESS-MODERATE VIGOUR	CONTROL	12.88	9,19Cycloergost24(28)en3ol
		9.86	Diphenyl sulfone
	PEG	17.64	Ethanol
		15.36	n-Hexadecanoic acid
	NaCl	18.35	Diphenyl sulfone
		16.28	n-Hexadecanoic acid

The GC-MS/MS based metabolite profiling revealed that certain compounds appeared to be common in most of the treatments but in different intensities and some others were specific to the particular treatments. Compounds like diphenyl sulfone appeared in the highest peak category except for some treatments.

9,19Cyclolanost24en3ol is another compound which appeared frequently in the GC-MS/MS analysis. The other forerunner metabolites include hexadecanoic acid, 2Propenenitrile2H1Benzopyran6ol, Lupeol, çSitosterol, Lup20(29)en3ol,acetate, (3á), Estra1,3,5(10)trien17áol *etc*.

DISCUSSION

5. DISCUSSION

Differential and dynamic response of rice vvariety Uma to drought stress imposition at vegetative stage and the associated impact on its physiological, morphological and biochemical parameters in successive growth stages as well as in seedling stage of second generation is discussed in this chapter.

5.1Effect of drought stress on physiological characters of Uma

Relative water content (RWC) estimated immediately after stress imposition revealed that stressed plants showed lesser RWC compared to the well watered ones (Table 2). According to Ha (2014), the relative water content in the leaves and water potential in the shoots of rice seedlings declined in response to drought, with a higher decrease at 60 hours time duration following treatment than at 48 hours duration. These findings were in agreement with the dehydration symptoms of leaf rolling in rice seedlings during non-watering periods of the present study. Another physiological parameter studied was membrane thermostability (MTS) which indicates the sustainability of cell membrane and relative electrolyte leakage (EL) against the stress exposure. MTS was examined in both non-stressed and stressed plants and the results revealed that the well-watered ones possessed higher membrane thermostability indicating the lesser electrolyte leakage and vice versa under drought stress, which hampered the membrane thermostability leading to high electrical conductivity or electrolyte leakage (Table 2). Similar results were obtained in a study conducted by the Agarie *et al.* (1998). The electrolyte leakage was assessed from leaf tissue caused by desiccation with polyethylene glycol (PEG) and high temperature to determine the integrity of cell membranes to evaluate the good impacts of silicon on rice plant stress tolerance. With the increase in the level of Si in leaves, it was observed that EL induced by 30% and 40% PEG solutions was diminished.

Chlorophyll content has prime importance when it comes to the case of drought stress. As a direct indication of the osmotic stress, stressed plants showed relatively lower chlorophyll content compared to the non-stressed ones (Table 2). It was quoted in the study of Singh *et al.* (1998), that the highest reduction in chlorophyll content was recorded in Swarna Sub-1 and lowest in Nagina among five rice genotypes exposed to seven days of continuous drought stress at reproductive stage.

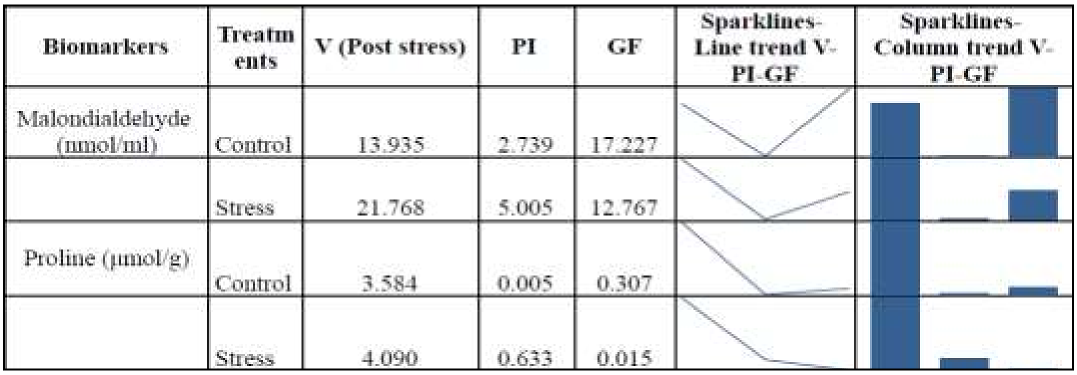
5.2 Effect of drought stress on biochemical characters of rice variety, Uma

Proline is one of the main amino acids which play key role in the osmolyte regulation when it comes to drought stress. Dynamics of this compound has been studied over successive growth stages starting from the vegetative stage where the drought stress was imposed. A differential dynamics of proline was observed in the different phenophases of the Uma variety. In the vegetative stage, stressed plants observed a higher amount of proline content compared to that of control plants. When it comes to the panicle initiation stage, there was no significant difference between the control and stressed plants. But in grain filling stage, control plants showed higher proline content than the stressed ones (Table 3). According to Jabasingh *et al.* (2013) increased proline buildup during water stress has a negative impact on *Oryza sativa* growth and development, as well as biochemical contents, due to lower photosynthesis in the leaves of water stressed plants. The buildup of proline in the leaves of water-stressed plants could be used as a stress indicator. Proline may operate as a free radical scavenger and may be more significant in overcoming stress than operating as a basic osmolyte in dealing with water stress. Increased proline buildup could be due to diminished proline oxidase. Increased proline levels in stressed plants could be an adaptation to cope with the stress. Proline accumulates in stressed plants and provides energy for growth and survival, allowing the plant to withstand stress. Proline may protect the structure of proteins and membranes from damage, as well as prevent enzyme denaturation.

Malondialdehyde (MDA) is one of the major indicators of lipid peroxidation process taking place inside plant cell especially under drought stress. MDA content estimated immediately after stress induction during the vegetative stage and at the panicle initiation post recovery exhibited significantly higher accumulation in the stressed tissues than the control, although the levels dropped drastically in the latter. However, in grain filling stage, the MDA content was lower in the same stressed plants compared to that of control ones (Table 4). According to Gusain *et al.* (2015), the amount of MDA, a natural result of oxidation of polyunsaturated fatty acids present in the membrane produced by the formation of free oxygen radicals, was utilised to detect lipid peroxidation. Drought and *Trichoderma* isolates had a substantial impact on MDA content. At all stress levels, the MDA content was greater in *Trichoderma* untreated (control) plants. Under drought stress, the buildup of MDA was the lowest in the treatment that reduced the accumulation of lipid peroxides. The levels of proline and MDA were found to drop and hike in a fashion, in the subsequent growth stages post stress exposure, possible due to their dynamic regulation in other developmental processes.

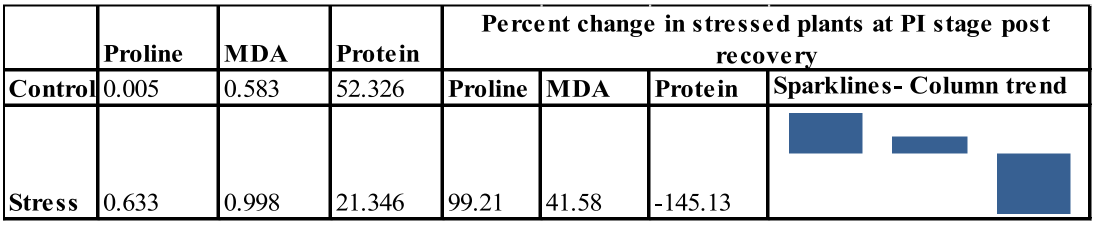
Proteins have significant role in attributing membrane persistence and other structural and biochemical parameters of the plant cell when it comes to the case of drought stress. The differential and dynamic variations in potential biomarkers such as proline and MDA over the different growth stages of rice variety Uma post stress recovery, led to the monitoring of total soluble protein content across the treatments during the panicle initiation stage. The results indicated that the stressed plants possessed lower total soluble protein in comparison with the well watered ones, even after the re-watering and recovery of stressed ones after the drought stress exposure in the vegetative stage (Table 5). This was in line with the findings of Hanif *et al.* (2021), wherein a pot experiment was carried out to determine the efficacy of foliar applied proline in reducing the effects of heat and drought on rice. It was observed that total soluble protein (TSP), glycine betaine (GB), and leaf proline levels were reduced under a stressful setting, but malondialdehyde (MDA) levels increased. In comparison to control, simultaneous heat and drought stresses resulted in the greatest decrease in osmoprotectants and an increase in MDA levels. The exogenous application of proline significantly increased TSP levels under multiple stress exposures.

The dynamics in the metabolite accumulation across the different phenophases revealed that the stressed tissues accumulated lesser candidate biomarkers, MDA and proline during the grain filling stage in comparison to the non-stressed control (Plate. 18). It was also interesting to note that in the panicle initiation stage immediately succeeding the stress exposure, the metabolite levels were still higher in the stressed plants with no concomitant increase of proteins (Plate. 19).



Biomarkers	Treatments	V (Post stress)	PI	GF	Sparklines- Line trend V-PI-GF	Sparklines- Column trend V-PI-GF
Malondialdehyde (nmol/ml)	Control	13.935	2.739	17.227		
	Stress	21.768	5.005	12.767		
Proline (µmol/g)	Control	3.584	0.005	0.307		
	Stress	4.090	0.633	0.015		

Plate 18: The dynamics in the metabolite accumulation across the different phenophases (V- Vegetative, PI- Panicle initiation, GF- Grain filling)



	Proline	MDA	Protein	Percent change in stressed plants at PI stage post recovery			
Control	0.005	0.583	52.326	Proline	MDA	Protein	Sparklines- Column trend
Stress	0.633	0.998	21.346	99.21	41.58	-145.13	

Plate 19: Percent change in stressed plants at panicle initation (PI) stage in post recovery

5.3 Effect of drought stress on yield attributes

Spikelet fertility was assessed for both treatments and the results revealed that control treatments exhibited higher spikelet fertility compared to those which experienced a set of stress during its vegetative growth (Table 6). This was in accordance with Rang *et al.* (2011) who assessed the response of five rice genotypes exposed to high temperature, water stress, and a combination of high temperature (HT) and water stress during flowering. In comparison to control conditions, water stress (WS) imposition by lowering the relative water content (RWC) to 50–60% resulted in a significant fall in spikelet fertility among the five genotypes. Similarly, it was discovered that under both WS and HT+WS exposures, spikelets inside the flag-leaf sheath were completely sterile, which contributed to the overall loss in spikelet fertility. Anthesis and fertilization were not possible because spikelets were confined within the leaf sheath.

There was a significant reduction in grain yield in stressed plants in comparison to control. The assessment of test weight in each set of seeds collected from the control as well as stressed plants indicated that there was no significant difference between them, probably due to the stress exposure in the vegetative stage. This was as interesting character which helped us to compare the response among se eds collected from stressed and control plants. Usually under water stress, grain size, shape, and ultimately weight decrease due to reduction in the amount of water in the plant, which inhibits reproductive development and grain growth as has been reported in a study where five rice genotypes exhibited considerable variation in test weight when water was withheld for seven days during the reproductive stage (Singh *et al.,* 2018). Swarna Sub1, NDR 102, NDR showed more change in test weight and minimal reduction was observed in Nagina 22. Five genotypes of rice were grown under pot culture and.

Correlation analysis between the post-stress characteristics of the stressed and non-stressed control plants revealed that chlorophyll content, relative water content and membrane thermostability had positive correlation with the yield attributes such as spikelet fertility and grain yield (Table 7). This was in accordance with Pirdashti *et al.* (2009), who assessed the plant response to treatments such as drought stress at different levels at the vegetative, blooming, and grain filling stages in four rice cultivars. Drought stress increased days to blooming and leaf rolling in different cultivars during the vegetative growth stage and increased proline levels at various stages of development. Drought stress reduced the amount of chlorophyll and the amount of water in different cultivars. There was a substantial and positive link between chlorophyll content, proline content, and relative water content, as well as between these features and grain yield, as has been observed in our study.

Candidate biomarker MDA (malondialdehyde) content at grain filling stage has a positive correlation between the yield attributes like spikelet fertility and grain yield (Table 7). Melandri *et al.* (2020) investigated central metabolism and oxidative stress status in the flag leaf of 292 indica rice (*Oryza sativa*) accessions using a mix of metabolomics and high-throughput colorimetric tests. Plants were grown in the field and exposed to either well-watered or drought conditions during the reproductive stage to discover the metabolic mechanisms linked to drought-induced grain yield decrease. From the analysis, two variables were identified having the highest correlation coefficients with grain yield loss, the first negative and the second positive, are dehydro ascorbate reductase (DHAR) and malondialdehyde (MDA) respectively. As a result, malondialdehyde (MDA) was a reliable biomarker for grain yield loss, implying that drought-induced lipid peroxidation is the primary restriction in stage specific drought stress exposure in rice which has also been proven to be true for rice variety Uma.

5.4 Next generation response under PEG (-4 bar) induced osmotic stress and NaCl (100 mM) induced salt stress

5.4.1 Growth parameters of the selected seeds

The speed of germination of seeds from control and stressed plants of the previous generation, as well as a new batch of fresh seeds of the variety Uma, was investigated. Surprisingly, based on the speed and vigour of the sprouted seeds, there were two distinct groups of seeds among those gathered from the first generation stress exposed plants (Experiment 1). The fresh set of seeds and the control set of seeds from the previous generation experiment both germinated at the same rate. The high vigour seeds among the stress-exposed class of plants germinated at a relatively fast rate (Table 12). It was recently reported that grain germination was slightly slower in fields where there was a water shortage than in fields where there was a plentiful supply of water and amid water shortage, the germination percentage and speed were reducedin rice (Chao *et al.,* 2021). Such genotypic variations in germination speed under favorable conditions are considered to impact early vigour and grain yield in drier conditions, which are similar to the concept of memory responses of a stress exposure.

The careful examination of the seedling vigour index of seeds collected from control and stressed of previous generation and a new set of fresh seeds of Uma was examined, indicated that it was significantly different for the two classes of progenies of the first generation stressed plants. High vigour seedlings showed a higher seedling vigour index and the lowest was recorded for the moderate vigor seedlings (Table 13). In a study by Salam *et al.* (2017) rice varieties/genotypes were screened for drought tolerance and shortlisted based on seedling vigour index, and characterized for drought tolerance using microsatellite markers. Uma was identified as one among the rice varieties maintaining optimum seedling vigour index even under stressed condition. It was opined that further research in marker based trait dissection is required to decipher the crucial drought tolerant mechanism(s).

5.4.2 Growth characterization under abiotic stresses in next generation as well as fresh set of seedlings

Plant parameters such as root length and shoot length of the seedlings across different treatments in the abiotic stress experiment, wherein the different sets of seeds viz. seeds from control and stressed (high vigour and moderate vigour) plants of first generation as well as a fresh set of seeds, were recorded.

The growth response of seedlings to PEG (-4 bar) and NaCl (100 mM) induced stresses was on par with each other with respect to both shoot length as well as root length in case of control set of seeds from first generation (Table 13). The non-stressed high vigour seedlings exhibited significantly higher root length as well as shoot length than PEG (-4 bar) and NaCl (100 mM) treatments, which were on par in terms of root growth. However, in case of shoot length, salt stressed as well as the control treatments was on par with each other (Table 13). In case of moderate vigour seedlings, both root and shoot parameters were significantly higher in control treatments than stressed seedlings, although the growth under PEG (-4 bar) and NaCl (100 mM) stressed was on par (Table 13). Root length and shoot length of seedlings belonging to the fresh set were significantly higher in control treatment than under stress; with each treatment being significantly different from each other in case of both these parameters (Table 13).

Percent reduction in growth over control reveals a lot of information when it comes to the comparison across the generations as well as the fresh seed lot, after osmotic and salinity stress induction. It was very interesting to note that percent reduction in growth over control of first generation seedlings (as part of standardization experiment) was higher in comparison to any of the treatments of its progeny under osmotic as well as salt stress (Fig. 1). However, the most significant effect was the drastic reduction in percent reduction in growth over control in high vigour set of seedlings (second generation) than its parent set of seedlings (first generation) under the same level of salt stress (100 mM) (Fig. 1). It was striking to note that this growth reduction was also lower than the moderate vigour set of seedlings which evolved under same circumstances of high vigour seedlings (Fig. 1). This highlights an indication of transfer of some memory or warning signal from the previous generation to the progenies when they were exposed to the same extent of stress. Furthermore, fresh set of seedlings did not show any specific trend towards the percent reduction over control data.

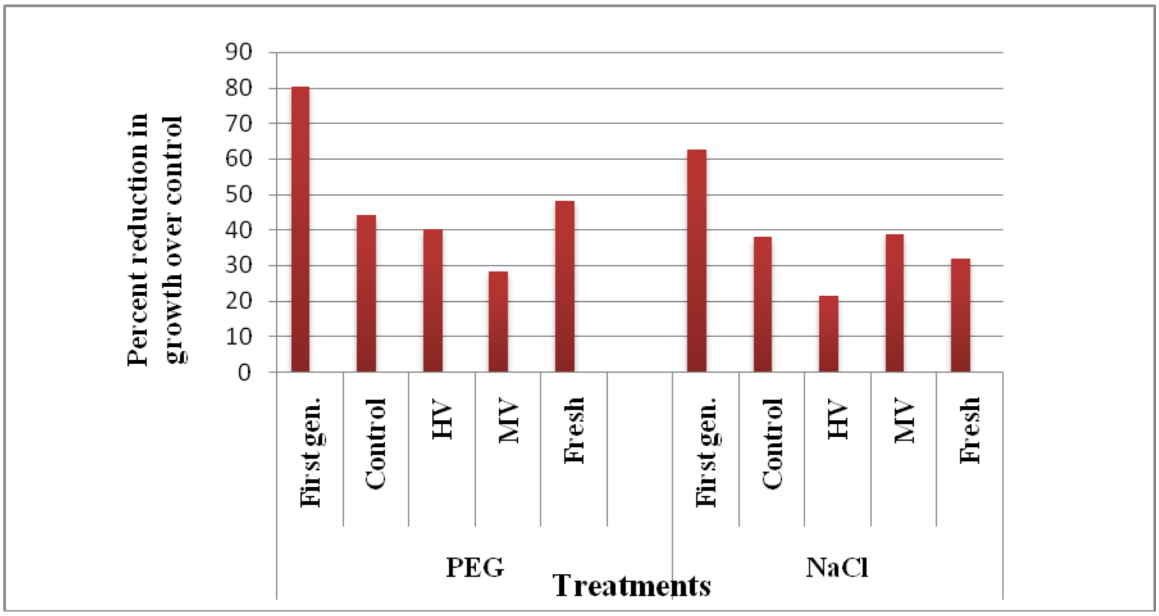


Fig. 1: Percent reduction in growth (total seedling length) over control under PEG (-4 bar) induced osmotic stress and NaCl (100 mM) induced salinity stress of first generation seedlings after stress level standardization experiment (First gen.), and seedlings of control, high vigour (HV), moderate vigour (MV) and fresh set of plants of Experiment 2.

Even under the recovery growth of plants after stress, percent reduction in growth over control was less under NaCl stress for those plants which exhibited high vigour in the next generation (Fig 3) compared to that of PEG stress (Fig 2) and also in comparison to plants of moderate vigour. This is probably due to the invisible exchange of memory in between generations. However, the percent reduction in recovery growth of seedlings raised from control (first generation) as well as fresh seed lots does not show such a tremendous variation under NaCl and PEG stresses (Table 18 and Table 21.)

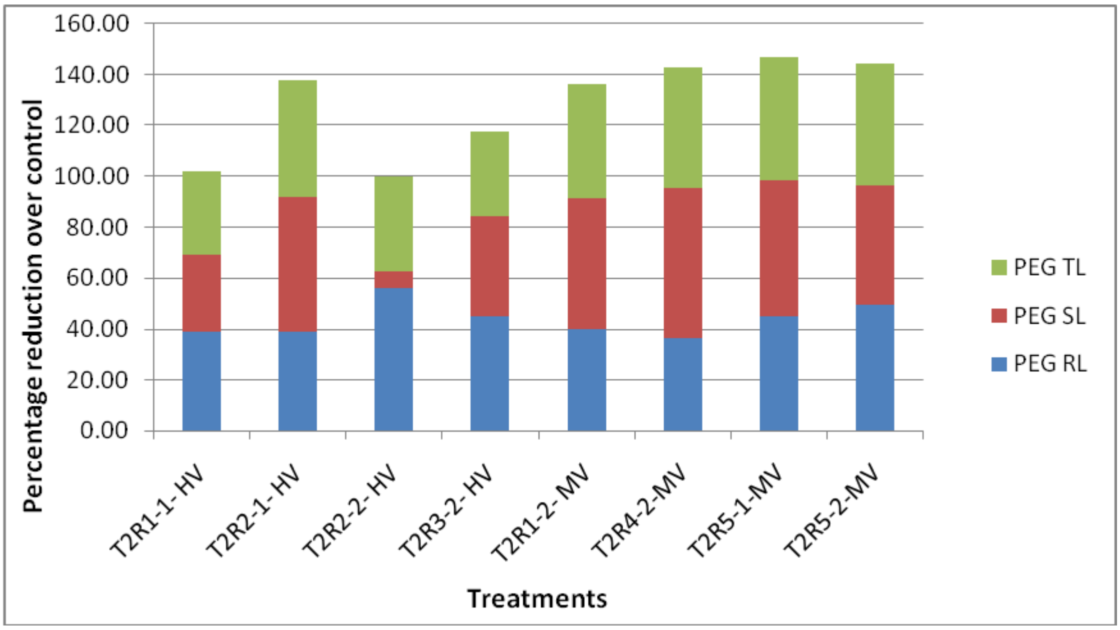


Fig 2: Percent reduction in recovery growth over control under PEG induced osmotic stress of high vigour and moderate vigour plants (SL-Shoot length; RL-Root length; TL-Total seedling length)

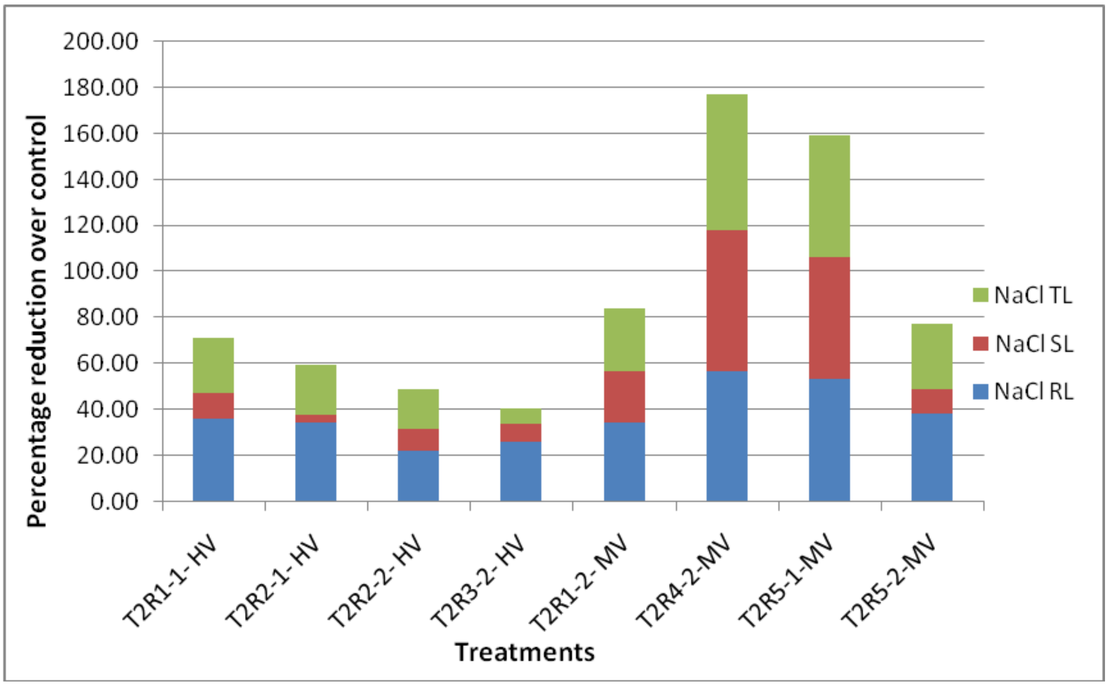


Fig 3: Percent reduction in recovery growth over control under NaCl induced salinity stress of high vigour and moderate vigour plants (SL-Shoot length; RL-Root length; TL-Total seedling length)

5.4.3 Biochemical characterization of the next generation seedlings

Malondialdehyde (MDA) estimated in high vigour and moderate vigour seedlings under the imposed abiotic stress treatments exhibited a bias in growth response. NaCl stressed seedlings accumulated significantly lesser amount of MDA in all sets of seed material used in comparison to control (Table 22). A similar trend was observed in the study conducted by Kumar *et al.* (2010) wherein the MDA content in transgenic and non-transgenic plants under 150 mM NaCl stress was compared to examine if free radical production and membrane damage levels were different. Under salt stress, all transgenic plants over producing a stress protectant metabolite, proline considerably reduced lipid peroxidation than non-transgenic plants, indicating that transgenic lines have lesser membrane damage.

Gas Chromatography-Mass Spectrophotometry (GC-MS/MS) study was performed on seedlings subjected to abiotic stresses using seeds obtained from control and stressed plants from the first generation, as well as a fresh batch of seeds of rice variety Uma, in order to determine the metabolite dynamics owing to stress exposures in the next generation. Due to the minimal derivatization stages included in the sample extraction, the metabolites examined were confined to volatiles and a smaller spectrum. The interpretations of the GC spectra were based on the relative abundance or area percentage, as well as the likelihood of the compound across the time period. The compound with the highest peak (H) has a substantially higher area percentage over time and unique peak (U) denoted those peaks that are specific to the treatment (Table 27).

Two compounds from each treatment were chosen based on the highest peak area percentage (Table 27). Diphenyl sulfone appeared as the highest or second highest peak in most of the treatments. Diphenyl sulfone is a defence related volatile chemical especially active against brown plant hopper (*Nilaparvata lugens*) which has been identified as feeding induced volatiles in rice cultivars (Navyashree *et al.,* 2019). Moreover, it possesses anti-bacterial activity, anti-oxidant activity by free radical scavenging and has also influence on the production of MDA levels in the plants (Shi *et al.,* 2015). 9,19Cyclolanost24en3ol is another compound which appeared frequently in the GC-MS/MS analysis. Another name of this compound is cycloartenol, which is a plant-derived triterpenoid that belongs to the sterol class. It is the starting step for the production of practically all plant steroids. Sterol synthesis has relationship between the reactive oxygen contents of the cell during stress condition according to Kolo (2016). As a biomarker, hexadecanoic acid stimulated root differentiation and boosted germination rates in accordance with Hu and Zhou (2014). 2Propenenitrile is an antimicrobial nitrogen compound which was profiled by GC-MS analysis of *Feronia elephantum Correa* (Muthulakshmi *et al*., 2012). 2H1Benzopyran6ol is a kind of alpha tocopherol which possesses anti-oxidant activity (Moongngarm *et al.,* 2012). Lupeol has been reported as a main constituent of epidermal wax (Liu *et al.,* 2021), which could be an important metabolite conferring stress tolerance. Moreover it has pertinent roles in helping in root nodulation (Phillips *et al.,* 2006). Lup20(29)en3ol,acetate, (3á) is a lupeol derivative which also appeared in the GCMS/MS analysis. çSitosterol has involvement in defense mechanism of plants (Griebel and Zeier, 2010), whereas β-sitosterol has influence on glucose and lipid metabolism. Estra1,3,5(10)trien17áol present in the essential oil of *Sesamum radiatum* has a role in supporting the plant's cardiovascular and estrogenic action, as well as its medicinal abilities for male infertility, constipation, fungal and bacterial infections, and bruising (Ogunlesi *et al.,* 2010). Ethanol has been reported to play a crucial role in detoxifying reactive oxygen species in salinity stress effected plants (Nguyen *et al.,* 2017).It was discovered that ethanol improved high-salinity stress tolerance in *Arabidopsis thaliana* and rice. Targeted microarray analysis in *A. thaliana* seedlings to investigate the molecular mechanism behind ethanol-induced tolerance revealed that under salt stress, ethanol increased the expression of genes encoding ROS scavengers and transcription factors linked with reactive oxygen species (ROS) signalling and high-salinity tolerance (Nguyen *et al.,* 2017). They used to undertake. In our study also a high peak of ethanol was detected in salt stress case of the moderate vigour plants (Table 25), which could probably be the reason why they accumulated less MDA content under stress in comparison with control (Table 22).

When it comes to unique peaks, amino acids such as glycine (Gly) appeared in case of treatments such as fresh set (Table 26) under salt stress and high vigour set (Table 24) of seedlings under osmotic stress. Proline was also found to be accumulated in minimal quantities in salt stressed seedlings in both moderate vigour (Table 25) and high vigour (Table 24) sets of seedlings. Alanine was also visualised in both salt stressed (Table 25) and osmotic stressed (Table 25) seedlings in moderate vigour seedlings. In accordance with Melandri*et al.* (2020) drought stress created a novel (stress-specific) cluster encompassing Asp, Glu, Gly, Ser, pyroglutamic acid, and allantoin which had a greater association within and between all leaf metabolite classes than under control conditions. Glycine and Ser, two amino acids from the stress-specific cluster, are well-known photorespiration markers. The presence of this cluster under drought conditions was reported to enhance physiological processes such as stress-induced senescence, nutrient recycling, and photorespiration (Melandri *et al*., 2020). The presence ester derivatives of important amino acids indicate their probable accumulation in response to the employed treatments.

Shi *et al.* (2015) professed about the relationship between the sulphone derived compounds and MDA content in the plants. A study on the influence of sulfone derivative 1,3,4-oxadiazole moieties were found to have good antibacterial activity against rice bacterial leaf blight caused by the pathogen *Xanthomonas oryzae pv. oryzae* (Xoo). Moreover, the MDA contents showed that a sulfone derivative might limit the increase in MDA content in rice, consequently improving the host's disease resistance.

In this context, the nature of sulfone derivative, diphenyl sulfone obtained from our GC-MS/MS analysis was assessed as a function of fold change of MDA as well as diphenyl sulfone contents across the different treatments.

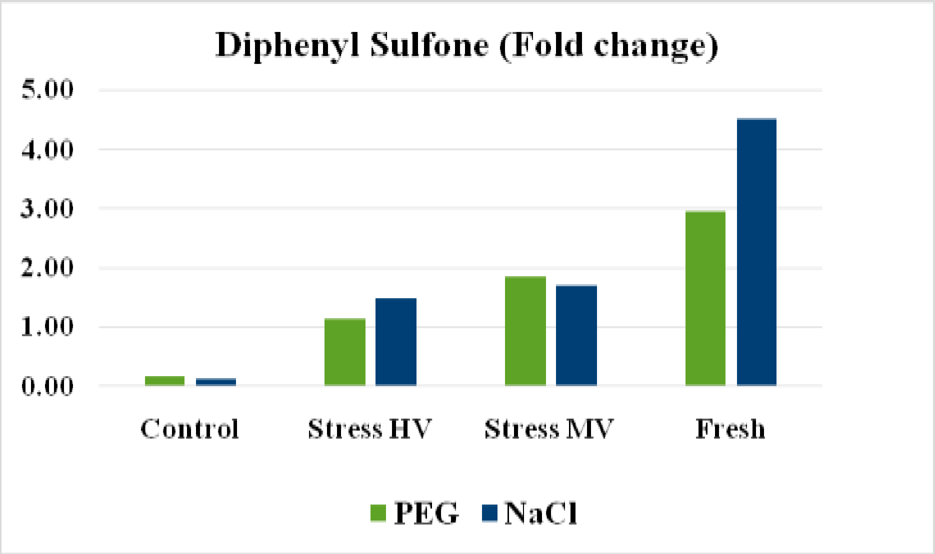


Fig 4: Fold change in diphenyl sulfone over control under different treatments- Control, High vigour (HV), Moderate vigour (MV) and fresh set of seedlings

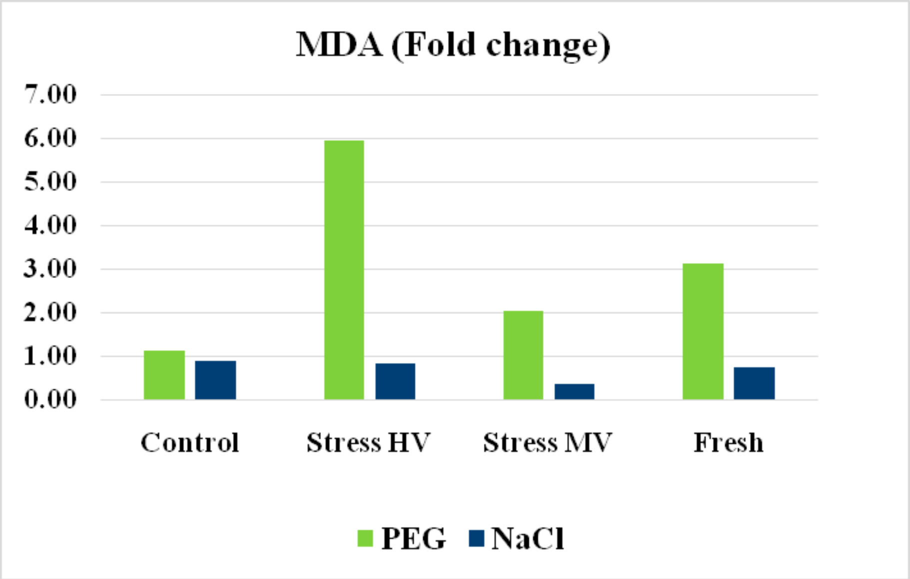


Fig 5: Fold change in malondialdehyde over control under different treatments- Control, High vigour (HV), Moderate vigour (MV) and fresh set of seedlings

The comparison between fold changes of MDA under PEG and NaCl induced stresses points towards the findings of Shi *et al.* (2015), that there were corroborative inferences from diphenyl sulfone contents as well as the MDA contents, wherein there were proportional variations in both the metabolites. There may be a prospective down regulation of MDA contents by the antioxidant potential of diphenyl sulfone as reported in the study of Shi *et al.* (2015). It was also interesting to note that NaCl induced salinity stress induced the variations in diphenyl sulfone and MDA contents; however, PEG induced osmotic stress exhibited a distorted pattern with no specific trend (Fig. 4 and 5).

The diphenyl sulfone levels were higher in moderate vigour seedlings, under both osmotic and salinity stresses (Fig. 4), with an inverse relation with that of the MDA contents (Fig. 5) specifically under NaCl induced salinity stress in comparison to that of stressed high vigour seedlings, suggesting a possible role of diphenyl sulfone in triggering a stress memory with a cross tolerance potential (Plate 20). Since no such inferences were drawn from the response to PEG induced osmotic stress, it can be assumed that the probable upstream/downstream regulatory signals are different for each distinct stress, as has been seen in our study with a drought stress exposure in one generation equipping the descendants for a better salinity stress response in the next generation (Plate 20 and Plate 21).

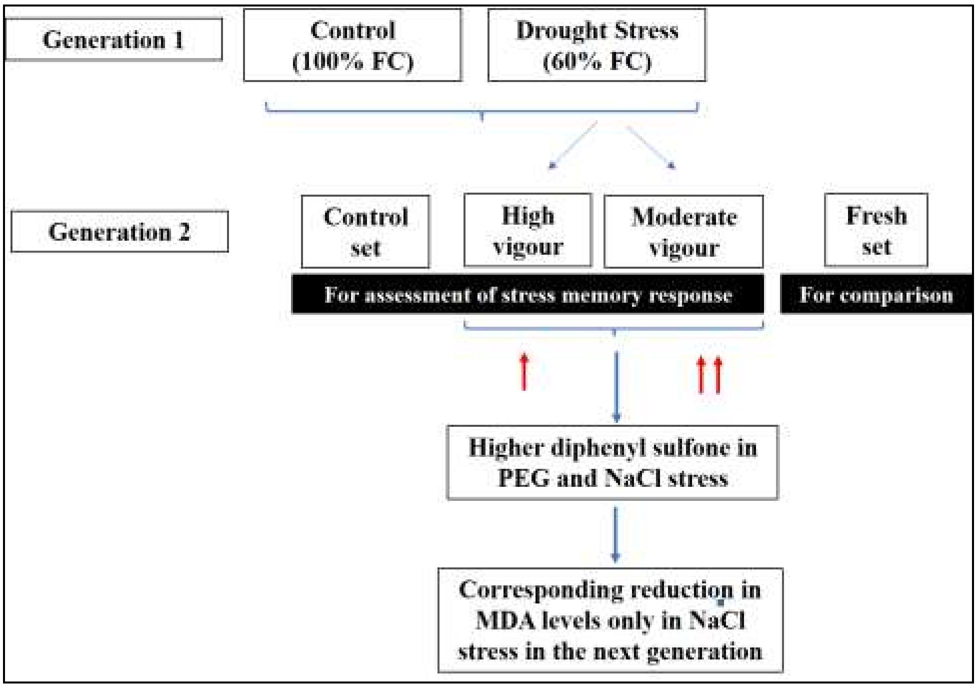


Plate 20: Probable cross generational memory response under multiple abiotic stress exposures

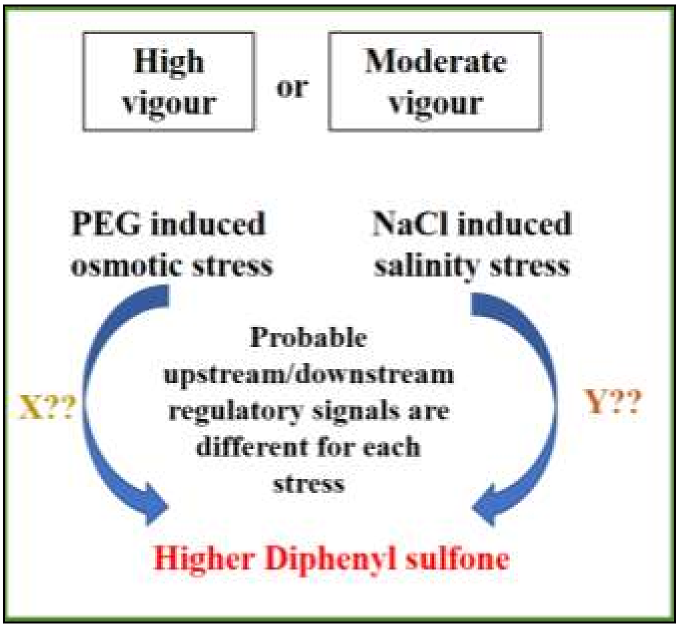


Plate 21: Possible dynamics of diphenyl sulfone across different abiotic stresses in modulating malondialdehyde (MDA) levels

SUMMARY

6. SUMMARY

Rice (*Oryza sativa* L.) is an important cereal crop consumed by millions of people as a staple meal. The rapid growth of the world's population necessitates increased rice production all throughout the world. Rice growth and development are influenced by environmental changes. Drought is one of the abiotic stressors that has the greatest influence on rice development and output. Plants have a number of physiological and biochemical responses to combat such stress exposures.

The present study has two experiments, in which first one was conducted in net house of Department of Plant Physiology, College of Agriculture, Vellanikkara. The two treatments included a set of plants were exposed to a gravimetric drought stress at vegetative stage and another set of non-stressed plants maintained under well-watered conditions throughout the lifespan. The second experiment was undertaken using the seeds obtained from the treatments of first experiment along with a new set of fresh seeds of rice variety, Uma. Standardized stress levels identified by a preliminary screening, were used to employ osmotic as well as salt stresses in the second experiment. Corresponding morphological, physiological and biochemical parameters were assessed in both the experiments so as to study the stress influence over the generations.

The salient findings of the study are as follows

1. Relative water content, membrane thermostability and chlorophyll contents of the stressed and non-stressed treatments assured the accurate stress imposition and served as ideal parameters for assessment of stress damage.
2. In the assessment of biochemical responses, the leaf proline content exhibited a differential pattern in its accumulation across the different growth stages post stress. Even though in the vegetative stage, immediately after stress, proline content was higher in the stressed plants compared to the non-stressed control, the levels dropped drastically post recovery, in the succeeding stages. Malondialdehyde content was also differentially regulated over the different

growth stages. Protein content assessed at panicle initiation stage revealed a lower level in stressed plants in comparison to non-stressed control.

3. Yield attributes such as grain yield and spikelet fertility were remarkably lesser in the stressed treatments compared to the control, with no variations in test weight.
4. Chlorophyll content, relative water content and membrane thermostability post stress and candidate biomarker MDA (malondialdehyde) content at grain filling stage had a positive correlation between the yield attributes like spikelet fertility and grain yield.
5. The speed of germination of seeds from control and stressed plants of the previous generation, as well as a new batch of fresh seeds of rice variety Uma, was investigated. Surprisingly, based on the speed and vigour of the sprouted seeds, there were two distinct groups of seeds among those gathered from the first generation stress exposed plants. The high vigour seeds among the stress-exposed class of plants germinated at a relatively fast rate. The fresh set of seeds and the control set of seeds from the previous generation experiment both germinated at the same rate. Seedling vigour index of the high vigour seedlings were higher compared to the other treatments. Moderate vigour seedlings from the first generation recorded lesser seedling vigour index.
6. In case of shoot and root parameters, high vigour seedlings managed to grow profusely in stress condition compared to other treatments. A similar trend was also observed in case of MDA (malondialdehyde) contents as well, especially in the salt stress treatment. This trend was evident even in the comparison of percent reduction in growth over control across generations. High vigour seedlings thrived well in the next generation stress screening experiment in comparison to the first generation standardization experiment under same salt stress levels. This can be an indication of transfer of some invisible messages across the generations to survive under the stress.
7. Metabolite profiling was done over the treatments to assess the metabolites in each treatment. Several metabolites exhibited abundance or specificity as evidenced by highest peak or unique peak respectively. Amino acids like proline, glycine, alanine *etc*. were also present in some treatments as the

indicators of stress damage repairs. Most noticeable presence was the appearance of sulfone derivative called diphenyl sulfone. There were corroborative inferences from diphenyl sulfone contents as well as the MDA contents, wherein there were proportional variations in the two especially in the NaCl induced salinity stress in all treatments.

Conclusion

A round of stress exposure at vegetative stage in rice variety, Uma influenced several morpho-physiological and biochemical parameters at different stages of the crop. There was an indication of the transmission of stress induced signal even in the next generation as evidenced by profuse growth and lower MDA contents under different stresses at seedling stage

Future line of work

Identification of novel potential biomarkers and physiological traits that aid in stress memory for better adaptation can form the basis for crop improvement initiatives.

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DIFFERENTIAL AND DYNAMIC RESPONSE OF RICE VARIETY UMA UNDER VARYING DROUGHT STRESS REGIMES

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Rice has the evolutionary advantage of being semi-aquatic, making it one of the world's most important crops. As a result, it has fewer water-scarcity responses and is especially vulnerable to drought stress. Climate change has put rice production in jeopardy today. According to recent studies, rice yield decreases as result of climate change such that when drought frequency and rainfall pattern changes, rice output is lowered. Rice is the principal staple grain grown in Kerala, with around 600 types used across the state's paddy fields. Uma (MO 16), developed by Kerala Agricultural University's Rice Research Station, Moncompu, is one of the most popular rice varieties in Kerala, which is currently planted in more than 60% rice growing areas in the state. It also has adaptability towards wide range of environmental conditions as well, even though environmental variations limit the potential yield of variety Uma.

In this context, the present study on differential and dynamic response of variety Uma under drought stress at vegetative stage and its impact on stress responses of progeny, were attempted with the main objective of identifying potential drought stress specific biomarkers representing a trend in metabolite dynamics across generations. This approach was aimed to demonstrate in a simple way that a natural drought stress exposure can influence stress responses of rice variety, Uma in the present as well as future generations, with an aim of identification and selection of potential traits for inclusion in crop improvement programmes. The study was carried out at the Department of Plant Physiology, College of Agriculture, Vellanikkara, Thrissur as two experiments- a drought stress imposition study in the net house facility and a laboratory study in the next generation. As part of the first experiment, drought stress (60% field capacity; FC) was imposed to rice variety, Uma at vegetative stage by gravimetric approach, simultaneously maintaining a non-stressed control at 100% FC by regular watering. The experiment was laid out in completely randomized design in customized rain-out shelter like structures in the net house. Physiological parameterswere assessed immediately after stress exposure

for assessment of stress damage and it was noted that there was a significant reduction in chlorophyll content (36.2%), membrane thermostability (78.5%) and relative water content (23.5%) under stress in comparison to non-stressed control tissues, ensuring effective stress exposure. Biochemical markers such as proline and malondialdehyde (MDA) estimated immediately after stress and after recovery across different phenophases, revealed a differential pattern in their regulation. The levels of both the biomarkers were significantly higher (14% and 70.7% respectively) under stress which dropped drastically at panicle initiation stage, followed by an increase in the grain filling stage. However, it was interesting to note that correspondingly both the biomarker metabolites exhibited lesser accumulation (95.1% and 25.2% respectively) in comparison to the non-stressed control plants in grain filling stage. The differential dynamics of proline as well as MDA over different phenophases when related to the protein levels estimated at panicle initiation stage, revealed that there was no concomitant recovery in protein levels even after stress recovery. The non-stressed control and stressed plants were raised to maturity and harvested seeds were used for the second experiment for assessing the stress memory response in the next generation. Yield attributes assessed also showed significant difference between the stressed and non-stressed plants.

The second experiment to assess the stress memory response aimed at comparative stress response assessment under salinity as well as osmotic stresses at seedling stage in the next generation. In this context, a standardization experiment was carried out using different concentrations of NaCl and polyethylene glycol (PEG 6000) to impose salinity and osmotic stresses respectively, to uniformly germinated seedlings of rice variety, Uma, to ascertain sub-lethal stress levels. From the standardization experiment, sub-lethal levels of salt (100 mM NaCl) and osmotic (-4 bar) stresses were arrived at based on the percent reduction in growth over control. The progenies of the first experiment along with a lot of fresh seeds were exposed to stress using the standardized doses of salt as well as osmotic stresses. Priorto stress exposure, germination speed and the vigour index of the seedlings were estimated, which led to the discovery of two classes of stress exposed plants of first experiment with their seeds differing in vigour viz. moderate and high vigour. Four classes of seed materials- control and stressed (high and moderate vigour) seed sets from first generation and a fresh set of seeds of rice variety, Uma were used for the stress response experiment using the sub-lethal stress levels ascertained. The high vigour seedlings from the stressed plants showed better growth under salt stress compared to control set of seedlings from first generation as evidenced by lesser percent reduction in growth as well as recovery responses. Subsequently, biochemical marker MDA was estimated in the different treatments revealing that moderate as well as high vigour seedlings of the stress class accumulated lesser (64.5% and 18.6% respectively) MDA levels in comparison to the non-stressed control, with a consistent pattern under salinity stress. GC-MS/MS analysis was performed using shoot tissues of the seedlings across the different treatments, with an attempt to unravel any possible stress response mechanism. GC spectra displayed a number of highly abundant as well as uniquely present compounds in the plants. Diphenyl sulfone, a potent sulfone derivative with antioxidant activity was differentially detected in all classes of plant materials used for the stress response study. There were corroborative inferences from the fold changes in diphenyl sulfone as well as the MDA contents, wherein there were proportional variations in the two especially in NaCl induced salinity stress in all treatments, indicating its probable role in maintaining lower MDA levels in the progenies of plants that were subjected to a prior drought stress exposure in the previous generation.

A round of stress exposure at vegetative stage in rice variety, Uma influenced several morpho-physiological and biochemical parameters at different stages of the crop. There was an indication of the transmission of stress induced signal even in the next generation as evidenced by profuse growth and lower MDA contents under different stresses at seedling stage. It will be rewarding to observe the stress response/acclimation behaviour of Uma, under different abiotic stress exposure regimes across different phenophases.