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PHENOTYPIC, GENOTYPIC, AND BIOCHEMICAL ASSESSMENTS OF ION BEAM-DERIVED RICE MUTANTS FOR DROUGHT TOLERANCE UNDER REPRODUCTIVE STAGE

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MSc

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MUHAMAD ADIB NAJMI BIN JA'AFAR

Thesis submitted in fulfilment
of the requirements for the degree of
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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 5 January 2026 to conduct the final examination of Muhamad Adib Najmi bin Ja'afar on his Masters of Science thesis entitled "Phenotypic, Genotypic, and Biochemical Assessment of Ion Beam-Derived Rice Mutants for Drought Tolerance Under Reproductive Stage" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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ABSTRACT

Drought stress is one of the most critical abiotic constraints to rice productivity, particularly at the reproductive stage where yield losses are most severe. With the help of mutation breeding, new rice lines that are tolerance towards drought can be developed. Despite the development of four advanced mutant lines in Malaysia demonstrating improved field performance, the molecular characterization of drought-tolerance markers in these lines remains limited. Integrating molecular data is crucial to confirm their genetic basis of drought tolerance and to enhance their value in breeding programs. Hence, study was carried out to evaluate the performance of seven rice genotypes, including four ion beam–induced mutants (NMR191, MINT1, MINT3, and MINT9), two tolerant checks (NMR151, NMR152), and a susceptible check (MR219), under well-watered and drought stress conditions across two growing seasons. A randomized complete block design was used, and data were collected on morpho-physiological, complemented by molecular marker analysis of qDTY loci using SSR markers and biochemical tests involving Catalase, Guaiacol Peroxidase and Ascorbate Peroxidase. Significant genotypic differences ($p < 0.05$) were observed with drought stress reducing yield-related traits overall. However, MINT3 maintained higher spikelet fertility, filled grain numbers, and chlorophyll content with lower unfilled grain per panicle under drought stress compared to MR219. Molecular screening confirmed that NMR151 carried three qDTY loci associated with drought tolerance, MINT1 and MINT3 carried two qDTY loci, while NMR191 and MINT9 carried one qDTY, validating their genetic potential for resilience. Biochemical testing on antioxidant and proline shows that both NMR191 and MINT3 performed better under drought stress compared to other advanced mutant lines and MR219. The integration of agronomic performance, physiological stability, and molecular evidence and also biochemical parameters highlight ion beam induced mutants as promising genetic resources for breeding programs especially MINT3 and NMR191. These lines, together with tolerant checks, demonstrate enhanced drought adaptability and contribute to strategies for achieving yield stability under water-limited conditions.

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LIST OF SYMBOLS

Symbols

A	Absorbance
bp	Base pairs
°C	Degree Celsius
GA	Genetic Advance
GCV	Genotypic Coefficient of Variance
σ_g^2	Genotypic Variance
g	Gram
Gy	Gray
h^2	Heritability
%	Percent
PCV	Phenotypic Coefficient of Variance
σ_p^2	Phenotypic Variance
k	Selection Differential Constant
U	Units
V	Volts

LIST OF ABBREVIATIONS

Abbreviations

A	Absorbance
C	Well-watered
D	Drought-stress
ANOVA	Analysis of Variance
APX	Ascorbate Peroxidase
AsA-GSH	Ascorbate-glutathione
BSA	Bovine Serum Albumin
CAT	Catalase
CC	Chlorophyll Content
CTAB	Cetylmethylammonium Bromide
DAT	Days After Transplanting
DNA	Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
DOSM	Department of Statistics Malaysia
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food and Safety Authority
EGA	Estimated Genetic Advance
FAO	Food and Agriculture Organizations of the United Nations
FGP	Number of Filled Grains per Panicle

GPX	Guaiacol Peroxidase
H	Broad-sense Heritability
HSD	Honest Significant Differences
IRRI	International Rice Research Institute
LEA	Late Embryogenesis Abundant
LEA	Late Embryogenesis Abundant
LET	Linear Energy Transfer
LSD	Least Significant Differences
LVT	Local Verification Trial
MAS	Marker Assisted Selection
MLT	Multi Location Trial
PCR	Polymerase Chain Reaction
PH	Plant Height
PN	Number of Panicles
PVP	Polyvinylpyrrolidone
qDTY	Quantitative Trait Loci Associated with Grain Yield Under Drought Stress
QTL	Quantitative Trait Loci
RCBD	Randomized Complete Block Design
RMK	Rancangan Malaysia
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species

RSD	Reproductive-stage Drought Stress
SAHN	Sequential Agglomerative Hierarchical and Nested
SF	Spikelet Fertility
SNP	Single Nucleotide Polymorphism
SSL	Self Sufficiency Level
SSR	Simple Sequence Repeat
TBE	Tris-borate-EDTA
TN	Number of Tillers
UFGP	Number of Unfilled Grains per Panicle
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultraviolet

LIST OF NOMENCLATURES

Nomenclatures

CO_2	Carbon Dioxide
H_2O_2	Hydrogen Peroxide
$MgCl_2$	Magnesium Chloride
O_2	Oxygen
NaCL	Sodium Chloride
H_2O	Water

CHAPTER 1
INTRODUCTION

1.1 Research Background

Rice (*Oryza sativa* L.) is the principal staple for more than half of world's population, providing over 60% of daily caloric intake (FAO, 2024). Asia dominates global rice production and consumption, accounting for nearly 90% of the total. In Malaysia, rice is not only a key dietary component but also a strategic commodity for national food security with average production between 1994 and 2020 recorded at 2.32 million tons. However, production has shown a worrying decline from 2.74 million tons in 2016 to 2.57 million tons in 2017, stabilizing at 2.32 million tons in 2020 (FAO, 2024). This production shortfall is of particular concern given Malaysia's rice self-sufficiency level (SSL) remains below the targeted 100%, making the country increasingly vulnerable to external supply disruptions.

These considerable multiple factors contribute to the rice yield gap can be attributed to various environmental strains that exert adverse effects on rice cultivation. These strains encompass both biotic stresses factors, such as pests, insects' infestation, and diseases, and abiotic stresses factors, including such as floods, salinity, drought stress, extreme temperatures, air pollution, mineral nutrient deficiencies, adverse pH levels, and heavy metal toxicity. Cumulatively, these factors impose significant stress on plant growth, resulting in a discernible reduction in rice yield. Among these, environmental stressors, drought stress emerges is recognized as one of the most severe devastating abiotic constraints factors, with global projections suggesting that more indicating than 50% of the world's arable land could face drought threats by 2050, according to research by Tang (2019). The implications of drought are notably severe for rice farmers in Malaysia, as it significantly impedes plant growth and agricultural productivity, leading to decreased crop yields. In Malaysia, drought event has become more frequent and severe due to climate change. In Malaysia, drought events have increased in both frequency and severity. Although the climate was historically relatively predictable, climate change has introduced greater variability and uncertainty, resulting in more drought occurrences, particularly during El Niño conditions. This climatic shift results in widespread drought in rice-cultivating areas, inflicting

significant financial losses on farmers. Notably, the 2016 El Niño event resulted in significant agricultural losses, with paddy farmers in Kerian, Perak, incurred damages estimated at RM 56 million (Shaiful Shahrin, 2016).

Drought exerts its most damaging effects when it occurs during the reproductive stage of rice growth encompassing panicle initiation, anthesis, and grain filling. Water deficit during this stage causes spikelet sterility, reduced grain set, incomplete grain filling, and substantial yield losses. Physiologically, drought stress induces a cascade of responses, including reduced relative water content, stomatal closure, decreased photosynthetic efficiency, oxidative stress, and impaired enzymatic activity (Z. Wang et al., 2018). In severe cases, metabolic and photosynthetic pathways are disrupted, leading to irreversible damage and crop failure.

To safeguard rice production under water-limited conditions, breeding drought-tolerant varieties is an essential strategy. Traditional breeding relies on recombination within existing germplasm, but progress is often slow due to limited genetic variability for complex traits like drought tolerance. Mutation breeding provides an alternative pathway by creating novel allelic variations that can be exploited in breeding programs. Among physical mutagens, ion-beam irradiation has emerged as a powerful tool due to its high linear energy transfer (LET), which induces a wider range of mutations, including large deletions, insertions, and chromosomal rearrangements, leading to unique phenotypic variations not easily achieved with conventional methods (Nor'Aishah et al., 2020; Oladosu et al., 2019). Globally, mutation breeding has contributed to the development of over 3,200 improved rice varieties, enhancing yield potential, stress tolerance, and grain quality (Johnson et al., 2017).

Identifying drought-tolerant mutants requires a multidimensional screening approach. Phenotypic markers such as days to flowering, plant height, spikelet fertility, and grain yield under stress provide direct evidence of performance in field conditions. Genotypic markers, particularly drought-related Simple Sequence Repeat (SSR) markers, enable the detection of specific alleles associated with tolerance, facilitating marker-assisted selection. Biochemical markers such as proline accumulation, malondialdehyde (MDA) concentration, and antioxidant enzyme activity offer insights into physiological mechanisms underlying stress adaptation. Integrating these three approaches increases selection accuracy, accelerates breeding progress, and reduces the risk of advancing false positives.

The outcomes of developing new crop varieties through induced mutations have been substantial, leading to a noteworthy increase in crop production. Consequently, ensuring equitable access to these improved crops, which exhibit observable characteristics that are easier to produce, becomes essential. Through mutation induction, more than 3200 varieties of rice have been developed worldwide, resulting in significant enhancements in food security, as highlighted by Johnson et al. (2017).

Drought stress presents itself as a complex occurrence in plants, demanding scrutiny across multiple aspects, including morphology, physiology, and biochemistry. These responses exhibit discernible changes in the plant's behaviour, notably evident in the reduction of water content within the plant and the closing of stomata to conserve moisture. Additionally, cell growth and elongation decrease under drought conditions, and gas exchange and enzymatic reactions experience disruptions. These combined effects are crucial for the plant's ability to endure water scarcity.

In more severe drought environments, the consequences are even more pronounced, often leading to significant disturbances in metabolism and photosynthesis, ultimately culminating in plant mortality (Z. Wang et al., 2018). Appreciating the physiological transformations that occur in plants during drought stress is immensely important, as it serves as a pivotal factor in crafting high-yield varieties that can prosper in dry conditions. Empowered by this knowledge, researchers can finely adjust breeding and cultivation techniques, endeavouring to create crops that exhibit resistance to drought and thrive in unfavourable weather conditions.

Dedicating attention to the physiological shifts transpiring during drought stress offers scientists invaluable insights into the intricate mechanisms that enable specific plant varieties to adapt and endure water-limited circumstances. This understanding serves as a robust foundation for making strides in agriculture, enabling the development of resilient crop systems capable of sustainably fulfilling the global food requirements, particularly in regions susceptible to water scarcity and erratic climatic patterns.

1.2 Problem Statement

Drought is one of the most critical abiotic constraints limiting rice (*Oryza sativa* L.) productivity, particularly in water-restricted environments such as rainfed lowlands and upland ecosystems (Bernier et al., 2007; Korres et al., 2017; S. S. Mishra & Panda,

2017). Under drought stress, rice plants experience shortened growth reduction, reduced biomass accumulation and impaired development of photosynthesis and storage organs, ultimately reducing yield potential (Kamoshita et al., 2008; Korres et al., 2017). The reproductive stage is particularly vulnerable, as water deficit during panicle initiation, flowering, or grain filling disrupts pollen viability, stigma receptivity and seed set, leading to spikelet sterility and substantial yield loss (Barnabás et al., 2008; Korres et al., 2017; Okami et al., 2015).

Globally, rice demand is projected expected to increase substantially due to population growth, with much of the required production coming from rainfed systems that are inherently more prone to water deficit (Panda et al., 2021). Most of this rice production would come from rainfed lowlands and upland rice which becoming the main concern for Asian and African rice breeders to cultivate drought-tolerant varieties. An estimated of 27 million hectares (ha) of rainfed rice field supplied mainly of rainwater affected by drought annually, with Southeast Asia among the hardest-hit regions has long suffered from severe drought (Rao et al., 2017). Southeast Asia has long suffered from severe drought. Over the past decade, severe drought episodes linked to climate variability and El Niño events have caused major agricultural losses across ASEAN member states (UNESCAP, 2020). In Malaysia alone, drought between 2017 and 2021, resulted in an loses in rice production due to drought is estimated at RM21.6 million loss from 9336.45 ha of paddy land (Bernama, 2021), threatening national food security and self-sufficiency targets.

Breeding drought-tolerant rice cultivars is therefore a strategic priority. However, conventional breeding is often constrained by the limited genetic variability for complex traits like drought tolerance. Mutation breeding offers a complementary approach by creating novel allelic variations within elite backgrounds in a shorter timeframe (Oladosu et al., 2019). Among mutagenesis techniques, ion-beam irradiation is particularly effective in generating unique mutations with high frequency and diversity, some of which may confer improved drought tolerance. In Malaysia, ten advanced mutant rice lines have been developed through ion-beam irradiation derived from Pongsu Seribu 2 at 120 Gy of radiation dose (Sobri et al., 2020). The genetic basis, quantitative trait loci (QTLs), and physiological mechanisms underlying their performance under drought has not been characterized yet to date.

Although significant progress has been made globally in breeding drought-tolerant rice, most research in Malaysia has focused on conventional varieties or

crosses, with very limited work on using ion-beam irradiation to develop mutant rice lines (Hasan et al., 2012). Moreover, comprehensive evaluations at the reproductive stage, the most yield-sensitive growth phase are scarce. Integrated screening approaches combining phenotypic performance, genotypic marker analysis (particularly drought-related SSR markers), and biochemical profiling have not been systematically applied to these advanced mutant lines. This knowledge gap limits the ability to identify stable, heritable drought tolerance traits and to map QTLs associated with yield stability under water deficit. Therefore, a systematic evaluation of advanced ion-beam-induced rice mutant lines at the reproductive stage, integrating phenotypic, genotypic, and biochemical markers, is crucial. Such an approach will enable the identification of promising drought-tolerant mutants, elucidate their physiological and molecular mechanisms of tolerance, and provide a scientific basis for accelerating the development of climate-resilient rice cultivars for drought-prone environments.

1.3 Research Objectives

The general objective of the study is to characterize advanced ion-beam-induced rice mutant lines for drought tolerance traits through integrated phenotypic, genotypic, and biochemical analyses.

Specific objectives of this study are:

a) To evaluate the morpho-physiological traits of advanced mutant rice lines under drought stress at the reproductive stage.
b) To screen advanced mutant rice lines for the presence of selected simple sequence repeat (SSR) markers associated with drought tolerance.
c) To determine biochemical responses of advanced mutant rice lines to drought stress.

1.4 Limitations of Study

The present study has several limitations that should be considered when interpreting the findings. Firstly, the evaluation focused on morpho-physiological, genotypic, and biochemical responses related to drought tolerance, without assessing other important traits such as grain nutritional quality, cooking and eating characteristics, or tolerance to other abiotic stresses (e.g., salinity, heat, and

submergence). Incorporating these parameters in future studies would provide a more comprehensive assessment of the overall agronomic value and market potential of the developed mutant lines.

Secondly, drought screening was conducted exclusively at the reproductive stage, the most yield-sensitive phase of rice growth. While this stage provides critical insights into drought impacts on yield components, it does not capture tolerance mechanisms that may operate during the vegetative or grain-filling stages. Multi-stage evaluation across the rice growth cycle is therefore recommended to better understand the temporal dynamics of drought tolerance in these lines.

Thirdly, performance assessment was carried out under controlled field conditions at a single location. This limits the ability to predict the stability and adaptability of the mutant lines across different agroecological zones. To confirm their suitability for large-scale cultivation, multi-location trials (MLT) and local verification trials (LVT) should be conducted under diverse environmental conditions. Such trials would allow evaluation under a broader range of abiotic and biotic stress combinations, thereby strengthening recommendations for varietal release.

Fourthly, the molecular analysis relied on a targeted set of previously reported drought-associated simple sequence repeat (SSR) markers. While the presence or absence of these markers provides preliminary insight into potential genetic variation related to drought tolerance, it does not constitute definitive quantitative trait locus (QTL) validation. Confirmation of QTL effects would require additional approaches such as fine mapping, high-density marker analysis, genome-wide association studies, or gene expression profiling under drought stress conditions.

Addressing these limitations through expanded trait evaluation (i.e., grain quality traits, nutritional attributes, and tolerance to additional abiotic stresses), multi-stage screening and wide adaptability testing will enhance the applicability of the findings and facilitate the effective deployment of drought-tolerant ion-beam-induced rice mutants in sustainable agricultural systems.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction of Rice

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world, serving as the primary source of calories for more than half of the global population (FAO, 2024). In Asia, where nearly 90% of rice is produced and consumed, it is not only a dietary staple but also a critical component of economic stability and rural livelihoods. In Malaysia, rice plays a strategic role in ensuring food security, with annual consumption averaging 80 – 85 kg per capita. The Malaysian Agricultural Policy recognizes rice as a politically sensitive commodity, and the government maintains a rice SSL target to reduce dependence on imports.

Despite its global and national importance, rice production faces numerous constraints, particularly from abiotic stresses such as drought, salinity, and extreme temperatures. Among these, drought stress is one of the most severe and widespread, posing a significant threat to both irrigated and rainfed rice ecosystems. Drought affects rice growth by reducing water availability for critical physiological processes, impairing photosynthesis, and altering nutrient uptake. Its impact is most devastating when it coincides with the reproductive stage, causing spikelet sterility, reduced grain filling, and substantial yield loss.

The challenge of ensuring stable rice production under increasingly variable climate conditions underscores the need for developing drought-tolerant rice varieties. Conventional breeding has made progress, but improvement for complex traits like drought tolerance is often slow due to the polygenic nature of the trait and limited genetic variability in existing germplasm. Mutation breeding, particularly through ion-beam irradiation, offers an effective strategy for creating novel alleles associated with stress tolerance. When combined with phenotypic, genotypic, and biochemical screening, this approach provides a powerful framework for identifying superior lines that can maintain productivity under water-limited conditions.

2.1.1 Morphology and Growth Stages of Rice

The rice plant is an annual grass that grows to about 1.2 meters (4 feet) tall, featuring long, flattened leaves on hollow stems and a broad, fibrous root system. Its inflorescence, or panicle, consists of spikelet that bear flowers producing the grain. Rice varieties vary in panicle length, shape, weight, and overall productivity.

Rice plant consists of three phases of agronomic development, which are vegetative phase (which includes germination, seedling, tillering and jointing stage), reproductive phase (consists of booting, heading and flowering stage) and grain filling and maturation phase (Moldenhauer et al., 2021) (Figure 2.1).

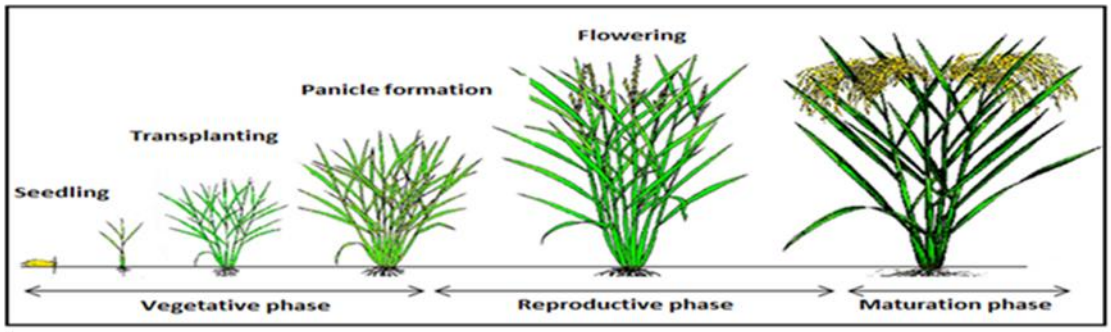

Figure 2.1 Growth phases of *Oryza sativa.*

Source: Hasan et al. (2012).

During vegetative phase, vegetative development starts with the germination of seeds and continues to the stage of tillering. Active tillering is a sign of vegetative phase, where a steady increase in height and growth of leaf at a frequent interval, where the length of this phase is mainly determined by the duration for the cultivars to grow. According to Parul (2017), rice plants will undergo four stages under vegetative phase, which are:

a) Germinating phase: the germination of seed of rice occurred with the emergence of radicle or coleoptile by germination of embryo.
b) Seedling stage: seeds germinate and develop seminal root, where this stage can be considered from the start of germination until the growth of the fifth leaf.
c) Tillering stage: the appearance of the first tiller from the axillary bud indicates the start of this stage, where upon reaching active tillering stage, the number of tillers will increase rapidly.

d) Inter node elongation stage: in this stage, inter node starts to elongate.

The vegetative phase of rice growth is marked by active tillering, during which the plant height gradually increases, and new leaves emerge. A rice tiller is a specialized, grain-bearing branch that forms on the unelongated basal internode and grows independently from the main stem (culm) using its own adventitious roots. The tillering process in rice occurs in two stages: first, the formation of an axillary bud at each leaf axil, followed by its subsequent outgrowth (X. Li et al., 2003).

This phase starts with the germination stage, where the seed coat absorbs enough water to become smooth and elastic. The coleorhiza, a sheath covering the embryonic primary root, elongates slightly, allowing the radicle to penetrate the soil. The coleoptile, meanwhile, elongates based on environmental conditions: root emergence precedes the coleoptile under aerobic or dry-seeded conditions, whereas the reverse occurs under watery or anaerobic conditions (Moldenhauer et al., 2021).

In the second stage of the vegetative phase, called seedling germination, the tip of the first sheathing leaf, known as the epiblast, breaks through the soil surface due to the elongation of the mesocotyl. When transplanting, an additional phase known as the recovery period begins when the seedling is uprooted and continues until it fully recuperates (Parul, 2017).

Following seedling germination, tillering usually starts during the fifth leaf stage when the first leaf from the axillary bud of the second leaf appears. The sixth leaf then emerges, and the second leaf falls from the axillary bud of the third leaf, signalling the start of tillering. Upon flooding, with oxygen and nutrients at the soil-water interface, secondary roots develop both vertically and laterally (Moldenhauer et al., 2021; Parul, 2017).

The vegetative phase progresses to the internode elongation stage before transitioning to the reproductive phase, as noted by Parul (2017). However, Moldenhauer et al. (2021) considers internode elongation a stage within the reproductive phase, occurring after panicle initiation. During this stage, internodes lengthen from the start of panicle initiation until the rice plant reaches its maximum height.

The reproductive phase is more critical than the vegetative stage for rice production, as it establishes essential agronomic traits. This phase includes panicle initiation (PI), booting, heading, and flowering stages (Parul, 2017). It is marked by culm elongation, a reduction in tiller number, booting, the emergence of the flag leaf,

heading, and flowering, which are vital for communicating beneficial traits (Moldenhauer et al., 2021).

Panicle initiation involves the start of panicle production through panicle primordia, which appears as a green band not yet visible to the naked eye (Moldenhauer et al., 2021). Previous studies often focus on panicle length, the number of panicles, and grain content, whether filled or unfilled.

The reproductive stage lasts around 20 to 30 days before panicle differentiation begins. In this period, the green band at the top of the node, as illustrated in Figure 2.2, appears for just a few days. Panicle differentiation, or "jointing" as described by Moldenhauer et al. (2021), marks the start of internode elongation. This phase spans about 20 to 30 days from the initiation of panicle development to booting, during which the application of herbicides is halted to avoid damaging the rice (Moldenhauer et al., 2021).

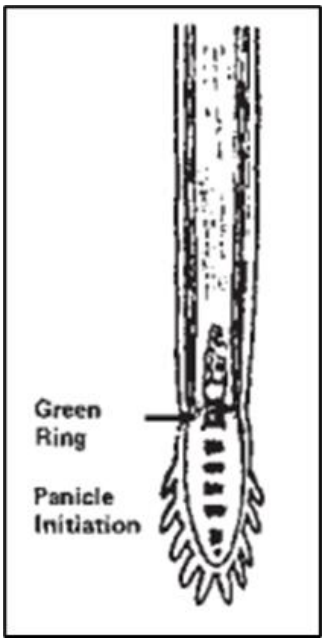

Figure 2.2 Formation of green ring during panicle initiation.

Source: Moldenhauer et al. (2021).

2.1.2 Global and National Rice Consumption Pattern

According to Food and Agriculture Organization of the United Nations (FAO, 2024), rice is ranked as the third-highest commodities produced in the world with 755.72 million tonnes production averaging from 2013 until 2022 while sugar cane with 1.89 billion tonnes in the first place, and maize at 1.12 billion tonnes in the second place.

In 2022, the production of rice worldwide is at 776.46 million tonnes, showing decrease in production compared to the previous year (789.05 million tonnes) (Figure 2.3).

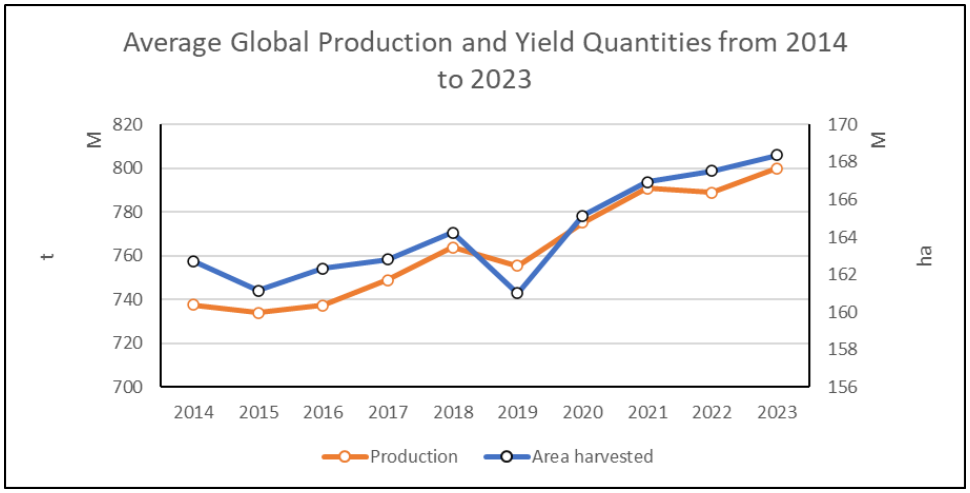

Figure 2.3 Average Global Rice Production from 2014-2023.

Source: FAO (2024).

Table 2.1 listed the top 10 countries that have the highest number of rice production in 10 years from 2014 until 2023 (FAO, 2024). Asian countries are dominating the world chart with China becoming the champion of producers. Rice is produced in large number every year within these countries as it is the main source of carbohydrate and calories for the citizens.

Table 2.1
Top 10 Countries with the Highest Number of Rice Productions on Average from 2014 to 2023.

Rank	Country	Yields (million tons)
1	China	210.71
2	India	177.85
3	Indonesia	56.10
4	Bangladesh	54.90
5	Vietnam	43.63
6	Thailand	31.97
7	Myanmar	26.78
8	Philippines	19.10
9	Pakistan	11.65
10	Brazil	11.36

Source: FAO (2024).

A study by Baldwin et al. (2012) found that Southeast Asia region had been the world's rice economy centre. Indonesia, Vietnam and Thailand became the top

contributor in Southeast Asia region in exporting rice with 24.5% of the world's rice export, with Vietnam following at 12.9% (Sarena et al., 2019). In 2024, Malaysia was ranked as the eighth largest rice producer in Southeast Asia, following countries like Indonesia, Vietnam, Thailand, Myanmar, Philippines, Cambodia, and Laos (Table 2.2). According to FAO (2024), Malaysia's rice production for that year reached 2.91 million tonnes, which marked the highest output in the past decade.

Table 2.2
ASEAN Ranking in Rice Production in 2023.

Countries	Production (1000 metric tons)
Indonesia	53,733.52
Vietnam	43,492.38
Thailand	31,807.77
Myanmar	27,990.92
Philippines	20,059.56
Cambodia	12,497.23
Laos	3,900.20
Malaysia	2,430.03
Brunei Darussalam	4.08

Source: AFSIS (2024).

Nor Lelawati et al. (2010) highlights on the factors contributing to the increase in rice production in Malaysia where one crucial factor is the availability of workers in the agricultural sector, which has supported the cultivation and harvesting processes. Additionally, there has been a distressed demand for rice commodities, leading to a reduction in government subsidies expenses and a decrease in rice imports. This situation has played a significant role in supporting local rice growers, enabling them to thrive and contribute to the rise in domestic rice production.

Reduces in rice yield in Malaysia has caused an uphill battle in achieving desired SSL (Table 2.3). Despite measures to enhance farmers' incomes and raise production, experts caution that attaining a 75% SSL by 2025 is increasingly improbable, due to current SSR is only at 56.2% in 2024. The current total population of Malaysia is estimated at 34.2 million, with an annual population growth rate of 0.5% (DOSM, 2025), and each individual consumes an average of 76.7 kilograms of rice per year in 2023 (DOSM, 2024). This accounts for approximately 26% of daily calorie intake and costs an average of RM 40 per month per household (DOSM, 2023). Notably, rice consumption varies across states, with Sabah having the highest average cost of rice

consumption per month per household at RM 57 and Pulau Pinang having the lowest at RM 29 (DOSM, 2023).

Table 2.3
Malaysia's Rice Production for the Past 10 Years.

Year	Production (million tonnes)
2014	4.63
2015	4.02
2016	3.98
2017	3.75
2018	3.77
2019	3.50
2020	3.65
2021	3.77
2022	3.58
2023	3.52

Source: FAO (2024).

Malaysia's current average rice yield of 3.54 tons per hectare (Department of Agriculture, 2024) enables the country to achieve around 56.2% SSL, leaving approximately 43.8% of rice to be imported to meet local demand. As a result, Malaysia relies on importing rice from countries such as Thailand to bridge the gap. Additionally, the import of specialty rice, such as Jasmine and Basmati, has been on the rise, reaching a value of RM 500 million as per reported by Jamal et al. (2014).

To obtain the 100% self-sustainability ratio (SSR) or at least reaching 80% SSR set under RMK13 plan (Table 2.4) in line with the increasing population, researchers need to explore and implement more technologically advanced methods to enhance rice production for local consumption and meet the growing demands. This will require continuous research and innovation in agricultural practices to optimize productivity and efficiency in rice cultivation, ultimately working towards achieving food security and reduced reliance on imports.

Table 2.4
SSR Based on the First until Thirteenth Malaysia Plan.

Plan	Period	SSR (%)
First Malaysia Plan (RMK1)	1966–1970	80.0
Second Malaysia Plan (RMK2)	1971–1975	87.0
Third Malaysia Plan (RMK3)	1976–1980	92.0
Fourth Malaysia Plan (RMK4)	1981–1985	76.5
Fifth Malaysia Plan (RMK5)	1986–1990	75.0

Sixth Malaysia Plan (RMK6)	1991–1995	76.3
Seventh Malaysia Plan (RMK7)	1996–2000	71.0
Eighth Malaysia Plan (RMK8)	2001–2005	71.0
Ninth Malaysia Plan (RMK9)	2006–2010	72.0
Tenth Malaysia Plan (RMK10)	2011–2015	71.4
Eleventh Malaysia Plan (RMK11)	2016–2020	100.0
Twelfth Malaysia Plan (RMK12)	2021-2025	75.0
Thirteenth Malaysia Plan (RMK13)	2026-2030	80.0

Source: Firdaus et al. (2020); MAFI (2021).

2.1.3 Malaysia's Rice Varieties and Breeding Strategies

In a study conducted by Raina et al. (2020), Malaysia successfully developed two rice varieties, namely NMR151 and NMR152, which possess drought tolerance and high yield attributes. These varieties were achieved through the use of gamma rays, resulting in rice plants that require minimal water for growth while maintaining high yields. Additionally, NMR152 exhibits an extra desirable trait, having longer panicle length compared to NMR151 (Table 2.5). This achievement was made in 2015, marking a significant milestone in rice breeding in Malaysia.

Table 2.5
Characteristics of NMR151 and NMR152.

	Description	
Paddy type	NMR151	NMR152
Plant height (excluding panicle)	73 cm	82 cm
Panicle: length of main axis	29 cm	30 cm
Panicle: number per plant	21	17
Day to maturity	105-110 days	100-105 days
Weight of 1000 fully developed grains	31.6 g	31.1 g
Length of grain	9.85 mm	11.10 mm
Width of grain	2.67 mm	2.75 mm

Source: Sobri et al. (2020).

However, despite this progress, Mohd Ikmal et al. (2019) expressed concerns that the development of drought-resistant rice in Malaysia seems to be stagnant. While efforts have been undertaken worldwide to cultivate drought-resistant rice, the project's advancement in Malaysia has been limited. Several challenges contribute to this situation, including the effects of climate change, the polygenic nature of drought-related characteristics in rice plants, and the lack of sufficient benchmarks for selecting

and breeding drought tolerance traits. These difficulties pose significant obstacles in the successful cultivation of drought-resistant rice varieties in the country. Addressing these challenges would require further research, collaboration, and innovative breeding strategies to achieve the goal of developing drought-resistant rice varieties that can withstand adverse environmental conditions and contribute to food security in Malaysia.

Given the increasing frequency and severity of drought events in Malaysia, future breeding strategies must place greater emphasis on resilience traits. This includes integrating drought-tolerant genes/QTLs into high-yielding backgrounds, exploiting local landrace diversity, and applying mutation breeding coupled with molecular marker analysis to accelerate varietal development. Ion-beam irradiation presents a promising approach to generate mutants with improved drought tolerance, which can be validated using integrated phenotypic, genotypic, and biochemical screening, which is the central focus of the present study.

2.2 Abiotic Stress in Rice

Abiotic stress is one of the most critical problems facing crop production which may result from a lack of vital materials, excess levels of harmful chemicals or changes in the environment. Across the globe, biotic and abiotic stress such as dryness, salinity, temperature, are major limiting factors in plant yields and their effect is still on the rise, particularly in the tropics due to climate change. Extended droughts, heavy rainfall and floods, inappropriate soil salinity, heat waves and damages due to frost are expected to escalate more in the future due to climate change that negatively impacts plant health and crop productivity. According to Zaidi et al. (2014), usually drought, flooding and soil salinity are the key causes of crop losses worldwide, which not only negatively impact plant growth but also productivity.

2.2.1 Drought in Malaysia: Occurrence, Severity, and Impact

Malaysia is one of the countries that are located on the equator, this its climate can be categorized as equatorial. It has a uniform temperature with abundant of rainfall and high humidity throughout the year (Nor Adawiyah et al., 2015) with spatial rainfall at the peninsular Malaysia at 2400 mm annually, 3600 mm for Sarawak and 2500 mm

for Sabah. Spatial rainfall variability can be defined according to N. Peleg et al. (2017) as variability resulting from multiple rainfall field that are spatially dispersed at a given point of time. Haliza (2009) in her study stated that although Malaysia can be considered as region that is free from climate related disaster, mild climate disasters such as heavy rainfall and drought may pose a big threat to the nations socio-economic. This can be supported by report from the World Bank Group & Asian Development Bank (2021) which stated that sectors such as fisheries, agriculture and societies' development are experiencing major damages from the rise of extreme weather in recent years. An estimated 9% to 10% of grain yield will be declined with every 1℃ of temperature rise from the optimal temperature of the surrounding air, which between 24℃ to 36℃ (Haliza, 2009). She also noted that the current flooded rice ecosystem cannot be sustained if drought prolonged, in which by developing dry land and non-flooded rice ecosystem is necessary to reach the national self-sustainability ratio.

Mohd Ikmal et al. (2019) demonstrated that while major granary areas benefit from established irrigation systems, smallholder rice fields remain vulnerable due to limited irrigation infrastructure and high associated costs. The study also reported rainfall reductions of 40 – 50% below the average during February 2017, coinciding with the main growing season in the southernmost region of Peninsular Malaysia. These conditions emphasize the necessity of developing high-yielding, drought-tolerant rice varieties to enhance production stability.

2.2.2 Morphological and Physiological Responses of Rice Towards Drought Stress

Drought stress is known to be a mild shortage of water contributing to the closure of stomatal and the restriction of plant gas exchanges (Singh et al., 2012). Among the factors that contribute plants to experience drought stress are when there is hardly water supplied to the root and the rate of transpiration of the plant becomes very high (Pandey & Shukla, 2015). According to Ahmed Basher Omer (2018), early maturity, early vigour and rapid in development are some morphological reactions, results from drought stress exerted on the plants while a study carried out by Wu et al. (2011) stated that if the drought strain occur in the anthesis and filling stage, it will severely have an influence on the production of the rice as it will causes the

degeneration of the spikelet, spikelet sterility, reduce in amount of grain per plant and rising the unfilled grain.

Plant height particularly during mid-tillering stage are severely reduced from the drought stress effect, which in return the elongation of cell and cell division (Davatgara et al., 2009). This study is supported by Shamsul Haque et al. (2016) where most genotypes experiencing severe reduction in plant height and root dry weight, although some genotypes managed to recover greatly. Cell division and cell elongation activities will be affected by these conditions and will eventually reduce plant height (Singh et al., 2018). Davatgara et al. (2009) also cited that drought stress during mid-tillering will severely reduce the number of panicles per hill.

Drought also causes sterility in plant as peduncle elongation during panicle emergence will be prevented, in which will hinder spikelet exertion (Nahar et al., 2016). It is also stated that both drought tolerant and intolerant cultivars increased in their root length, although the cultivars that are tolerant have deeper roots as it is their adaptive characteristics to combat drought stress (Figure 2.4).

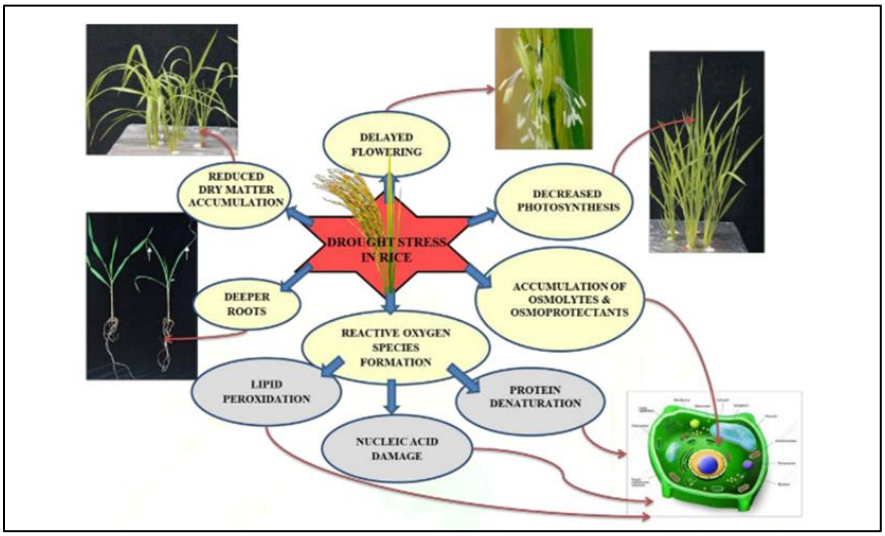

Figure 2.4 Diagrams of Morphological and Physiological Effects of Drought Stress on Rice.

Source: Nahar et al. (2016).

2.2.3 Effects of Drought Stress on Rice During Reproductive Stage

Reproductive-stage drought stress (RSD), in which water deficit occurring from panicle initiation/booting through heading/flowering and into grain filling, is widely recognized as one of the most yield-damaging stress windows in rice, particularly in

rainfed systems. According to Bhandari et al. (2020), genome-to-phenotype studies and reviews consistently describe RSD as a major constraint because yield formation is highly sensitive to disruptions in flowering, fertilization, and assimilate supply during this period. Evidence syntheses also indicate that the reproductive stage is often the most drought-sensitive growth phase, with large reductions reported in multiple yield components (e.g., panicle length, filled grains, seed-setting rate, grain weight) and, in severe cases, very large yield penalties (Dwiningsih et al., 2023).

A primary pathway by which RSD reduces grain yield is through impaired anthesis quality and spikelet fertility. Controlled reproductive-stage study by Salleh et al. (2022) on susceptible rice IR64 shows that drought at booting/heading depresses grain yield mainly via poor spikelet fertility. This is driven by disrupted anthesis-related traits such as spikelet opening, anther exertion/dehiscence, pollen-related traits, and that the impacts can vary strongly depending on whether stress occurs at booting versus heading.

Mechanistically, reproductive tissues are strongly influenced by plant and spikelet water status. For example, work under heading-stage drought emphasizes that spikelet moisture status is critical for maintaining functional anthesis; once spikelet moisture collapses, fertility losses can become difficult to reverse even after re-watering, contributing directly to spikelet sterility and reduced seed set (Salleh et al., 2022).

At the crop level, Jarin et al. (2024) described that these reproductive disruptions as presenting downstream outcomes such as increased spikelet infertility, higher proportions of unfilled grains, and reduced harvest index under RSD. Beyond fertilization failure, drought during reproductive development also compromises grain filling. He also remarked that water stress during grain filling reduces photosynthetic carbon supply and weakens source-to-sink transport, producing poor assimilate translocation to grains, increased empty grains, and shortened grain-filling duration due to accelerated senescence. Recent integrative drought-physiology review by Hu et al. (2025) framed this as a coupled disruption of photosynthesis and respiration/energy metabolism, which ultimately reduces carbohydrate availability for developing grains and limits final yield formation.

Under RSD, rice plants typically exhibit classical drought responses, like declines in leaf relative water content, stomatal conductance, and canopy photosynthetic capacity, often accompanied by leaf rolling and altered phenology (e.g., delayed

heading/flowering in some backgrounds). Quantitative genetic studies of drought resistance indices by Yi et al. (2023) reported broad reductions across yield-associated traits under drought and note shifts in flowering timing as part of the stress response. Physiological reviews connect these patterns to reduced CO_2 assimilation (stomatal and non-stomatal limitations), altered canopy temperature regulation, early senescence ("stay-green" vs non-stay-green behaviour), and reduced biomass partitioning to panicles (Jarin et al., 2024).

RSD is also associated with increased reactive oxygen species (ROS) production as drought disturbs photosynthetic and metabolic homeostasis. Reviews emphasize that drought triggers coordinated biochemical adjustments, including antioxidant defence activation, osmoprotectant accumulation, and membrane stabilization processes to mitigate oxidative damage (Jarin et al., 2024). In practical phenotyping, drought-tolerant genotypes often maintain better redox balance and cellular integrity than sensitive genotypes, consistent with the broader oxidative-stress framework described for rice under drought (Geng et al., 2024).

At the molecular level, drought responses in rice involve coordinated signaling through abscisic acid (ABA) pathways, Ca^{2+}/MAPK signaling, aquaporin regulation, transcription factor networks (e.g., DREB/NAC/MYB families), and protective proteins such as LEAs. Recent mechanistic reviews summarize these pathways and highlight their roles in regulating water relations, stress-responsive gene expression, and drought adaptation (Geng et al., 2024). The reproductive stage has additional regulatory complexity because stress response must be integrated with floral development and grain set. Contemporary reviews on reproductive responses under stress emphasize that reproductive traits are shaped by stress impacts on developmental timing, resource allocation, and reproductive tissue viability (Dwiningsih et al., 2023).

Because RSD impacts yield via multiple interacting traits, the genetic architecture is typically polygenic with major-effect and background-dependent loci. Large-scale association studies in diverse rice panels have identified marker–trait associations under managed reproductive-stage drought environments, reinforcing that grain yield under RSD is genetically complex and environment-sensitive (Bhandari et al., 2020). More recent multilocation genome wide association study (GWAS) reports numerous loci and candidate genes linked to yield under reproductive-stage drought and notes overlap of some associations with known drought-yield QTL regions (qDTY), alongside many potentially novel loci (Singh et al., 2025). Complementary mapping

studies by Venkateshwarlu et al. (2024) continue to report stable drought-yield QTLs (including known qDTY regions) and candidate genes, supporting ongoing marker-assisted and genomic breeding pipelines targeting reproductive-stage drought resilience.

2.2.4 Biochemical Responses to Drought Stress

Pandey & Shukla (2015) stated that the occurrence of water shortage had caused different organic type of solutes and inorganic type of solutes accumulate in the plant cytosol, which will maintain the cell turgor by lowering then osmotic potential. Osmotic adjustments, which is the above biochemical process, is strongly depends on the plant water stress rate, and can be achieved by accumulating multiple solutes in the cytoplasm, such as sucrose, proline, glycinebetaine, in which will cause the uptake of water in dry to improve.

Proline is a type of proteinogenic amino acid and have an extraordinary conformational stiffness and it is very important for plant main metabolism (Szabados & Savoure, 2009). Proline acts as a control for osmotic modification, alleviates cell membranes and protein's structure and serves as enzymes' defensive agent, aside from acting as antioxidant and free radical scavenger (Dien et al., 2019). According to Dien et al. (2019), albeit proline had been widely studied under water shortage condition, its true role still remain controversial in drought tolerance. The accumulation of protein had been reported as the effect from drought condition, high light and UV radiations, oxidative stress, heavy metals, and as well as a type of response to biotic stress (Szabados & Savoure, 2009). Proline concentration changes had been observed in rice that are exposed to drought, where the accumulation of proline (which acts as osmolyte) resulted in better drought tolerance and performance (Lum et al., 2014; Pandey & Shukla, 2015). According to Hayat et al. (2012), proline plays three key roles beside acting as an excellent osmolyte, which are as a metal chelator, a signalling molecule, and a molecule of antioxidative defence. Increasing antioxidant activity due to proline accumulation may increase the ability of plant to repair plant damage (Pandey & Shukla, 2015). The use of proline as a biochemical marker during drought stress also important to select cultivars that resist to drought condition (Mostajeran & Rahimi-Eichi, 2009).

Antioxidant also plays a key role in combating drought stress in rice. Among the typical outcome of water scarcity in rice are disruption of generation and removing reactive oxygen species (ROS) such as superoxide ($O_2 \cdot -$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) (Faize et al., 2011; Pandey & Shukla, 2015). According to Cruz de Carvalho (2008), under abiotic stress settings, ROS appear to have a twofold impact that is dependent on their overall cellular quantity. They are expected to operate as components of a stress-signalling system, prompting stress defence/acclimation responses if held at relatively low levels. However, if a certain threshold of phytotoxicity is reached, ROS become exceedingly toxic, triggering uncontrolled oxidative cascades that damage cellular membranes and other cellular components, resulting in oxidative stress and protein denaturation and can cause DNA mutation (Cruz de Carvalho, 2008; Faize et al., 2011).

Plants naturally create reactive oxygen species ROS as part of their normal metabolic activities and while singlet oxygen and the hydroxyl radical are produced at low levels, superoxide and hydrogen peroxide (H_2O_2) are produced at a faster rate. One of the important cellular locations that is responsible to synthesise ROS is the chloroplast. During photosynthesis, sunlight is gathered and transported to two light-harvesting complexes termed photosystem II and photosystem I, which are situated in the chloroplast's thylakoid membranes. A succession of redox events occurs inside the electron transport chain in the presence of light until the electrons finally reach carbon dioxide (CO_2) during the dark processes. However, it's not uncommon for other electron acceptors, such as oxygen, to be utilized along this pathway. Cruz de Carvalho (2008) stated that singlet oxygen can be produced when energy is transferred from a triplet excited state of chlorophyll to oxygen. Furthermore, in the presence of oxygen, certain components of the thylakoid electron transport system, such as Fe-S centres and reduced thioredoxin, can undergo auto-oxidation, resulting in oxygen reduction and the generation of superoxide and hydrogen peroxide (H_2O_2). The Mehler reaction is responsible for around 10% of the flux of photosynthetic electrons (Noctor et al., 2002).

Interestingly, this "leakage" of electrons to oxygen and the generation of reactive oxygen species (ROS) is actually beneficial for the electron transport chain. It helps maintain the balance of electron carriers, making them more efficient. This phenomenon contributes to the overall functionality of the photosynthetic process. The harmful ROS effect can be avoided by plant cells with the help of a complex systems of antioxidants consist of enzymatic antioxidants such as catalase (CAT), guaiacol

peroxidase (GPX) and ascorbate peroxidase (APX) and for antioxidants that are non-enzymatic are glutathione (GSH) and ascorbate (Asa). CAT, APX and GPX helps in combating ROS in plant by converting it into water but each antioxidants have their own mechanism of reaction of scavenging H_2O_2 (Sofo et al., 2015) (Figure 2.5).

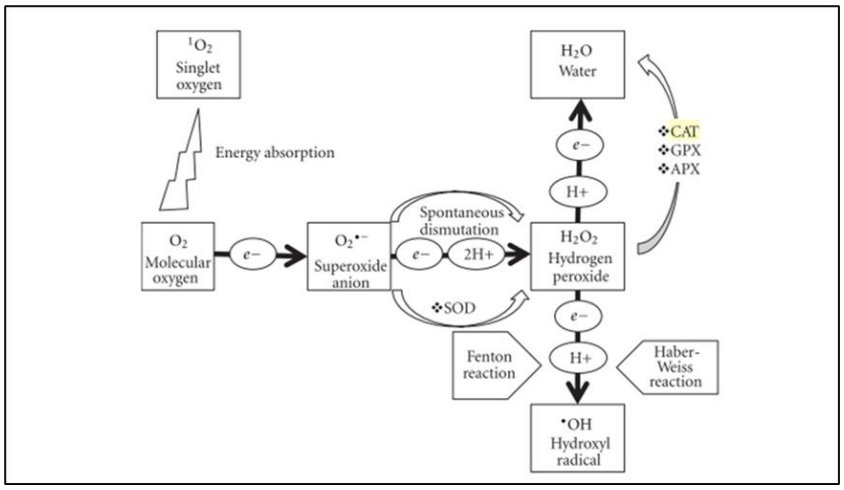

Figure 2.5 Schematic Diagram of ROS Reaction with Enzymatic and Non-Enzymatic.

Source: Sharma et al. (2012).

According to Sharma et al. (2012), APX operates within the ascorbate–glutathione cycle, an ascorbate- and GSH-regenerating system that sustains APX activity during oxidative stress. Under abiotic stress, this cycle constitutes one of the primary H_2O_2-scavenging mechanisms in plant cells, in which APX isoenzymes catalyse the initial reaction by reducing H_2O_2 to H_2O using ascorbate as the specific electron donor; this detoxification pathway is particularly important in chloroplasts (Sofo et al., 2015) (Figure 2.6).

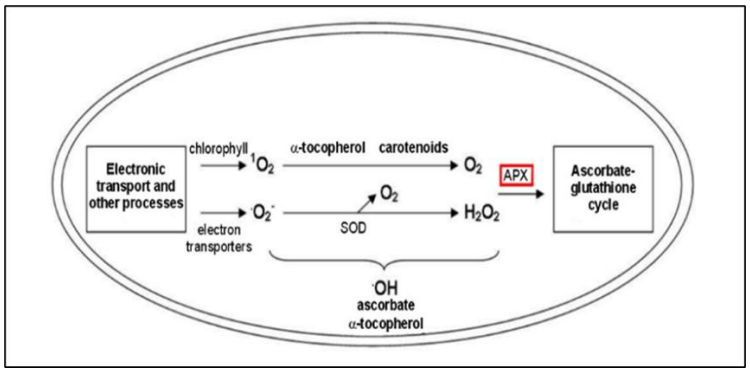

Figure 2.6 Key Role of APX in Production and Scavenging ROS in Plant Chloroplasts Under Abiotic Stresses.

Source: Sofo et al. (2015).

APX is composed of many isoenzymes encoded by a multi-gene family and may be found in a variety of cell compartments. It is essential in catalysing the transition of hydrogen peroxide (H_2O_2) into water (H_2O). Ascorbate (Asa) acts as an electron donor in the ascorbate-glutathione (ASH-GSH) and water-water cycles to achieve this. The ASH-GSH and water-water cycles are important antioxidant mechanisms in cells that help to neutralise and detoxify harmful reactive oxygen species like H_2O_2 (Wu & Wang, 2019).

Compared to APX, CAT have lower affinity toward H_2O_2 but higher reaction rate, which in contrast to regulating as a signalling molecule, CAT is more active in H_2O_2 detoxification than regulating (Cuypers et al., 2011). H_2O_2 is directly converts into H_2O and ½ O_2 by CAT (Sofo et al., 2015) (Figure 2.7).

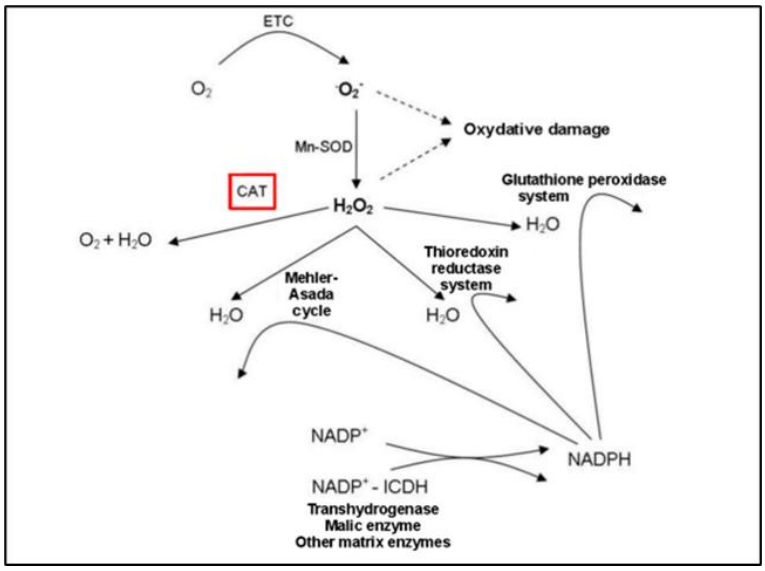

Figure 2.7 CAT in Scavenging Pattern of ROS.

Source: Sofo et al. (2015).

2.2.5 Mechanisms of Drought Tolerance in Rice

Drought tolerance in rice is a complex trait governed by the interaction of physiological processes, biochemical adaptations, and genetic factors that enable the plant to maintain growth and yield under water deficit. The degree of tolerance depends on drought intensity, duration, and growth stage, with the reproductive stage being the most yield-sensitive.

Rice plants cope with drought through a combination of drought escape, avoidance, tolerance, and recovery mechanisms (Blum, 2011; Gowda et al., 2011).

Drought escape involves early flowering and short growth duration to complete the life cycle before severe stress sets in while drought avoidance is achieved by maintaining favourable plant water status through deeper and more prolific root systems, reduced leaf area, leaf rolling, cuticular wax deposition, and stomatal regulation to minimize water loss while sustaining photosynthesis.

Drought tolerance refers to the plant's ability to sustain metabolic activity at low water potential via osmotic adjustment, which is supported by the accumulation of compatible solutes such as proline, soluble sugars, and glycine betaine to maintain cell turgor and enzyme stability (Bhandari et al., 2023; Serraj & Sinclair, 2002). Antioxidant defence systems, which includes superoxide dismutase, catalase, and peroxidases, help mitigate oxidative damage from reactive oxygen species (ROS), while protective proteins such as late embryogenesis abundant (LEA) proteins and dehydrins stabilize cellular structures during dehydration. Drought recovery occurs when plants resume growth and grain filling after rehydration, depending on their ability to restore photosynthetic function and remobilize stored carbohydrates.

These physiological and biochemical mechanisms are highly interconnected; for example, deep rooting (physiological) supports osmotic adjustment (biochemical), while stomatal closure (physiological) can induce oxidative stress that requires enhanced antioxidant activity (biochemical). Understanding these integrated mechanisms is essential for breeding programs, as it provides the basis for selecting genotypes with stable drought tolerance through combined phenotypic, genotypic, and biochemical screening.

2.3 Mutation Breeding for Drought Tolerance

In plant mutation breeding, gamma-rays are widely used as the principal source of mutagenic radiation. However, the exact properties of the altered DNA sequences caused by gamma-ray radiation have not been well explored and comprehended. In contrast, the use of recently emerging physical mutagens, such as heavy-ion beams, has piqued the interest of geneticists and breeders. Numerous researches have been carried out to evaluate the mutation effectiveness of these physical mutagens as well as the properties of the modified DNA sequences they cause.

The quality of mutation breeding has substantially increased as mutation technologies have advanced and new mutagens have emerged. It is now well recognised

as a versatile and selective strategy for creating genetic variants, akin to hybridization breeding. The unique qualities of the mutagenic agents and the characteristics of the biological organisms involved determine the success of mutation procedures in developing variants. It is critical to consider all relevant factors when deciding on the best mutation method to use for creating genetic variations, including the characteristics of the mutagenic factor, the properties of the biological object being studied, and the correlations between these factors and the desired outcomes.

According to Acquuah (2012), mutation can be classified into three categories based on the structural type produced during the mutation which are ploidy variation (involves the change of chromosomal number, whether gained or lose complete set or part of sets), chromosome structure variation (involves structural changes of chromosome from duplication, or translocation of segments), and gene mutation (changes in DNA confirmation from substitution or deletion of segment). Mutagenesis is a mechanism by which the genetic material of the organism is altered, resulting in a mutation, a hereditary alteration in the genetic make-up of the individual, which results in new characteristics which are transferred from parent to offspring, thus guiding evolution. Mutagenesis can affect the growth and development of plants by causing cytological, genetic, biochemical and physiological changes in cells and tissues. Several forms of ionizing radiation are accessible for inducting plant mutations. Each has a common function of releasing ionizing radiation. There are, however, a variety of variations in ionizing radiation in terms of the energy required for penetrating power and the degree of risk associated for operators (Table 2.6).

Table 2.6
Commonly Used Physical Mutagens in Plant Mutations.

Mutagens	Characteristics	Hazard
X-ray	Electromagnetic radiation; Able to penetrate tissues in between few millimetres to many centimetres	Dangerous, Penetrating
Gamma (γ) rays	Electromagnetic radiation formed by radioisotopes and nuclear reactors; Are very tissues penetrating	Dangerous, Very penetrating
Alpha (α) particles	Derived from isotopes; Capable of heavy ionization from a small helium nucleus; Penetrating very shallowly	Very dangerous
Beta (β)	Produced in particle accelerators or from radioisotopes;	May be

particles	Can ionize; Shallowly penetrating	dangerous
Ion beam	Positively charge ions were produced and are accelerated at a high speed (20% to 80% of light speed); Deposit high energy on the target	Dangerous

2.3.1 Types of Mutagens: Physical and Chemical Agents

There are two types of mutagens used to induce mutations in plant according to Spencer-Lopes et al. (2018) which are physical mutagenesis, and chemical mutagenesis (Figure 2.8). Nuclear radiations and all sources of radioactivity can be considered as physical mutagens (Spencer-Lopes et al., 2018). Example of nuclear radiations is ultraviolet rays, which is a type of non-ionising agent. Some type of ionising agents also can be categorised under physical mutagens, which are X-ray, gamma ray, alpha and beta particles, protons and neutrons. All of these mutagens are very dangerous and can cause cancer to human if exposed in high dosage (Table 2.6) as cited by Oladosu et al. (2016).

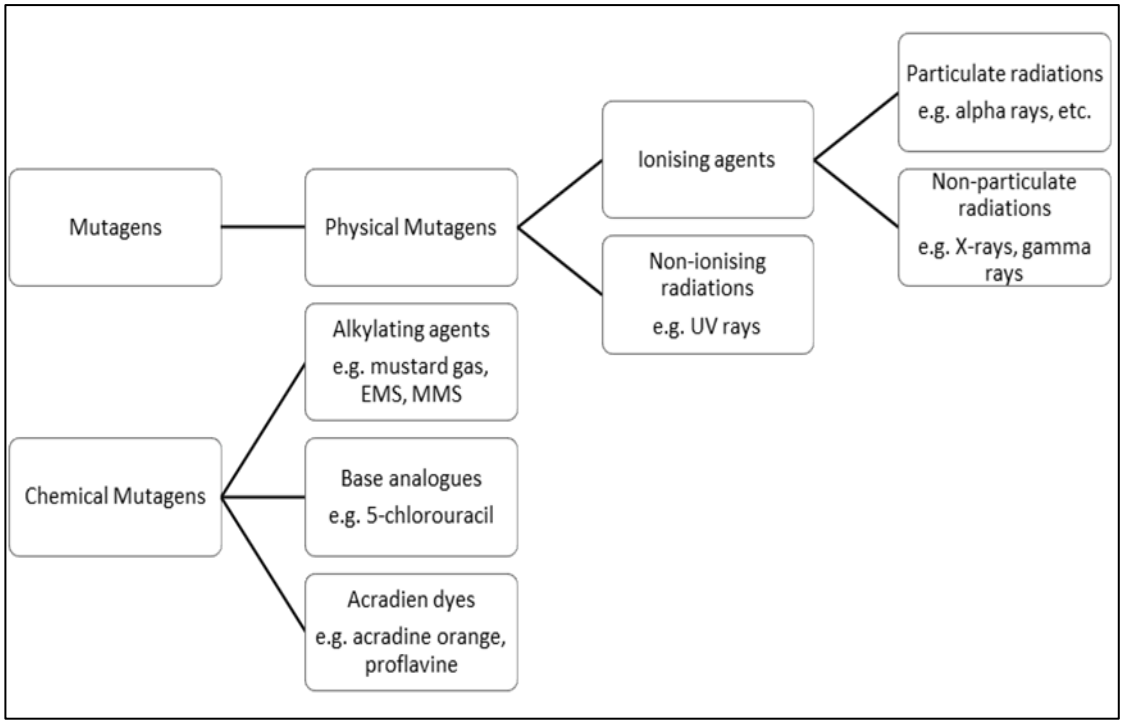

Figure 2.8 Common Mutagens Commonly Used in Inducting Plant Mutations.

Source: Spencer-Lopes et al. (2018).

Physical mutagens, often ionizing radiation, have been commonly used for causing genetic anomalies in the last 80 years and it have produced more than 70% of mutant varieties (Oladosu et al., 2016). In this study, four of the rice variety, which are NMR191, MINT1, MINT3, MINT9 had been exposed with ion beam. Physical mutagen is directly applied to the target without the use of any chemical compared to chemical mutagens, such as alkylating agent and acridine dyes, which need to be applied first to the target in order to mutate the target. In plant mutation breeding, both techniques are good, but physical mutagenesis is better because it of the higher degree of accuracy and sufficient reproducibility, especially gamma rays which have uniform penetrating power into tissue (Oladosu et al., 2016).

2.3.2 Ion-Beam Irradiation: Principles and Advantages

Li et al. (2019) conducted a study using whole genome sequencing to characterize mutations induced by carbon (C)- ion beams and gamma rays in rice plants at M5 generation. The result shows that gamma-ray irradiation resulted in a greater number of minor alterations, whereas C-ion irradiation resulted in the development of complex structural variations (SVs). The differing radiation characteristics of gamma-rays and C-ion beams may explain these differential mutation patterns.

Ion beam implantation is mostly used in the industry, but can also be applied on plant tissues, by where atoms are injected on the surface of the tissues, which resulted in an efficient tool for inducing mutation (Spencer-Lopes et al., 2018). Ionizing radiation induces mutations by splitting the molecular bonds in the DNA molecule, removing the nucleotide, or replacing the nucleotide for the new molecule, and it should be delivered at the right dosage, which depends radiation intensity and period of exposure (Acquuah, 2012).

According to Oladosu et al. (2016), DNA double strands damage caused by ion beam are less repairable compared to using gamma rays. This is because of the deletion of different sizes of DNA fragment during the irradiation of ion beam. Although the damage in unrepairable, ion beam method has been shown to exhibit many outstanding benefits such as having lower damage rate, higher rate of mutation, and mutational spectrum newer and wider (Spencer-Lopes et al., 2018). Moreover, he also remarked that mutant rice that have been applied ion beam irradiation have higher yield, resistance towards broader type of diseases, higher grain quality with shorter growing period.

2.3.3 Application of Ion-Beam Irradiation in Crop Improvement

With the use of ion beam irradiation in mutation breeding in plants, multiple unique varieties of flowers and trees had been commercialized, for example carnation (*Dianthus caryophyllus*), rose (*Rosa* sp.), and chrysanthemum (*Chrysanthemum* spp.) (Yamaguchi, 2018). This is due to the fact that mutant varieties produced by mutagens, especially ion beam irradiation, are categorized as a non-transgenic plant, and consumers are easily accepted it, thus becoming a practical choice worldwide in terms of rice production. Ion beam irradiation method has been hailed as a new mutation method for the past two decades for inducing mutation in rice and has been widely exercised in many varieties, including Hitomebore (Yamaguchi et al., 2009) and Nipponbare (Hayashi et al., 2007), Indonesian Toraja (Sjahril et al., 2018), and Indian rice variety, Indira Barani Dhan-1 (Chauhan et al., 2019). A study conducted by Yamaguchi et al. (2009) have concluded that ion beams have been demonstrated to be highly effective in inducing mutations while causing minimal radiation harm. Surprisingly, no significant changes in the relative frequency of each kind of mutation were identified between the three types of ion beams and gamma rays. According to Nor'Aishah et al. (2020), compared to other countries, research on the use of ion beam irradiation in rice breeding in Malaysia is still limited.

2.4 Molecular Marker in Rice Breeding

Drought tolerance in rice is a complex trait controlled by multiple physiological processes and many loci with environment- and background-dependent effects (Mohd Ikmal et al., 2020; Panda et al., 2021; Sandhu et al., 2018; Selamat & Nadarajah, 2021). Marker-assisted selection (MAS) is therefore widely used to increase selection efficiency by tracking genomic regions linked to drought-adaptive traits while minimizing reliance on phenotype alone, especially for traits expressed strongly only under managed stress environments. Sandhu & Kumar (2017) emphasize that large-effect drought-yield QTLs (qDTY loci) identified under reproductive-stage drought can be introgressed and pyramided into elite varieties to stabilize yield under stress while preserving performance under non-stress conditions.

In order for quantitative trait loci (QTLs) associated with drought resistance in rice to be mapped, the SSR technique combined with selective genotyping was used

(Lang & Buu, 2010). Simple sequence repeats (SSRs) remain a practical marker system in many rice breeding programs because they are PCR-based, typically multi-allelic, and co-dominant, enabling differentiation of homozygotes and heterozygotes, which is an advantage for tracking segregation in backcross and pyramiding pipelines. In applied MAS pipelines, SSR markers are typically deployed in three complementary roles: foreground selection (confirming target QTL presence), recombinant selection (using flanking markers to reduce linkage drag), and background selection (accelerating recovery of the recurrent parent genome). Arra et al. (2024) recommend SSR/SNP markers for foreground selection and outline dense genome-wide SSR panels for background selection and genome recovery estimation.

Their broad adoption in plant breeding reflects practical advantages, including high genomic abundance, substantial allelic diversity, and straightforward gel- or capillary-based scoring. Consequently, integrating SSR genotyping with phenotypic screening strengthens selection by enabling the identification of QTLs controlling drought-related traits such as root architecture and associated adaptive characteristics, which can then be deployed through marker-assisted selection to accelerate the development of improved rice varieties (Lang & Buu, 2010).

Report by Sandhu & Kumar (2017) catalyzed the identification of qDTY loci with measurable effects on grain yield under reproductive-stage drought and demonstrated that combining loci (QTL pyramiding) can deliver stronger and more stable yield advantages than single-locus introgression in many backgrounds. This is because drought-yield QTL effects can be background-dependent, a key principle emphasized in drought breeding is local validation of the QTL and its linked markers in each recipient background and target ecosystem before wide deployment. Meta-analysis of rice drought QTLs reinforces this point by compiling hundreds of reported drought-related QTLs and identifying “stable” regions that are more plausible candidates for routine MAS (Selamat & Nadarajah, 2021).

Malaysia provides a clear case study of SSR-enabled drought breeding through the introgression of qDTY loci into locally important varieties, especially MR219 (mega-variety background) and MRQ74 (high-quality/aromatic background). In MR219, three qDTY loci (qDTY2.2, qDTY3.1, qDTY12.1) were pyramided into the recipient background using marker-assisted breeding. Noraziyah et al. (2016a) used peak SSR markers RM236 (qDTY2.2), RM520 (qDTY3.1), and RM511 (qDTY12.1) to confirm hybrids and advance selections, followed by background selection using

additional SSRs to recover the MR219 genome. In MRQ74, the same three qDTY loci were pyramided by Noraziyah et al. (2016b) to improve grain yield under reproductive-stage drought stress using marker-assisted breeding across multiple seasons.

The ability of rice to tolerate drought depends on the root system and its genotype will continue to increase root length and number of lateral roots under drought stress (Uttam, 2019). Multiple efforts had been carried out in order to identify donors for the drought tolerance traits and at the same time developing suitable criteria for breeding drought tolerance cultivars (Kumar et al., 2014). Steele et al. (2006) reported that numerous QTLs that are associated with the ability of root length, root penetration, and root thickness had been discovered and assimilated into rice varieties to enhance drought tolerance traits. Large effect QTLs, associated with root penetration index and root pulling force had been detected in chromosome 4 of rice (J. Zhang et al., 2001). Zheng et al. (2003) reported that 18 QTLs linked to seminal root length, adventitious root number, lateral root length, and lateral root number had been detected.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Experimental Procedure

This study was carried out from March 2021 to December 2021 and the screening of drought tolerance in seven rice varieties was carried out in Sekinchan, Selangor. Evaluation of antioxidant and biochemical analysis was carried out at the Plant Biochemistry and Biotechnology Laboratory, Universiti Putra Malaysia; while DNA genotyping work was carried out at the Laboratory of Molecular Biology of Bioscience and Agrotechnology Division, Malaysia Nuclear Agency, Selangor located at Dengkil, Selangor.

3.2 Media and Chemicals

The chemicals used in this study were Ancom Thiram 80 fungicide (Ancom Berhad, Malaysia), NPK Green fertilizer (Golden Harvest Agricultural Supply, Malaysia), Malathion 57 (Hextar Chemicals Sdn. Bhd., Malaysia), Antracol 70WP (Bayer Co., Germany), Storm® rat bait (BASF (Malaysia) Sdn. Bhd., Malaysia), and Siputox® (Agricultural Chemicals (M) Sdn. Bhd., Malaysia), Score (Syngenta Sdn. Bhd., Malaysia), Armure 300EC (Syngenta Sdn. Bhd., Malaysia), Match 050EC (Syngenta Sdn. Bhd., Malaysia), Takumi 20WG (Agricultural Chemical (M) Sdn. Bhd., Malaysia), and Plenum 50WG (Syngenta Sdn. Bhd., Malaysia). These chemicals were used as fertilizers and also for controlling rat and as pesticide.

For molecular characterization, the media and chemicals used included the agarose powder (Sigma-Aldrich, US), Tris-borate-EDTA (TBE) solution (1st Base, Singapore), FloroSafe fluorescent dye (1st Base, Singapore), 1kbp DNA Ladder (Promega, US), 50 bp DNA Ladder (VWR Amresco, US), Gel Loading Dye Blue (Promega, US), 5x Green GoTaq Flexi Buffer (Promega, US), $MgCl_2$ (Promega, US), dNTP (Promega, US), and Taq DNA Polymerase (Promega, US).

3.3 Investigating Morpho-Physiological Characteristics of Advanced Mutant Rice Lines under Drought Stress

3.3.1 Plant Materials

A total of four advanced mutant lines developed using ion-beam irradiation namely NMR191, MINT1, MINT3, MINT9 were selected for drought screening. These lines were previously developed through 320 MeV carbon ion-beam irradiation at an absorbed dose of 120 Gy at the ion beam facility in Takasaki, Japan (Sobri et al., 2020). All seeds were provided by the Malaysian Nuclear Agency. All rice control varieties were selected based on their high yield performance, good agronomic traits and high preference among local farmers while the advance mutant lines were developed based on high yielding, drought tolerance and good agronomic traits from the parent. However, these advance mutant lines lack of information on field study. Both NMR151 and NMR152 were represented as positive control whereas MR219 act as negative control. Table 3.1 presented the information on the tested varieties.

Table 3.1
Resistancy of Selected Rice Varieties and Lines.

Variety	Resistance to drought	Source	Method of development
NMR151	Moderate tolerant	Harun et al. (2012)	Mutation induction using gamma irradiation
NMR152	Moderate tolerant	Harun et al. (2012)	Mutation induction using gamma irradiation
MR219	Susceptible	Evamoni et al. (2024)	Conventional breeding
NMR191	Moderate tolerant	Agensi Nuklear Malaysia	Ion beam irradiation mutation breeding
MINT1	Moderate tolerant	Agensi Nuklear Malaysia	Ion beam irradiation mutation breeding
MINT3	Moderate tolerant	Agensi Nuklelar Malaysia	Ion beam irradiation mutation breeding
MINT9	Moderate tolerant	Agensi Nuklear Malaysia	Ion beam irradiation mutation breeding

3.3.2 Seed Germination

The seeds were placed inside the oven at 60℃ for 24 hours to break the seeds' dormancy. Meanwhile, Thiram fungicide solution (Ancom, Malaysia) was prepared earlier to prevent fungal growth during the germination process. Each variety was divided into individual petri dish and the fungicide solution was poured until all seeds were fully immersed. The seeds were left for 24 hours at room temperature (24 - 27 °C). The seeds were then rinsed with distilled water and germinated in a petri dish filled with a moist filter paper for seven days at 28°C. The filter paper was moistened daily to ensure sufficient water supply for all seeds. After seven days, the seeds from petri dishes with 80% of germination were subjected to subsequent treatments.

3.3.3 Field Screening

Field screening was carried out in two seasons, which are the off season and the main season. The off season begins on the month of January until June 2021, while for main planting season, the season starts from July until December 2021. The seeds were sown first to encourage germination. The seeds were then planted individually according to the designated block using randomized complete block design (RCBD) (Figure 3.1). Each row of blocks was represented as replicate (4 blocks for 4 replicates). Genotypes from first replicate were arranged randomly in block 1. The same goes for replicate 2, 3, and 4. This method was also used for the plot that were subjected to drought stress. During the development phase, the plants were supplemented with standard amount of fertilizer according to the Department of Agriculture’s Rice Check (Appendix 1). Tensiometers were installed in four points inside to plot to monitor water tension in the experimental plots.

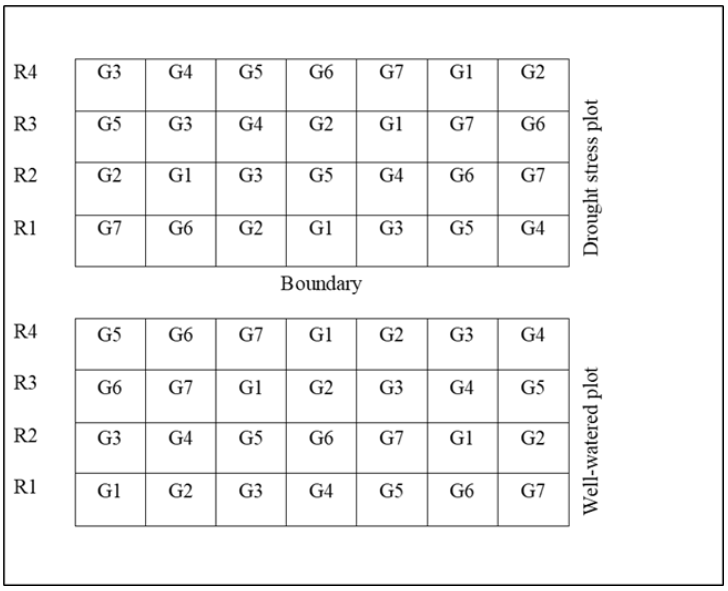

Figure 3.1 The Layout of Experimental Design.

Soil based drought model will be used in this study as it has close similarity between experimental conditions and actual drought in natural and agriculture (Osmolovskaya et al., 2018). The well-watered plot was flooded with water until 14 days before harvesting while the drought stress plot was only supplied with water up until 30 DAT and irrigation was withdrawn after 30 DAT. Figure 3.2 shows the on-site simulation of drought stress in this study.

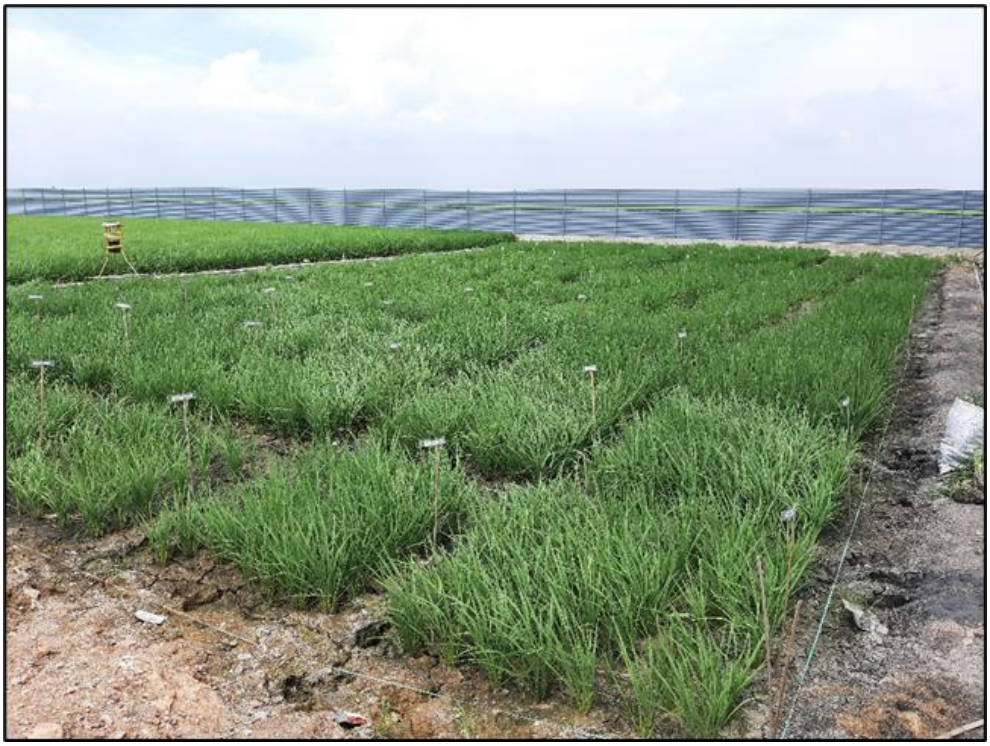
Figure 3.2 On-Site Simulation of Drought Stress.

3.3.4 Evaluation of Morpho-physiological Traits and Performance.

The agronomic performances were examined in all four advanced mutant lines in comparison to the control varieties. Data were recorded for morphological traits of plant height (PH, cm), tiller numbers (TN), panicle length (PL, cm); agronomic traits consist of number of panicles (PN), number of filled grains per panicle (FGP), number of unfilled grains per panicle (UFGP) and spikelet fertility (SF, %); and the physiological changes which is chlorophyll content (CC) by using SPAD meter. The measurement procedure of all these agronomic traits is shown in Table 3.2.

Table 3.2
Morphology, Agronomic and Physiology Traits that were Measured.

Traits	Unit	Description
Height of plant	cm	Maximum vertical distance from soil surface to the tip of the tallest panicle
Number of tillers	n	Number of specialized bearing branch that holds the panicles
Number of panicles	n	Number of spikelets bearing branches
Length of panicle	cm	Maximum vertical distance from panicle base to the tip
Number of filled grains per panicle	n	Number of filled grains within the whole spikelet of a panicle
Number of unfilled grains per panicle	n	Number of empty grain(s) within the whole spikelet of a panicle
Chlorophyll content	SPAD unit	Relative amount of chlorophyll presents by measuring the absorbance of the leaf in two wavelength regions
Spikelet fertility	%	The ratio between the number of grains that are filled, including both fully and partially filled ones, and the total count of florets within a spike.

3.3.5 Statistical Analysis

Data were analysed by using the Statistical Tools for Agricultural Research (STAR) version 2.0.1 software and obtained data will be subjected to a two-way and three-way analysis of variance (ANOVA) and the mean differences are compared by least significant differences (LSD) and Tukey's Honest Significance Differences (HSD).

3.3.6 Genetic Analyses

In order to quantify and calculate genetic variations between genotypes and to evaluate genetic and environmental effects on various traits, genetic parameters are used. In order to compute genetic parameters, some formulas are given. Burton & DeVane (1953), Johnson et al. (1955) & Bitew (2016) have calculated and accepted the formula for genotypic variance (1.1), phenotypic variance (1.2), genotypic coefficient of variance (GCV) (1.3) and phenotypic coefficient of variance (PCV) (1.4). For broad sense of Heritability (H) (1.5), all characters and traits were computed by using a formula as cited from Falconer & Mackay (1996) and Bitew (2016). For genetic advance of selection intensity (k) at 5% (2.06) (1.6), it was estimated using formula from Johnson et al. (1955) and Bitew (2016).

$$\sigma_g^2 = \frac{GMS - EMS}{r} \quad (1.1)$$

$$\sigma_p^2 = \sigma_g^2 - \sigma_e^2 \quad (1.2)$$

$$\frac{\sigma_g \times 100}{\text{Population mean}} \quad (1.3)$$

$$\frac{\sigma_p \times 100}{\text{Population mean}} \quad (1.4)$$

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} * 100 \quad (1.5)$$

$$EGA = k * \sigma_{ph} * H_b^2 \quad (1.6)$$

3.4 Molecular Characterization: Identifying QTLs Linked to Drought Stress

For molecular characterization, the rice varieties IR 77298-14-1-2-10 and IR 84984-83-15-18 B were used as positive controls, as these lines have previously been reported to contain drought-responsive quantitative trait loci (QTLs). The earlier positive controls, NMR151 and NMR152, were not included because no prior studies have validated these varieties for the presence of the selected drought-related genes. The commercial cultivar MR219, known to be susceptible to drought, was used as the negative control.

3.4.1 Genomic DNA Extraction

Genomic DNA was extracted using a cetyltrimethylammonium bromide (CTAB) method. The CTAB extraction buffer contained 3% w/v CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, and 1% w/v PVP.

Fresh and young leaves of three weeks seedlings were used to extract the genomic DNA. All leaf samples were cut into smaller pieces by using sterilized pair of scissors. An approximated 100 mg of the sample was transferred into 2 mL labelled tube. After that, two sterilized metal beads were added into each tube. The tubes were closed, inverted 2 to 3 times and placed into the Tissue Lyzer II machine (Retsch® Company, Germany) for cell lysation. The process was set at a frequency of 27 shakes per second for 3 minutes. The tubes were incubated for 25 minutes at 65 °C. Phase separation was performed by adding 500 µL chloroform: isoamyl alcohol (24:1), mixing by inversion for 1–2 min, and standing for 5 min. After 5 minutes, the lysate was centrifuged for 5 minutes at 13 200 rpm. Then, 200 µL of the lysate was pipetted into the Qiashredder mini spin column (colour: lilac) placed in a 2 mL collection tube. All columns were then centrifuged for 2 minutes at 13 200 rpm. The flow through fraction was then transferred into a new tube without disturbing the pellet. The volume for the next step was determined. Typically, 200 - 400 µL of lysate was recovered. Isopropanol was then added according depending on the supernatant volume into a tube and store at -20 ℃ for 60 minutes. The samples were then centrifuged for 15 minutes at 13200 rpm. After centrifuged, the supernatant was then discarded and 70% ethanol was added to clean the DNA pallet. The tube was then centrifuged and the ethanol was discarded from the tube and the tube was let dry. Next, ~100 µL of TE buffer was added into the tube. The finished sample was then kept in -20 ℃ freezer for storage.

3.4.2 DNA Quantification and Quality Assessment

The quality and quantity of the extracted genomic DNA were assessed prior to polymerase chain reaction (PCR) amplification. DNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Scientific, USA) at absorbance ratios of A260/280 and A260/230. An absorbance ratio of ~1.8 at A260/280 was considered indicative of pure DNA, while a ratio between 2.0–2.2 at A260/230 was taken as acceptable for low contamination with polysaccharides or phenolic

compounds. DNA concentration was recorded in ng/µL, and samples were diluted to working concentrations of 20–50 ng/µL for PCR reactions.

To verify DNA integrity, agarose gel electrophoresis (0.8% w/v agarose in 1× TBE buffer) was performed. Approximately 5 µL of each DNA sample was loaded with loading dye and run alongside a 1 kb DNA ladder (Promega, USA) at 80 V for 45 minutes. The gels were stained with FloroSafe dye (1st Base, Singapore) and visualized under a UV transilluminator. Intact high-molecular-weight DNA with minimal smearing was considered suitable for further downstream applications.

Samples meeting the minimum quality standards were used for SSR marker amplification in molecular characterization, while degraded or impure DNA samples were re-extracted to ensure reliability of genotyping results.

3.4.3 SSR Marker Amplification

Polymerase Chain Reaction (PCR) amplification was performed to detect polymorphisms at drought-responsive SSR loci linked to quantitative trait loci (QTLs) associated with reproductive-stage drought tolerance in rice. A set of validated SSR primers targeting qDTY2.2, qDTY3.1, and qDTY12.1 were used (Kumar et al., 2014a).

PCR reactions were carried out in a final volume of 20 µL consisting of 50 ng of genomic DNA template, 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each forward and reverse primer, 1 U of Taq DNA polymerase (Promega, USA), and nuclease-free water to make up the volume. Amplification was conducted in a thermal cycler (Bio-Rad, USA) using the following program: initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, primer annealing at the respective optimized temperature (50 – 67 °C depending on the marker; see Table 3.3), and extension at 72 °C for 1 minute. A final extension was carried out at 72 °C for 7 minutes before holding at 4 °C.

Table 3.3
Identified QTLs for High-Yielding Rice in Lowland Habitats Under Extreme Drought in Reproduction-Stage.

QTLs	Ch	Marker/Interval	Annealing Temperature
$qDTY_{2.2}$	2	RM236, RM12460, RM12569, RM555, RM110, RM233A, RM573	55℃
		RM154	61℃

		RM109	67℃
$qDTY_{3.1}$	3	RM520, RM416, RM16030, RM15935	55℃
$qDTY_{12.1}$	12	RM1261	50℃
		RM28166, RM28048, RM28089, RM28099, RM28130, RM511, RM28172, RM28076	55℃

Source: Gramene (n.d.).

PCR products were resolved on 3% agarose gels prepared in 1× TBE buffer, stained with FloroSafe dye (1st Base, Singapore) and 50 bp DNA ladder (Promega, USA) was used as size markers to determine allele sizes. The gel was then run under 75V for at least 65 minutes with the expected PCR product (Table 3.4) visualized using an AlphaImager Gel Imaging System (Alpha Innotech Corporation, USA) prior exposure under UV illumination. Clear and reproducible bands were observed and scored using AlphaEaseFC™ software, with the presence or absence of specific alleles recorded for each genotype. Table 3.4 shows the expected PCR products and the primer sequence of the selected markers used in this study.

Table 3.4
Simple Sequence Repeat (SSR) Markers with the Expected PCR Product.

Gene	SSR markers	Primer sequence	PCR product
$qDTY_{2.2}$	RM236	F:5'-GCGCTGGTGGAAAATGAG-3' R:5'-GGCATCCCTCTTTGATTCCTC-3'	191bp
	RM12460	F:5'-TGGCACTACAGTGACAACAAACC-3' R:5'- AGGGACTTTATCCAAAGGACACG-3'	168bp
	RM12569	F:5'-GCTCATCATCATCATCGCAGTGG-3' R:5'- ATCCATGTGGCAGACACACTTGC-3'	129bp
	RM555	F:5'- TTGGATCAGCCAAAGGAGAC-3' R:5'- CAGCATTGTGGCATGGATAC-3'	223bp
	RM110	F:5'-TCGAAGCCATCCACCAACGAAG-3' R:5'-TCCGTACGCCGACGAGGTCGAG-3'	156bp
	RM233A	F:5'-CCAAATGAACCTACATGTTG-3' R:5'-GCATTGCAGACAGCTATTGA-3'	162bp
	RM573	F:5'-CCAGCCTTTGCTCCAAGTAC-3' R:5'-TCTTCTTCCCTGGACCACAC-3'	201bp
	RM154	F:5'- ACCCTCTCCGCCTCGCCTCCTC-3' R:5'- CTCCTCCTCCTGCGACCGCTCC-3'	183bp
	RM109	F:5'- GCCGCCGGAGAGGGAGAGAGAG-3' R:5'- CCCCGACGGGATCTCCATCGTC-3'	97bp
$qDTY_{3.1}$	RM520	F:5'- AGGAGCAAGAAAAGTTCCCC-3' R:5'- GCCAATGTGTGACGCAATAG-3'	247bp
	RM416	F:5'- GGGAGTTAGGGTTTTGGAGC-3' R:5'- TCCAGTTTCACACTGCTTCG-3'	114bp
	RM16030	F:5'- GCGAACTATGAGCATGCCAACC-3' R:5'- GGATTACCTGGTGTGTGCAGTGTCC-3'	99bp
	RM15935	F:5'- GACTTTGATTAGACAGAGGCCAAGC-3' R:5'- GCGCCAGGACTAAACTAAACAGC-3'	143bp
$qDTY_{12.1}$	RM1261	F:5'- GTCCATGCCCAAGACACAAC-3' R:5'- GTTACATCATGGGTGACCCC-3'	167bp
	RM5364	F:5'- GTATTACGCTCGATAGCGGC-3' R:5'- GTATCCTTTCTCGCAATCGC-3'	148bp

	RM28166	F:5’- TGCTTGCAAACATTGCTTCTGG-3’ R:5'- ACTGATGTACTGAACACGGGAAGG-3'	194bp
	RM28048	F:5’- TTCAGCCGATCCATTCAATTCC-3’ R:5'- GCTATTGGCCGGAAAGTAGTTAGC-3'	93bp
	RM28089	F:5’- GGGAGGACACCTGTGTAAGTAGG-3’ R:5'- GGTTCAAATGAGACCCAATTCC-3'	260bp
	RM28099	F:5’- TGTGCGGATGCGGGTAAGTCC-3’ R:5'- CCACCTGTCAACCACCGAAACC-3'	120bp
	RM28130	F:5’- CAGCAGACGTTCCGGTTCTACTCG-3’ R:5'- AGGACGGTGGTGGTGATCTGG-3'	175bp
	RM511	F:5’- CTTCGATCCGGTGACGAC-3’ R:5'- AACGAAAGCGAAGCTGTCTC-3'	130bp
	RM28172	F:5’- GGCTCTGTTCATCACACAACACG-3’ R:5'- ATGGCGAGGTAAGCAGAGATGC-3'	171bp
	RM28076	F:5’- GGGACTTGGGACCAGTTTATGG-3’ R:5'- TCAGGTCTGTTGGATTCCATGC-3'	289bp

Note: F= Forward primer, R= Reverse primer; Source: Gramene (n.d.)

Allelic data generated from SSR markers were subsequently used to identify polymorphisms between advanced mutant rice lines and control varieties, and to assess marker-trait associations with drought tolerance at the reproductive stage.

3.4.4 Cluster Analysis

To evaluate the genetic relationships among advanced mutant rice lines and control varieties, cluster analysis was performed based on SSR marker data. Alleles were scored as binary data, with “1” indicating the presence of a band and “0” indicating its absence. The binary matrix was used to calculate pairwise genetic similarity coefficients using Nei & Li (1979) similarity index.

A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm implemented in NTSYSpc version 2.10e (Rohlf, 2000). This method groups genotypes according to their genetic similarity, allowing visualization of relationships among drought-tolerant mutant lines, positive controls, and the susceptible check. The robustness of clustering was assessed through cophenetic correlation analysis, which measures the goodness-of-fit between the similarity matrix and the dendrogram structure.

3.5 Biochemical Changes of Advanced Mutant Rice Lines in Response to Drought Stress.

3.5.1 Determination of Proline Content.

Method to measure proline followed Bates (1973) and Zarifth Shafika et al. (2018). In 10 mL aqueous sulfosalicylic acid (3%), 0.5 g of plant content was homogenized and the homogenate purified onto filter paper. In a test tube, 2 mL of filtrate was reacted with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid for 1 hour at 100 °C, and the reaction was finished in an ice bath. The reaction mixture would then was extracted with 4 mL of toluene and stirred vigorously for 15-20 seconds with a test tube agitator. The toluene-containing chromophore (pink colour; Figure 3.3) was withdrawn from the aqueous stage, then warmed until reaching to room temperature and the absorption is read at 520 nm by using toluene as a blank. The proline concentration was then determined from a standard curve and calculated on a fresh weight basis using the formula as stated by Bates (1973).

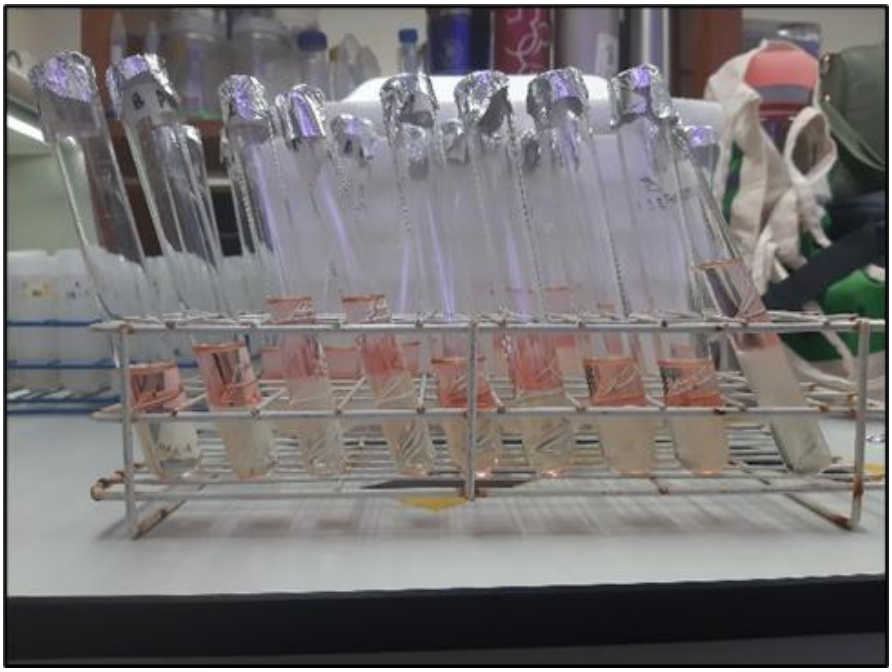

Figure 3.3 Formation of the Proline Ninhydrin Chromophore During Proline Determination.

3.5.2 Evaluation of Enzyme Extract and Antioxidant Enzyme Assay

3.5.2.1 Protein Extraction

Enzyme extraction was determined using the method of Aebi (1984) and Zarifth Shafika et al. (2018). Third or fourth fully expanded leaves from the tip of shoots in four plants per genotype in each treatment were sampled for enzyme extraction at peak flowering stage (85 DAT) and immediately snap-frozen in liquid nitrogen and the samples were transferred into an ice box containing ice cubes and brought to the laboratory. A total of 0.5 g of samples was crushed to a fine powder using mortar and pestle in the presence of liquid nitrogen and homogenized with 5 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 0.4 M EDTA, 1 M ascorbate and 2% (w/v) polyvinylpyrrolidone. The crude mixture was homogenized at 15000 rpm for one minute and centrifuged at 15000 × g for 20 minutes. The supernatants were used for the determination of antioxidant enzyme activity.

3.5.2.2 Determination of Protein Content

In order to measure the total soluble protein in the enzyme extract, the Bradford protein assay was used. Six of a BSA standard with a range of 0 to 100 µg protein were prepared in the same extraction buffer used for the samples. The enzyme extract was appropriately diluted to fall within the linear range (0 - 100 µg protein/ 30 µL). Each 30 µL of standard solution or rice protein sample was added to an appropriately labelled test tube. Two blank sets of tubes were prepared; for the standard curve, 30 µL of H_2O was added instead of the standard solution, and for the rice protein samples, 30 µL of protein preparation buffer was added. The protein solutions were assayed in triplicate. Next, 1.5 mL of Bradford reagent was added to each tube and mixed well. Protein samples were then incubated for at least 5 min at room temperature. Over time, the absorbance increased, and samples were incubated for no more than 1 h at room temperature. Absorbance was then measured at 595 nm. The supernatant product from Section 3.5.2.1 was used for the enzyme activity assay.

3.5.3 Determination of Enzyme Extract (Catalase, Ascorbate Peroxidase, and Guaiacol Peroxidase)

3.5.3.1 Catalase

Catalase (CAT) activity (EC 1.11.1.6) was measured following the method described by Aebi (1984) and Zarifth Shafika et al. (2018). The assay mixture consisted of 2.8 mL of 50 mM potassium phosphate buffer (pH 7.0, without EDTA), 120 µL of enzyme extract, and 80 µL of 0.5 M H_2O_2.

The reaction was started by adding H_2O_2, and the decrease in absorbance was monitored at 240 nm for one minute using a spectrophotometer. The activity was calculated using an extinction coefficient (ε) of 36 mM^{-1} cm^{-1} and expressed as unit per mg of protein (U mg-1). Four replicates were analysed for each genotype under each treatment.

3.5.3.2 Ascorbate Peroxidase

Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was determined according to the method of Rao et al. (1997) with minor modifications by Zarifth Shafika et al. (2018). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 1 mM ascorbic acid, 0.2 mM H_2O_2, and 20 µL of enzyme extract.

The reaction was initiated by adding H_2O_2, and the decrease in absorbance was recorded at 290 nm over three minutes. The activity was calculated using an extinction coefficient (ε) of 2.8 mM^{-1} cm^{-1} and expressed as U mg^{-1}. One unit of APX activity was defined as the amount of enzyme required to oxidise 1 nmol of ascorbate per minute. Four replicates per genotype per treatment were analysed.

3.5.3.3 Guaiacol Peroxidase

Guaiacol peroxidase (GPX) activity (EC 1.11.1.7) was measured following the method of Nakano & Asada (1981). The assay mixture consisted of 0.6 mL of 7.14 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 1 mM EDTA, 0.1 mL of 1 mM H_2O_2, and 0.1 mL of 100 mM guaiacol. The reaction was initiated by adding 0.1 mL of enzyme extract, and the increase in absorbance due to tetraguaiacol formation was monitored at

470 nm for 30 seconds. The activity was calculated using an extinction coefficient (ε) of 26.6 mM^{-1} cm^{-1} and expressed as U mg^{-1} protein. Four replicates for each genotype under each treatment were measured.

3.5.4 Data Analysis for Biochemical Analysis

The biochemical data obtained from proline content determination and antioxidant enzyme assays (catalase, ascorbate peroxidase, and guaiacol peroxidase) were subjected to statistical analysis to evaluate the effects of drought stress and genotype. All assays were performed in triplicates, and the mean values for each genotype under both water regimes (well-watered and drought stress) were calculated.

The data were analysed using two-way analysis of variance (ANOVA) with water treatment as the main factor and genotypes as the subplot factor, in order to detect significant differences between treatments and among rice genotypes. Where significant effects were observed, mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test at a 5% probability level to separate treatment means.

All statistical analyses were carried out using the Statistical Tools for Agricultural Research (STAR) version 2.0.1. Significance was set at $p \leq 0.05$.

CHAPTER 4
RESULTS AND DICUSSIONS

4.1 Morpho-Physiological Responses of Advanced Mutant Lines to Drought Stress at Reproductive Stage.

Table 4.1 presents the combined ANOVA results across seasons, while Table 4.2 details the ANOVA results within each season. In the combined analysis (Table 4.1), all traits showed significant differences at $p < 0.05$ except for replication (R) and the three-way interaction (G × S × T). Seasonal effects (S) were significant for plant height (PH), filled grains per panicle (FGP), and panicle length (PL), indicating that environmental differences between seasons influenced these traits. These results highlight the importance of season-specific environmental factors, such as temperature and rainfall on growth and yield-related traits, consistent with earlier studies by Kovi et al. (2011) on rice adaptability under different growing conditions.

Treatment effects (T), however, were not consistently significant across traits, suggesting that drought-stress responses were more genotype-dependent than treatment-dependent. For the interaction between season and treatment (S × T), only chlorophyll content (CC) was significant, highlighting its sensitivity to combined environmental and drought effects. This agrees with reports by Havrlentová et al. (2021) that CC is one of the most responsive physiological indicators of stress in cereals, reflecting changes in photosynthetic efficiency under limited water supply.

Genotypic effects (G) were significant for all traits, confirming strong genetic variability among the tested lines. The genotype × treatment (G × T) interaction was significant for tiller number (TN), panicle number (PN), and unfilled grains per panicle (UFGP), suggesting that genotypes responded differently to imposed drought conditions for these traits. Such genotype-specific responses are important for identifying tolerant lines that maintain tillering and panicle production under stress, which are critical determinants of yield stability. Meanwhile, the genotype × season (G × S) interaction was significant for PH, FGP, and UFGP, reflecting that these traits were influenced by seasonal environments in addition to genetic control.

Table 4.1
Combined Analysis of Variance (ANOVA) Across Seasons for Selected Agronomic Traits in Advanced Mutant Rice Lines.

Sources of variation	df	Mean Square							
		PH (cm)	TN	PN	PL (cm)	FGP	UFGP	SF (%)	CC
Seasons (S)	1	4567.83*	10.69	43.44	38.05*	3798.83*	9.94	38.11	6.94
Replications within seasons (R/S)	6	91.77	10.49	8.77	2.55	522.26	46.74	33.71	2.58
Treatment (T)	1	553.31	262.30*	174.88*	2.51	1501.63	7185.77*	2401.40*	43.70*
S × T	1	100.21	6.32	8.16	0.07	1067.16	66.42	0.38	13.97*
Genotypes (G)	6	158.53*	11.93*	11.27*	11.22*	1795.22*	2302.78*	683.07*	12.14*
G × T	6	66.23	17.97*	8.77*	0.96	467.76	253.76*	71.76	1.28
G × S	6	184.06*	7.15	5.04	3.47	602.29*	193.39*	34.51	2.11
G × S × T	6	13.20	5.64	2.54	0.88	340.4	149.92	50.31	0.18
Error	72	41.10	4.97	3.00	1.92	254.23	79.37	37.27	2.34

Note: * indicates significant differences at $p < 0.05$; PH = plant height, TN = number of tillers, PN = number of panicles, PL = length of panicle, FGP = filled grain per panicle, UFGP = unfilled grain per panicle, SF = spikelet fertility, CC = chlorophyll content.

Table 4.2
Season-Wise Analysis of Variance (ANOVA) for Selected Agronomic Traits in Advanced Mutant Rice Lines.

Source of variation	Mean Square							
	PH (cm)	TN	PN	PL	FGP	UFGP	CC	SF
Season 1 (Offseason)								
Replications (R)	147.38	15.90	15.16	0.26	184.00	15.73	3.05	45.19
Treatment (T)	562.23	175.02*	129.29*	0.88	2550.29	2935.24	53.55*	1231.22*
Genotypes (G)	292.69*	7.69	9.22*	6.00*	592.77	1288.14*	4.93*	325.42*
T × G	62.83	6.08	1.54	1.30	48.82	116.81	0.76	48.79
Error	74.76	3.43	2.20	0.80	360.20	102.98	0.89	41.24
Season 2 (Main season)								
R	36.15	5.09	2.38	4.83	860.52	77.75	2.11	22.23
T	91.29	93.60*	53.74*	1.70	18.50	4316.95*	4.13	1170.56*
G	49.89*	11.39	7.08	8.69*	1804.74*	1208.03*	9.31*	392.16*
T × G	16.60	17.54*	9.77*	0.54	759.34*	286.87*	0.70	73.29*
Error	7.44	6.50	3.80	3.04	148.26	55.77	3.79	33.30

Note: * indicates significant differences at $p < 0.05$; PH = plant height, TN = number of tillers, PN = number of panicles, PL = length of panicle, FGP = filled grain per panicle, UFGP = unfilled grain per panicle, SF = spikelet fertility, CC = chlorophyll content.

This indicates the presence of genotype × environment interactions that need to be considered in breeding programs, echoing the findings of Senguttuvel et al. (2021) who emphasized the role of multi-environment testing in identifying stable drought-tolerant rice varieties.

In the seasonal analyses (Table 4.2), trait-specific responses were more evident. Plant height (PH) was significantly affected by genotype (G) in both Season 1 (off-season) and Season 2 (main season) ($p < 0.05$). This indicates that PH differences were largely genetically determined, with minimal influence from drought stress or interaction effects. Similar results were reported by Zhou et al. (2016), suggesting that PH is a relatively stable trait under water-limited conditions.

For TN and PN, both treatment and genotype effects were significant in both seasons, with additional T × G interaction effects observed in Season 2. This suggests that tillering and panicle production were responsive to drought but with genotype-dependent variation under stress, supporting the view that TN and PN are reliable selection indices for drought tolerance (Bhattacharjee et al., 2023; Jarin et al., 2024).

Panicle length (PL) showed significant genotypic effects across both seasons, but treatment effects remained non-significant, suggesting PL was a relatively stable trait under drought stress similar to the finding by Eragam et al. (2023) and Ji et al. (2012).

Filled grains per panicle (FGP) was strongly influenced by genotype in both seasons, while unfilled grains per panicle (UFGP) showed significant effects from treatment in Season 2, highlighting stress-induced sterility under drought. These results indicate that reproductive-stage drought primarily disrupts grain filling, aligning with earlier findings by Jarin et al. (2024) that spikelet sterility is one of the most critical limitations to rice yield under drought.

Chlorophyll content (CC) demonstrated significant treatment and genotype effects in both seasons, and in Season 1, it was particularly responsive to drought stress, confirming its role as a physiological indicator of tolerance as used by Zhang et al. (2024).

Finally, spikelet fertility (SF) was highly sensitive to both treatment and genotype effects in both seasons, with significant T × G interactions observed in Season 2. This indicates that SF was the most drought-responsive trait and a critical determinant of yield stability under stress (Senguttuvel et al., 2021).

Overall, the combined and seasonal analyses revealed that while some traits such as PH and PL were primarily under genetic control, key yield-related traits including TN, PN, FGP, UFGP, CC, and SF were strongly influenced by genotype × environment interactions. These findings emphasize the importance of multi-season evaluations to capture trait stability and the differential performance of genotypes under drought stress. The identification of traits and genotypes showing consistent performance across environments provides valuable insights for breeding programs aimed at developing drought-resilient rice varieties.

4.1.1 Morphological Traits (Growth-related)

4.1.1.1 Plant Height

Table 4.3 shows the mean comparison of genotypes between the off-season (Season 1) and main planting season (Season 2). In Season 1, mean comparison using Tukey's HSD test (Table 4.3) showed that PH ranged from 107.62 to 122.20 cm. The tallest genotype was NMR152 (122.20 cm), followed closely by MR219 (120.80 cm) and MINT3 (120.06 cm). In contrast, NMR191 (107.62 cm) and MINT9 (108.33 cm) recorded the lowest plant heights during this season.

Table 4.3
Tukey's HSD Mean Comparison of Genotypes for Plant Height (PH) During Off-Season and Main Season.

Genotype	Off-Season Mean (cm)	Main Season Mean (cm)
NMR151	119.60 ab	133.59 a
NMR152	122.20 a	127.66 bc
MR219	120.80 ab	125.58 c
NMR191	107.62 b	130.20 ab
MINT1	117.22 ab	129.51 abc
MINT3	120.06 ab	130.25 ab
MINT9	108.33 b	128.45 bc

Note: Means with the same letter are not significantly different according to Tukey's HSD at $\alpha = 0.05$.

In Season 2, PH values were generally higher, ranging from 125.58 to 133.59 cm. NMR151 (133.59 cm) exhibited the greatest plant height, followed by NMR191 (130.20 cm) and MINT3 (130.25 cm). The shortest genotype in the main season was MR219 (125.58 cm). These results indicate that several advanced mutant lines, particularly MINT3 and NMR191, were statistically comparable to the positive control NMR151 in both seasons, and all mutant lines except MINT9 (in Season 1) performed

similarly or better than the negative control MR219. Only MINT9 remained significantly shorter in Season 1 but demonstrated improved PH in Season 2.

Across both seasons, the advanced mutant line NMR151 consistently recorded the tallest plant height and outperformed all other genotypes, including the positive controls, in Season 2. Several other advanced mutant lines particularly MINT3, MINT1, and NMR191 showed comparable or superior performance relative to the negative control MR219, and were statistically similar to the positive control NMR152, especially in the main season. Only MINT9 remained significantly shorter in Season 1 but demonstrated improved PH in Season 2.

The lack of treatment effect across both seasons is likely due to the timing of stress, as PH is largely determined during the vegetative stage and stabilizes before reproductive development begins. Yang et al. (2019) elucidates that drought imposed at the reproductive stage typically affects reproductive and physiological traits more than vegetative morphology, which supports the lack of treatment effect on PH in this study. The non-significant G × T interaction further supports the stability of genotypic performance under varying water availability. Across both seasons, genotypic rankings were relatively stable: NMR151 and NMR152 consistently exhibited greater height, while MINT9 and NMR191 remained among the shorter genotypes. This stability supports the use of PH as a morphological selection trait in breeding programs targeting consistency across environments as noted by Xu et al. (2021).

Additionally, rainfall patterns may have mitigated drought stress severity, especially in Season 2. Rainfall data obtained from myMETdata using the nearest weather station, which is at MARDI Tanjong Karang (Appendix 2), shows Season 1 (March – July) received approximately 124.1 mm/month, while Season 2 (August – December) received around 265.54 mm/month, which may have reduced the physiological impact of drought treatments.

4.1.1.2 Tiller Number (TN)

For TN, significant differences were observed for T in both seasons, but during Season 2, significant differences were also detected for the T × G interaction. In Season 1, only the T effect was significant ($p < 0.05$), while G and T × G effects were not. This indicates that drought stress uniformly reduced tiller number across all genotypes during the offseason, with no statistically meaningful variation among them, similar to the

general findings by Pervin et al. (2023). This suggests that drought during the reproductive stage may have disrupted tiller retention regardless of genotype, likely due to physiological stress responses such as reduced assimilate availability or altered hormonal signalling that suppress further vegetative development (Liu et al., 2020; Patil et al., 2022). The post hoc LSD analysis (Table 4.4) further confirmed this effect, with plants under drought exhibiting a significantly lower mean TN compared to the well-watered control.

Table 4.4
LSD Result for Treatment Effect on Tiller Number (TN) During the Off-Season.

Treatment	Mean
Control ©	22.98 a
Drought (D)	19.44 b

Note: Mean with the same letter are not significantly different at $p < 0.05$.

In Season 1, under well-watered conditions, the highest TN values were recorded in NMR151 (25.25), MINT9 (24.60), NMR152 (23.50), and NMR191 (22.45), while MR219 also produced a relatively high TN (23.25). However, all genotypes exhibited reductions under drought stress. MR219 experienced the most reduction of TN during drought stress with 23.87% (17.70) while the positive control reduced by 19.41% (20.35) and 14.04% (20.20) for NMR151 and NMR152 respectively. Among the advanced mutant lines, NMR191 reduced the lowest at 5.04%, followed by MINT3 (10.14%), MINT1 (11.24%) and MINT9 (21.54%) in order.

In Season 2, under control conditions, TN ranges from 21.45 to 25.60 with the highest TN exhibited by MINT9 (25.60) followed by NMR191 (24.45), and NMR151 (24.00) while the lowest TN exhibited by MR219 (21.75). Under drought stress, TN ranges from 17.30 to 23.35 with the highest TN exhibited by MINT1 (23.35) and NMR152 (22.95) while MR219 has the lowest TN (17.30). Post hoc analysis using Least Significant Differences (LSD) test (Table 4.5) revealed significant genotypic variation under both water regimes where only NMR151, MR219, and MINT9 are significantly different between well-watered and drought stress plot. Interestingly, MINT1 and NMR152 recorded slight increases in TN under drought, with 8.86% and 1.55% change, respectively, while NMR191 experienced a modest decline of 12.47%. In contrast, MINT9 showed the most severe decline (26.37%), followed by MR219 (19.91%) and NMR151 (17.29%). These contrasting responses highlight the broad phenotypic variation among the ion-beam-derived mutant genotypes. Such variation is commonly observed following ion-beam mutagenesis because irradiation can generate

a wide mutation spectrum, producing both beneficial and neutral or deleterious phenotypes (EFSA et al., 2021; Feng et al., 2023).

Table 4.5
LSD Result for Treatment × Genotype Interaction on Tiller Number (TN) During the Main Season.

Genotype	Treatments	
	Control	Drought
NMR151	24.00 a	19.85 b
NMR152	22.60 a	22.95 a
MR219	21.75 a	17.30 b
NMR191	24.45 a	21.40 a
MINT1	21.45 a	23.35 a
MINT3	22.00 a	20.05 a
MINT9	25.60 a	18.85 b

Note: Mean with the same letter indicate no significant differences at $p < 0.05$.

These results confirm the negative impact of drought stress on tillering in both seasons. However, the advanced mutant lines NMR191, MINT3 and MINT1 demonstrated superior stability and drought adaptability, with minimal in TN under stress. These lines consistently outperformed the drought-sensitive check MR219 and showed performance on par with or superior to the positive controls, highlighting their potential as promising candidates for breeding programs aimed at improving drought tolerance in rice suggesting inherent physiological advantages, such as robust root systems (Kim et al., 2020) or hormonal regulation supporting tiller survival (Wang et al., 2022), making them promising drought-resilient genotypes. MINT1 and MINT3's moderate declines further support their potential as stress-tolerant lines, while MINT9's vulnerability highlights the need for careful selection among mutant populations. These findings confirm the finding by Khitka et al. (2021) that ion beam irradiation can produce lines with variable stress adaptability, and underscore the importance of validating performance under both stressed and non-stressed environments. Additionally, the observed interaction between genotype and treatment in the main season but not in the off-season may be influenced by more favourable baseline environmental conditions, which allowed genotypic differences to be more clearly expressed. Overall, these results highlight the importance of multi-season screening and demonstrate the utility of TN as a drought-adaptive trait in rice breeding programs, especially when advanced mutant lines are evaluated alongside standard checks.

4.1.1.3 Panicle Number

For panicle number (PN), during Season 1, both T and G effects were significant ($P < 0.05$), while the T × G interaction was not, indicating that genotypic rankings remained consistent across water regimes. In contrast, Season 2 showed significant effects for T and T × G interaction, suggesting that genotypic responses to drought varied depending on the water availability, although the main effect of G was not significant. This aligns with studies by Pantuwan et al. (2002) and Jarin et al. (2024) reporting PN as a drought-sensitive trait, particularly under terminal drought stress.

In Season 1, the mean PN under well-watered conditions (19.05) was significantly higher than under drought (16.01), reflecting an average reduction of 15.96%. By comparing the PN between well-watered plot and drought stress plot, MR219 drops the most at 21.96%, averaging from 17.86 in well-watered plot to 13.94 in drought stress plot followed by MINT9 at 21.54%. NMR191, MINT1 and MINT3 experienced below 15% reduction in drought stress plot compared to well-watered plot. Post hoc analysis using Tukey's HSD test (Table 4.6) revealed that MINT3 (18.81) was the highest-yielding genotype and significantly different from MR219 (15.90) and MINT1 (16.45) and not significantly different to the positive controls, NMR191 and MINT9. All of the advanced mutant lines performed better than MR219 indicated by the higher PN.

Table 4.6
Mean Comparison of Genotypes for Panicle Number (PN) During Off-Season (Tukey's HSD Test).

Genotype	Mean PN
NMR151	18.65 ab
NMR152	17.93 abc
MR219	15.90 c
NMR191	17.41 abc
MINT1	16.45 bc
MINT3	18.81 a
MINT9	17.56 abc

Note: Mean with the same letter are not significantly different.

In Season 2, although G differences were not significant overall, the significant T × G interaction warranted closer examination. Under well-watered conditions (Table 4.7), NMR191 (18.34), NMR151 (18.00), and NMR152 (16.95) showed the highest PN values. Under drought stress, NMR152 (17.21) and MINT1 (17.51) maintained their PN respectively. Conversely, MINT9 showed decline from 19.20 (well-watered plot) to

14.14 (drought stress plot), and MR219 decreased from 15.78 to 12.98. These results highlight the superior drought tolerance of NMR152 and MINT1, which not only retained panicle number under stress but also matched or exceeded the performance of the positive controls.

Table 4.7
Mean Comparison of Treatments for Each Genotypes for Panicle Number (PN) During Main Season Using Least Significant Differences Test.

Genotype	Control	Drought
NMR151	18.00 a	14.88 b
NMR152	16.95 a	17.21 a
MR219	15.78 a	12.98 b
NMR191	18.34 a	15.38 b
MINT1	16.09 a	17.51 a
MINT3	16.50 a	15.04 a
MINT9	19.20 a	14.14 b

Note: Mean with the same letter show no significant differences at $p < 0.05$.

Notably, MR219 again recorded the lowest values under drought, affirming its status as a susceptible check (Noraziyah et al., 2016a). NMR151, though showing a reduction under stress, remained among the higher-performing genotypes, consistent with its role as a positive control (Ahmad et al., 2023). This interaction pattern highlights the utility of PN as a discriminating trait for drought adaptability, consistent with reports by Jarin et al. (2024), which noted declines in PN under reproductive-stage drought serve as a practical early yield-component signal of reproductive stress in rice.

Furthermore, genotypes like NMR191, MINT1 and MINT3 demonstrated PN values under drought that were statistically comparable to their positive control means (Table 4.7). Such performance parallels known drought-tolerant varieties like Sahbhagi Dhan (Anantha et al., 2016) and IR64-DTY lines (Swamy B. P. et al., 2013), which maintained stable PN under terminal drought stress. Meanwhile, the response of MR219 closely mirrors known susceptible varieties like Swarna, which experience notable PN reductions under similar stress conditions (Grondin et al., 2018).

4.1.2 Reproductive Traits (Yield-contributing)

4.1.2.1 Panicle Length

Panicle length (PL) was significantly affected by season (S) and genotype (G) ($P < 0.05$), while treatment (T) and interaction effects were not significant, as indicated

by the combined ANOVA. The significant seasonal effect suggests that environmental variation between the offseason and main season such as temperature (Mohamaed et al., 2021), photoperiod, and solar radiation (Lee et al., 2016) contributed to overall differences in PL across genotypes. In both individual seasons, genotype had a significant effect on PL ($P < 0.05$), while T and T × G interaction remained non-significant. This indicates that differences in panicle length were genetically determined and relatively stable under both well-watered and drought conditions.

During the off-season (Season 1), the longest PL was observed in MINT9 (29.20 cm), (Table 4.8), followed by MINT1 (28.73 cm), NMR152 (28.53 cm), and NMR191 (27.79 cm). The shortest panicles were recorded in MR219 (27.12 cm) and NMR151 (26.96 cm), significantly lower than the top performers. All of the advanced mutant lines have longer PL and outperformed the drought-sensitive check MR219 and the positive control NMR151. During the main season (Season 2) (Table 4.8), the trend remained consistent. NMR191 produced the longest panicles (30.51 cm). Other lines including MINT1 (28.81 cm), MINT3 (29.37 cm), MINT9 (30.25 cm), NMR152 (29.09 cm), and NMR151 (28.29 cm) while MR219 (27.55 cm) recorded the shortest panicle length.

Table 4.8
Tukey's HSD Comparison of Genotypes for Panicle Length (PL) in Both Planting Season.

	Mean PL (cm)	
Genotype	Off-Season	Main Season
NMR151	26.96 c	28.29 ab
NMR152	28.53 ab	29.09 ab
MR219	27.12 c	27.55 b
NMR191	27.79 bc	30.51 a
MINT1	28.73 ab	28.81 ab
MINT3	27.39 bc	29.37 ab
MINT9	29.20 a	30.25 ab

Note: Mean with the same letter show no significant differences.

Comparisons across seasons showed slight increases in PL for all genotypes in the main season, further supporting the significant seasonal effect. For instance, NMR191 increased from 27.79 cm (Season 1) to 30.51 cm (Season 2), while MINT9 improved from 29.20 cm to 30.25 cm. In contrast, MR219 only slightly increased from 27.12 cm to 27.55 cm and remained the lowest performer.

The significant genotypic and seasonal effects observed for PL highlight the strong genetic basis and environmental responsiveness of this trait. The lack of a

treatment effect indicates that PL was relatively stable across moisture regimes, which is consistent with previous report by Eragam et al. (2023) suggesting that PL is less sensitive to drought stress than other yield components such as grain number or spikelet fertility. Longer panicles are often associated with higher yield potential due to an increased number of grain-bearing spikelets (Heng et al., 2018).

Advanced mutant lines consistently produced longer panicles than MR219, regardless of season or water regime. This aligns with earlier studies suggesting that mutation breeding can enhance yield-related traits like panicle length by altering regulatory or hormonal pathways involved in inflorescence development (Agata et al., 2020; Sobri et al., 2020). The superior performance of the advanced mutant lines suggests these lines carry favourable alleles that could be leveraged in breeding programs to improve panicle architecture.

The seasonal increase in PL, particularly in the main season, may be attributed to more favourable climatic conditions that promote reproductive growth. This observation is consistent with literature by Fang et al. (2024) indicating that environmental factors during the panicle development stage can significantly influence final panicle length. Overall, the consistent performance of the advanced mutant lines supports their suitability for incorporation into breeding strategies aimed at improving panicle morphology and yield stability under varying field conditions.

4.1.2.2 Filled Grains per Panicle

Significant differences in filled grain per panicles (FGP) were observed for genotypes (G) effects in Season 2 (main season), along with a significant T × G interaction ($P < 0.05$). In contrast, Season 1 (offseason) showed no significant differences for any source of variation. The combined ANOVA also revealed significant seasonal effect, indicating different planting season exhibits FGP differently.

From the descriptive statistics, the range of FGP under control (C) conditions was 66.00 to 166.67 grains per panicle in Season 1, and 105.31 to 161.78 grains in Season 2. Under drought stress (D), FGP ranged from 66.00 to 156.00 grains in Season 1, and from 72.78 to 199.89 grains in Season 2. These data confirm an overall decline in FGP due to drought, but also show that some genotypes maintained relatively high values under stress.

In Season 1, although differences were not statistically significant, NMR152 (78.50), MINT1 (76.75), and NMR191 (74.00) maintained high FGP under drought, with minimal reductions of 7.93%, 6.11%, and 7.21%, respectively. In contrast, MR219 showed a greater reduction of 16.25%, indicating weaker tolerance under drought stress.

In Season 2, where statistical differences were significant, post hoc mean comparison using Tukey's HSD test revealed clear genotypic separation under drought stress (Table 4.9). While all genotypes showed no significant differences under control, significant differences emerged under drought. MR219 (89.38) was the most affected while all of the advanced mutant lines and the positive controls maintained high number of FGP under drought stress with the highest shown by MINT3 (170.11).

Table 4.9
Tukey's HSD Grouping for Filled Grains per Panicle (FGP) in Season 2.

Genotype	Treatment	
	C	D
NMR151	147.58 a	146.61 ab
NMR152	145.81 a	137.67 ab
MR219	126.66 a	89.38 c
NMR191	137.72 a	138.20 a
MINT1	132.31 a	134.84 ab
MINT3	142.44 a	170.11 a
MINT9	130.86 a	138.53 b

Note: Mean with the same letter show no significant differences at $p < 0.05$.

These findings are in line with previous reports indicating that drought stress during the grain-filling stage reduces spikelet fertility and assimilate transport, resulting in lower FGP as cited by Salgotra & Chauhan (2023). Genotypes that maintain higher FGP under stress maybe due to possessing physiological traits such as improved source-sink translocation, hormonal regulation, or peduncle elongation and panicle exertion (He & Serraj, 2012; Jiang et al., 2022; Z. Peleg et al., 2011)

4.1.2.3 Unfilled Grains per Panicle

For unfilled grains per panicle (UFGP), the analysis of variance revealed that significant differences ($P < 0.05$) were observed among genotypes in both seasons. In Season 2 (main season), significant effects were also detected for T, G the G × T interaction, indicating that genotypes responded differently to drought stress. In

contrast, only genotypic differences were significant in Season 1 (offseason), with no significant effects from T or the G × T interaction.

In Season 1, UFGP under well-watered conditions ranged from 8.67 grains (NMR191) to 42.72 grains (NMR151), while under drought stress, values increased and ranged from 24.50 grains (NMR191) to 60.53 grains (MR219). By performing Pairwise Mean Comparison of the genotypes using Tukey's HSD test, it can be observed that NMR151 and MR219 are significantly different compared to NMR152 and the advanced mutant lines with the former shown high UFGP (Table 4.10). Notably, NMR191 maintained the lowest UFGP across both treatments in this season. The absence of significant treatment or interaction effects suggests that the imposed drought stress was either insufficient to differentially impact grain filling or that environmental conditions during this off-season allowed genotypes to compensate effectively. Despite this, UFGP values were still numerically higher under drought for all genotypes, highlighting some physiological sensitivity even in the absence of statistical significance.

Table 4.10
Pairwise Mean Comparison of Genotypes for Unfilled Grains per Panicle Using Tukey's HSD for UFGP During Season 1.

Genotype	Mean
NMR151	48.79 a
NMR152	31.60 b
MR219	49.33 a
NMR191	16.58 b
MINT1	23.59 b
MINT3	29.78 b
MINT9	23.62 b

Note: Similar letters indicate no significant differences between each other at $p < 0.05$.

In Season 2, UFGP under control conditions ranged from 12.71 grains (NMR191) to 38.12 grains (MR219), and under drought stress from 28.55 grains (MINT3) to 76.61 grains (MR219). The drought treatment led to a clear and consistent increase in UFGP across all genotypes, in line with the significant T effect. Additionally, the significant G × T interaction suggests that the magnitude of increase varied among genotypes. By comparing the genotypes at different treatment using Tukey's HSD test (Table 4.11), it was observed that under well-watered treatment, NMR151 and MR219 significantly different compared to the advanced mutant lines.

Table 4.11
Comparison of Genotypes at Different Treatment for UFGP Using Tukey's HSD During Season 2.

Genotype	Treatment	
	C	D
NMR151	37.75 a	37.03 b
NMR152	23.86 ab	41.69 b
MR219	38.12 a	76.61 a
NMR191	12.71 b	29.70 b
MINT1	14.13 b	36.68 b
MINT3	19.03 b	28.55 b
MINT9	20.41 b	38.97 b

Note: Mean with same letter shows no significant differences.

All of the advance mutant lines have lower UFGP compared to NMR151 and MR219. Under drought stress plot, MR219 differ significantly compared to the other tested genotypes by showing highest UFGP. The significant increase in UFGP under drought in Season 2 demonstrates a clear negative impact of water stress on spikelet fertility. This aligns with findings from previous studies reporting that drought stress disrupts pollination and reduces starch accumulation in developing grains, leading to spikelet sterility (Salgotra & Chauhan, 2023). The significant G × T interaction further indicates that the extent of this impact varied among genotypes. Positive control and the advanced mutant lines exhibited lower UFGP under drought, suggesting better maintenance of reproductive processes and sink strength under stress.

The stable performance of the advanced mutant lines across seasons under both control and drought conditions, is particularly notable. This advanced mutant line may possess enhanced physiological traits such as superior panicle exertion, improved pollen viability, or better translocation efficiency, all of which contribute to lower spikelet sterility under stress.

4.1.2.4 Spikelet Fertility (SF)

For SF, the analysis of variance in for both Season 1 and Season 2 revealed significant differences ($P < 0.05$) in both T and G. This indicates that although drought stress significantly reduced spikelet fertility overall, all genotypes responded similarly to the treatment in this season.

Across genotypes and seasons, SF under well-watered conditions ranged from 75.97% (MR219) to 92.66% (NMR191), while under drought stress, SF ranged from 59.62% (MR219) to 81.60% (NMR191). By performing pairwise mean comparison of

treatment, the LSD test confirmed that SF under drought (76.11%) was significantly lower than under control conditions (85.37%), indicating clear reproductive sensitivity to water deficit. By comparing genotypes across season using Tukey's HSD test, MR219 was significantly different compared to the positive control and the advanced mutant lines by showing the lowest SF among all of the tested genotypes.

During Season 1, the average mean of SF under well-watered plot is 84.84% while for drought stress plots, average SF is at 75.74%, which can be distinct into two different group by performing pairwise mean comparison of T using LSD test.

Tukey's HSD comparison among genotypes showed that NMR191 had the highest SF (86.81%) and was statistically distinct from the susceptible check MR219, which recorded the lowest SF (68.65%). Several other genotypes, including MINT1, MINT3, MINT9 also maintained relatively high SF values above 79%, suggesting partial tolerance to drought-induced reproductive stress. In contrast, NMR151 showed moderately reduced fertility (75.33%) and grouped statistically with both high and low performers.

Table 4.12
Pairwise Mean Comparison of Genotypes Using Tukey's HSD Test for SF Across Seasons.

Genotype	Mean
NMR151	75.33 bc
NMR152	79.41 ab
MR219	68.65 c
NMR191	86.81 a
MINT1	85.08 ab
MINT3	81.45 ab
MINT9	84.36 ab

Note: Mean with same letter signify no significant differences.

For Season 2, average SF for well-watered plot is 85.89%, while for drought stress plots, average SF is at 76.75%. By performing pairwise mean comparison of G using Tukey's HSD, all of the advanced mutant lines were grouped together with the positive control, while MR219 is located in its own distinct group.

SF is a critical component of yield, directly reflecting the success of pollination and grain set. In the present study, SF was significantly influenced by both genotype and water availability in Season 1 (off season), while the G × T interaction was not significant. This indicates that although drought stress reduced SF overall, the response trend was relatively consistent across genotypes during this season.

The significant reduction in SF under drought stress aligns with numerous studies reporting that water deficit during flowering disrupts processes such as anther dehiscence, pollen viability, and fertilization (He & Serraj, 2012; Salgotra & Chauhan, 2023). The observed drop in mean SF from 84.84% under control to 75.47% under drought reflects a moderate but statistically significant reproductive penalty due to limited water availability.

Among the tested genotypes, NMR191 consistently maintained the highest SF under both control and drought conditions. Its performance was better to that of the drought-susceptible check MR219, which recorded the lowest SF values across treatments. This suggests that NMR191 may possess enhanced physiological traits supporting reproductive resilience, such as efficient water retention, stronger spikelet sink strength, or improved flowering synchrony under stress (S. K. Dwivedi et al., 2023; Rang et al., 2011).

Several other advanced lines including MINT1, MINT3, MINT9 also maintained relatively high SF values under drought, grouping closely with NMR191 and outperforming MR219. In contrast, NMR151, while superior to MR219, showed moderately reduced fertility, suggesting some sensitivity to drought at the reproductive stage.

4.1.3 Physiological Traits (Indicator of Stress Tolerance)

4.1.3.1 Chlorophyll Content

For chlorophyll content (CC), significant differences ($P < 0.05$) were observed among genotypes in both seasons. In Season 1, genotype (G) and treatment (T) effects were significant, indicating that chlorophyll levels differed among genotypes and were reduced/altered by drought stress overall. The non-significant G × T interaction suggests that the drought effect on chlorophyll was generally consistent across genotypes, with no strong evidence of genotype-specific differences in the magnitude or direction of response. In contrast, only genotypic effects were significant in Season 2, suggesting more stable CC under different water regimes during this period.

In Season 1, CC under well-watered conditions ranged from 38.45 (MINT1) to 41.34 (NMR151), while under drought stress it ranged from 37.25 (NMR191) to 38.56 (MR219). By performing pairwise mean comparison of G using Tukey's HSD test, it

can be observed that NMR151 and MR219 were not significantly different, while the advance mutant lines NMR191, MINT1 and MINT9 differ significantly from the former two genotypes. MINT3 on the other hand is not significantly different to any of the genotypes.

In Season 2, CC under control conditions ranged from 37.12 (NMR191) to 40.37 (MINT3), and under drought stress from 36.58 (NMR191) to 39.70 (MINT3). Tukey's HSD analysis revealed that genotypes such as MINT3 and MR219 consistently recorded higher CC values. Conversely, NMR191 consistently recorded the lowest CC across both treatments, indicating lower chlorophyll retention.

CC is often used as an indicator of photosynthetic capacity and plant health under abiotic stress, including drought. In this study, significant genotypic differences in CC were observed across both seasons, aligning with previous findings by Yang et al. (2024) that chlorophyll content is largely genotype-dependent under stress conditions.

In Season 1, the significant effects of both T and G × T interaction suggest that drought stress caused differential chlorophyll degradation among genotypes. Some genotypes, such as MINT3 and MR219, maintained relatively higher CC under drought, suggesting better protective mechanisms for chlorophyll retention. This is consistent with studies reporting that tolerant genotypes exhibit delayed chlorophyll degradation as a drought adaptive trait (Ramkumar et al., 2019).

However, in Season 2, only G had a significant effect on CC, indicating that seasonal factors may have moderated the drought impact, or that plants experienced less physiological stress during the main season. The lack of significant treatment and interaction effects in Season 2 implies that CC was more stable and less influenced by mild and moderate drought, a finding also reported in similar agroecological conditions (Wang et al., 2025).

Among the advanced mutant lines, MINT3 consistently performed well across seasons, maintaining high chlorophyll levels under both control and drought conditions. This suggests that MINT3 may possess traits associated with improved oxidative stress management and photosynthetic stability. In contrast, NMR191 consistently recorded the lowest CC values and was grouped in the lowest significance category, highlighting its susceptibility to chlorophyll degradation under stress.

4.1.4 Multivariate Analysis Using Cluster Analysis (Trait Integration)

By using the data of agronomic and morpho-physiology of the selected varieties and line, agglomerative hierarchical clustering separated the seven genotypes into two principal group at 50% coefficient (Figure 4.1).

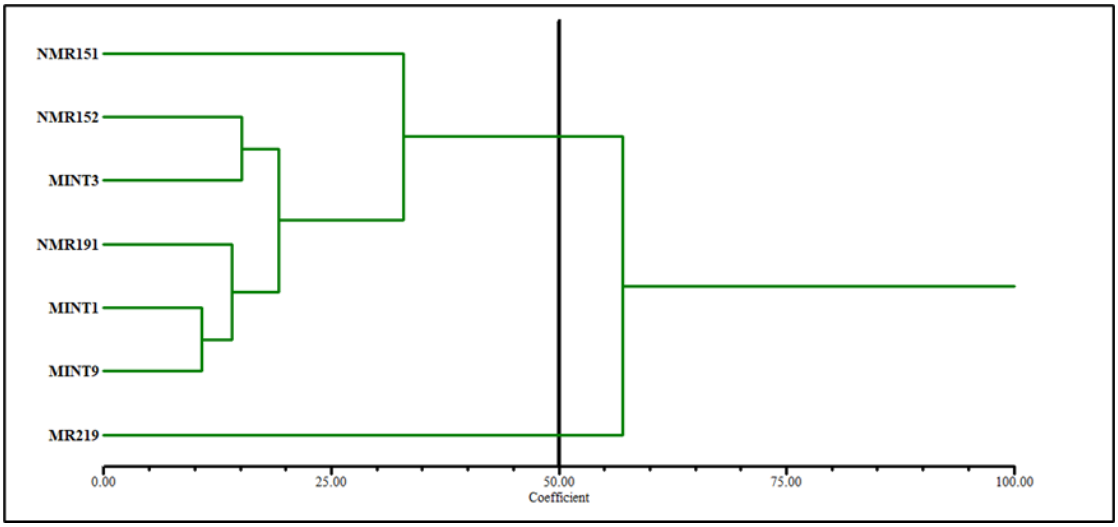

Figure 4.1 Cluster Analysis for Morpho-Physiological Traits.

Cluster 1 comprised the two drought-tolerant checks NMR151 and NMR152 together with the ion-beam–derived advanced mutant lines NMR191, MINT1, MINT3, and MINT9, while the drought-susceptible check MR219 formed a solitary cluster (Cluster 2), confirming its pronounced overall divergence from the remainder of the panel. Within the major cluster, MINT1 and MINT9 fused at the lowest level, indicating near-redundant profiles; NMR191 joined this pair next, forming a compact "mutant" subgroup. In parallel, MINT3 clustered tightly with NMR152, indicating higher drought tolerance compared to other advance mutant lines. NMR151 attached later than these subgroups but still within the same cluster, marking it as the most distinct member of the drought tolerant set.

This topology is consistent with prior characterizations of NMR151 and NMR152 as drought-tolerant checks and MR219 as a drought-susceptible check (Ahmad et al., 2023; Mohd Ikmal et al., 2019), and with the shared provenance of NMR191 and the MINT lines as advanced mutants derived via ion-beam irradiation, which plausibly explains their proximity in multivariate trait space (Hussein, 2021). MR219's separation as a drought-susceptible genotype supports its continuous role as

susceptible check and to maximize inter-cluster divergence in methodological evaluations (Nivedha et al., 2024). Given MR219's known drought susceptibility, caution is warranted when it is the recurrent parent in breeding focused primarily on drought resilience (Ahmad et al., 2023; Mohd Ikmal et al., 2019).

4.1.5 Correlations Among Traits Between Different Seasons

Table 4.13 shows the correlation between different observed traits across planting season. The correlation analysis revealed significant associations among the measured traits ($p < 0.05$), with the strength of relationships ranging from weak to strong.

Table 4.13
Correlations Between Observed Traits for Two Planting Seasons.

	PH	PL	TN	PN	FGP	UFGP	CC	SF
PH	1.00							
PL	0.21*	1.00						
TN	0.09	0.15	1.00					
PN	-0.13	-0.02	0.85*	1.00				
FGP	0.28*	0.30*	0.28*	0.19*	1.00			
UFGP	0.01	-0.25*	-0.28*	-0.33*	-0.39*	1.00		
CC	0.07	-0.17	0.19*	0.20*	0.08	0.04	1.00	
SF	0.05	0.27*	0.31*	0.34*	0.57*	-0.93*	-0.01	1.00

Note: * shows significant differences at $p < 0.05$.

Strong correlations were observed between TN and PN ($r = 0.85$), indicating that genotypes producing a greater number of tillers also developed more panicles. An equally strong but negative correlation was detected between SF and UFGP ($r = –0.93$), suggesting that higher fertility was closely associated with a substantial reduction in grain sterility.

Moderate correlations included the positive association between FGP and SF ($r = 0.57$), as well as between TN and SF ($r = 0.31$), PN and SF ($r = 0.34$), and PL and FGP ($r = 0.30$). Negative moderate correlations were recorded between FGP and UFGP ($r = –0.39$) and PN and UFGP ($r = –0.33$), indicating that higher PN and longer PL were generally associated with improved SF and grain filling, accompanied by a reduction in UFGP.

Weak correlations were found between PH and PL (r = 0.21), PH and FGP (r = 0.28), TN and FGP (r = 0.28), PN and FGP (r = 0.19), PL and SF (r = 0.27), TN and CC (r = 0.19), and PN and CC (r = 0.20). Weak negative correlations were also noted between PL and UFGP (r = –0.25) and between TN and UFGP (r = –0.28). Although these relationships were of lower magnitude, they still indicate that certain morphological traits, such as PH and PL, exert a measurable influence on reproductive performance.

The correlation analysis (Table 4.13) revealed several significant relationships among the measured traits, many of which have direct implications for yield improvement through breeding and agronomic interventions. The strong positive correlation between SF and FGP ($r = 0.57$, $p < 0.05$) indicates that genotypes with higher SF generally produce more filled grains per panicle. This result is supported by the finding from this study where all of the advanced mutant lines have higher FGP resulting in higher SF values when compared to MR219 which has the lowest FGP at an average of 109.71 grains with SF only an average of 67.79%. This finding highlights the central role of SF in determining grain productivity, as a greater proportion of fertile spikelets directly translates into higher yield potential (Cheabu et al., 2019; Thuy et al., 2023). Similar positive associations between SF and FGP have been reported in rice and wheat, where improving spikelet fertility enhanced both grain filling and final yield (Thuy et al., 2023). Conversely, the strong negative correlation between SF and UFGP ($r = –0.93$, $p < 0.05$) suggests that increased fertility substantially reduces grain sterility, reinforcing the value of SF as a selection target for improving yield stability as agreed by Chen et al. (2019).

SF was also positively correlated with TN (r = 0.31) and PN (r = 0.34), implying that genotypes with greater tiller production and more panicles per plant tend to have higher fertility. These results are consistent with previous reports by Mohd Ikmal et al. (2019) and Mai et al. (2021), which linked TN and PN with increased reproductive sink size and improved yield potential. The very strong correlation between TN and PN (r = 0.85) reflects their close physiological linkage, as greater tillering capacity often leads to higher panicle production, as also documented by Oladosu et al. (2018). TN was weakly but negatively correlated with UFGP (r = –0.28), indicating that higher tiller numbers can contribute to reducing sterility, provided assimilate supply remains sufficient. However, excessive tillering may lead to intra-plant competition for

resources, as noted by Sabri et al. (2020), potentially offsetting gains in grain filling efficiency.

PL exhibited a moderate positive correlation with FGP (r = 0.30) and a weak negative correlation with UFGP (r = –0.25), indicating that longer panicles tend to bear more filled grains and fewer unfilled grains. Similar trends have been observed in previous studies by Gunasekaran et al. (2023), which suggest that increasing panicle size can enhance yield capacity. However, longer panicles require adequate assimilate supply to fully support grain filling; otherwise, partial grain filling, particularly in basal spikelets, may occur (Parida et al., 2022).

PH showed weak positive correlations with PL (r = 0.21) and FGP (r = 0.28), but no significant relationship with SF, suggesting that plant height alone is not a strong determinant of reproductive success. PH has also been linked to CC (r = 0.29 in related data), which may indicate improved photosynthetic potential in taller plants, although this does not necessarily translate into higher fertility (Qu et al., 2017). This observation is consistent with Zarifth Shafika et al. (2018), who reported that while taller plants may accumulate more biomass, this does not always result in improved reproductive performance.

Although the relationships observed here align with much of the published literature, some contradictions exist. In certain “super” hybrid rice genotypes, excessive PN has been associated with source limitations, where the available assimilates are insufficient to fill all spikelets, especially under suboptimal nutrient management (Won et al., 2022). Furthermore, dense-panicle cultivars have been reported to suffer from poor basal spikelet filling due to asynchronous anthesis or limited assimilate flow (Sekhar et al., 2021; You et al., 2016).

Overall, the results indicate that improving SF should be a primary focus in breeding programs, as it is positively associated with FGP and inversely associated with UFGP. Selecting genotypes with optimal TN, PN, PL, and stable SF can increase yield potential while minimizing sterility. However, increases in sink size must be supported by adequate source strength to ensure complete grain filling. Agronomic practices such as optimized nitrogen management, regulation of tiller production, and timely irrigation, particularly during flowering, can help maintain a favourable source–sink balance, thereby enhancing the stability and productivity of high-yielding, stress-resilient rice varieties.

4.1.6 Genetic Variations

The phenotypic and genotypic coefficient of variation for the eight morphological, agronomical traits, and physiological change are shown in Table 4.14 and Table 4.15.

Table 4.14
Genetic Parameters for Eight Traits of Rice During Season 1 (Off season).

	Season 1					
Trait	σ_g^2	σ_p^2	GCV (%)	PCV (%)	h^2 (%)	GA
PH	27.24	102.00	4.48	8.67	26.71	5.56
PL	0.65	1.45	2.88	4.31	44.83	1.11
TN	0.53	3.96	3.47	9.48	13.44	0.55
PN	0.88	3.08	5.34	10.01	28.51	1.03
FGP	29.07	389.27	4.30	15.73	7.47	3.04
UFGP	148.15	251.12	38.16	49.68	58.99	19.26
CC	0.51	1.40	1.84	3.06	36.20	0.88
SF	35.52	76.76	7.35	10.81	46.28	8.35

Note: σ_g^2 = genotypic variance; σ_p^2 = phenotypic variance; GCV = genotypic coefficient of variance; PCV = phenotypic coefficient of variance; h^2 = heritability; GA = genetic advance.

During Season 1, σ_g^2 ranged from 0.51 (CC) to 148.15 (UFGP), while σ_p^2 ranged from 1.40 (CC) to 389.27 (FGP) and 251.12 (UFGP) (Table 4.11). For all traits, PCV exceeded GCV, indicating notable environmental influence on expression in rice (S. C. Roy & Shil, 2020; Tuhina-Khatun et al., 2015). GCV spanned 1.84% (CC) to 38.16% (UFGP), whereas PCV ranged 3.06%–49.68% for the same traits. Broad-sense heritability (h^2) was highest for UFGP (58.99%), followed by SF (46.28%) and PL (44.83%); FGP showed low h^2 (7.47%), consistent with stronger environmental modulation in the offseason (Demeke et al., 2022; Faysal et al., 2022; S. C. Roy & Shil, 2020). The largest GA occurred for UFGP (19.26), followed by SF (8.35); the combination of relatively high h^2 with substantial GA supports an additive genetic basis and the effectiveness of direct selection (Holland et al., 2003; Johnson et al., 1955).

During Season 2, σ_g^2 ranged from 0.41 (PN) to 207.06 (FGP), while σ_p^2 ranged from 4.21 (PN) to 355.32 (FGP) (Table 4.12). As in the offseason, PCV surpassed GCV for all traits (Roy & Shil, 2020; Tuhina-Khatun et al., 2015). GCV varied from 1.78% (PH) to 36.94% (UFGP), with corresponding PCV values of 2.76% – 43.51%. h^2 was highest for UFGP (72.09%), followed by FGP (58.27%) and SF (57.39). FGP also showed large GA (22.63), indicating a stronger additive signal under favourable

conditions (Demeke et al., 2022; Venkateshwarlu et al., 2022). UFGP exhibited high GA (20.99), reaffirming its value as a selection criterion closely tied to grain filling performance (Barik et al., 2019; S. C. Roy & Shil, 2020). By contrast, PN, TN and CC showed low h^2 (<20%), indicating slower expected progress via direct phenotypic selection alone, similar to the finding by Akinwale et al. (2011) and Mazid et al. (2013).

Table 4.15
Genetic Parameters for Eight Traits of Rice During Season 2 (Main season).

	Season 2					
Trait	σ_g^2	σ_p^2	GCV (%)	PCV (%)	h^2 (%)	GA
PH	5.31	12.75	1.78	2.76	41.63	3.06
PL	0.71	3.75	2.88	6.64	18.85	0.75
TN	0.61	7.11	3.58	12.22	8.60	0.47
PN	0.41	4.21	3.65	11.70	9.74	0.41
FGP	207.06	355.32	10.50	13.75	58.27	22.63
UFGP	144.03	199.80	36.94	43.51	72.09	20.99
SF	44.86	78.16	8.24	10.87	57.39	10.45
CC	0.69	4.48	2.16	5.49	15.40	0.67

Note: σ_g^2 = genotypic variance; σ_p^2 = phenotypic variance; GCV = genotypic coefficient of variance; PCV = phenotypic coefficient of variance; h^2 = heritability; GA = genetic advance.

By comparing seasons, several traits were environment-sensitive. FGP showed greater GCV, h^2 and GA in the main season relative to the offseason, implying clearer genetic expression under favourable conditions as drought during reproductive stage is known to reduce spikelet filling and increase spikelet sterility (Barik et al., 2019). Conversely, PH and PL displayed low genetic variability and modest GA across seasons, and are suggested that these traits may require strategies such as targeted hybridization to introduce novel alleles rather than relying solely on selection within the present population as suggested by Adhikari et al. (2018). Overall, UFGP and SF emerged as robust selection targets across seasons, combining higher h^2 with sizable GA and clear relevance to drought-affected yield formation; selecting for lower UFGP together with higher SF is therefore expected to enhance productivity under water limitation (Barik et al., 2019).

As emphasized by Johnson et al. (1955), the assessment of heredity and genetic advancement holds paramount importance when opting for selection grounded in phenotypic expressions. The success of identifying individuals based on morphological traits is anticipated because of strong heritability and significant genetic advancements

as cited by Mazid et al. (2013). In the current study, four traits, which are PL, UFGP, SF, and CC, exhibited moderate heritability alongside noteworthy genetic advancement, whereas no instances of elevated heritability aligning with high genetic advance were identified.

Parallel observations were documented in the research conducted by Abebe et al. (2017), Adhikari et al. (2018), Muhidin et al. (2018) and Awad-Allah et al. (2022) for UFGP, PL, CC, and SF, respectively. These findings suggest a presence of additive genetic regulation, an inference congruent with investigations into drought-tolerant rice genotypes.

To develop an effective breeding strategy for any crop, it is crucial to comprehend the interrelationships among different attributes. The characteristics influencing crop yield are influenced by genetic factors, the interplay of genotype and environment, and the inheritance of quantitative genetics. Because certain desired traits manifest later in the plant's growth cycle, directly selecting for enhanced yield can be complex and intricate. Consequently, adopting an indirect selection approach is notably more straightforward and advantageous. Therefore, it is important to identify and apply closely related traits in the selection process.

4.2 Molecular Characterization of Mutant Rice Lines Using SSR Markers

A total of 6 advanced mutant lines, including the ones previously used as positive controls in the morpho-physiological study (NMR151, NMR152, NMR191, MINT1, MINT3, MINT9) were proceed with genotyping analysis. Three drought-tolerant improved lines developed at IRRI, namely IR 77298-14-1-2-10, IR 81896-B-B-195 and IR 84984-83-15-18-B were used as positive check varieties (donor for qDTY2.2, qDTY3.2 and qDTY12.1) (Noraziyah et al., 2016a) and MR219 was act as negative control. Genotyping analysis was suggested to carried out with phenotypic screening to validate the tolerance of rice genotypes as mentioned by Roy et al. (2021). A total of twenty-three Simple Sequence Repeat (SSR) marker associated with qDTY2.2, qDTY3.2 and qDTY12.1 were used in this study to confirm the identification of tolerance.

4.2.1 Quantity and Quality Check of DNA Sample

The concentration of DNA sample was measured by using a Nano-Drop Spectrophotometer (Thermofisher, USA) whereas agarose gel electrophoresis (at 80 volts for 30 minutes) was used to assess the quality of DNA sample. Table 4.14 presented that the range of DNA purity (A260/A280) for all extracted DNA varied around 1.8 to 2.0.

Table 4.16
Purity and Concentration of All Extracted Samples.

Rice lines	Purity	Concentration
IR 77298-14-1-2-10	1.88	217.4
IR 81896-B-B-195	1.84	185.2
IR 84984-83-15-18-B	1.85.	137.9
MR219	1.82	197.1
NMR151	1.83	150.4
NMR152	1.88	182.5
NMR191	1.81	191.4
MINT1	1.81	175.2
MINT3	1.85	187.3
MINT9	1.91	177.5

It can be concluded that purity of all extracted DNA in this studied suit the minimal requirement needed for PCR analysis (purity = >1.8) as suggested by Wanere et al. (2023). High concentrations were also observed for all advanced mutant lines plus controls at a range above 90 ng/µL. According to Sajib et al. (2017), DNA samples with a very high concentration could cause a nonspecific amplification however very low concentration of DNA sample will affect the yields of PCR product. Therefore, dilution step was further carried out to all DNA sample from this study before proceed with PCR amplification process. For quality analysis, all the advanced mutant lines were found appeared at more than 10 000 bp indicating a large set of genome sequences in each of them in agarose gel. All DNA bands are located very close to the wells which indicate high purity of the extracted DNA with no RNA contamination (Wanere et al., 2023).

4.2.2 PCR Amplification with Markers Associated with Tolerance to Drought

Twenty-three SSR markers were used for the identification of qDTY2.2, qDTY3.1 and qDTY12.1 in the six advanced mutant lines studied. Those markers including RM109, RM236, RM154, RM12460, RM12569, RM555, RM110, RM233A, and RM573 for qDTY2.2; RM520, RM416, RM16030, RM15935 for qDTY3.1; also, RM28166, RM28048, RM28089, RM28099, RM28130, RM1261, RM511, RM28172, RM28076, and RM5364 for qDTY12.1 (Noraziyah et al., 2016a). As for control, three drought-tolerant improved lines developed at IRRI, namely IR 77298-14-1-2-10, IR 81896-B-B-195 and IR 84984-83-15-18-B were used as positive check varieties and MR219 was act as negative control. IR 77298-14-1-2-10, IR 81896-B-B-195 and IR 84984-83-15-18-B were widely used as positive control as donor for qDTY2.2, qDTY3.1 and qDTY12.1, respectively (Noraziyah et al., 2016a). On the other hand, MR219 was selected as the negative control due to its susceptibility and absence of the aforementioned genes as confirmed by Zarifth Shafika et al. (2019).

4.2.2.1 qDTY2.2

Drought-responsive QTLs (qDTYs) are among the most important genetic regions for stabilizing yield under water-limited environments. Among these, qDTY2.2 has been consistently reported as a major-effect QTL contributing to drought tolerance across diverse ecosystems. This locus has been highlighted in several studies as conferring stable performance under both lowland and upland conditions (Ndikuryayo et al., 2023; Noraziyah et al., 2016a; Zarifth Shafika et al., 2019). Specifically, qDTY2.2 has been predominantly observed in lowland rice populations (Noraziyah et al., 2016a; Site Noorzuraini et al., 2013), although validation studies have also identified it in upland populations such as MTU1010/Kali Aus (Sandhu et al., 2018) and under irrigated lowland environments (Palanog et al., 2014). Its significance lies not only in experimental populations but also in its wide introgression into popular elite rice varieties, including Swarna, IR64, MTU1010, TDK1-Sub1, MRQ74, MR219, Savitri, and Vandana (Kumar et al., 2018; Selamat & Nadarajah, 2021). This widespread use underscores the importance of qDTY2.2 as a target for marker-assisted breeding in drought-prone regions.

In the present study, nine SSR markers (RM109, RM236, RM154, RM12460, RM12569, RM555, RM110, RM233A, and RM573) (Plate 4.1) were used to screen advanced mutant rice lines for the presence of qDTY2.2. These markers were chosen based on their previous validation in multiple international studies (Ndikuryayo et al., 2023; Roy et al., 2021) and their successful use in Malaysian rice lines, particularly MRQ74 and MR219 mutants (Mohd Ikmal et al., 2021). Positive (IR 77298-14-1-2-10) and negative (MR219) checks were used as controls for allele identification. Overall, all nine markers exhibited polymorphism between tolerant and susceptible checks.

Detailed amplification results revealed that some markers provided consistent detection patterns, whereas others failed to discriminate clearly between the controls. For example, RM12460 (Plate 4.1) amplified a tolerant allele of approximately 133 bp and a susceptible allele of 139 bp, consistent with Noraziyah et al. (2016a). None of the tested mutant lines matched the tolerant allele of IR77298-14-1-2-10, suggesting that they do not harbour the exact segment detected by this marker, although drought tolerance may still arise from alternative QTLs or evolutionary adaptations (Kumar et al., 2014). Similarly, RM12569 produced a tolerant band at 173 bp and a susceptible band at 179 bp; NMR151 and NMR152 displayed amplification similar to the susceptible control, though this may reflect allele convergence rather than true susceptibility, as emphasized by Collard & Mackill (2008). Marker RM555, previously associated with the strongest additive effect on grain yield under severe drought stress (Dixit et al., 2012), failed to amplify tolerant alleles in any of the tested lines, indicating the absence of qDTY2.2 at this locus.

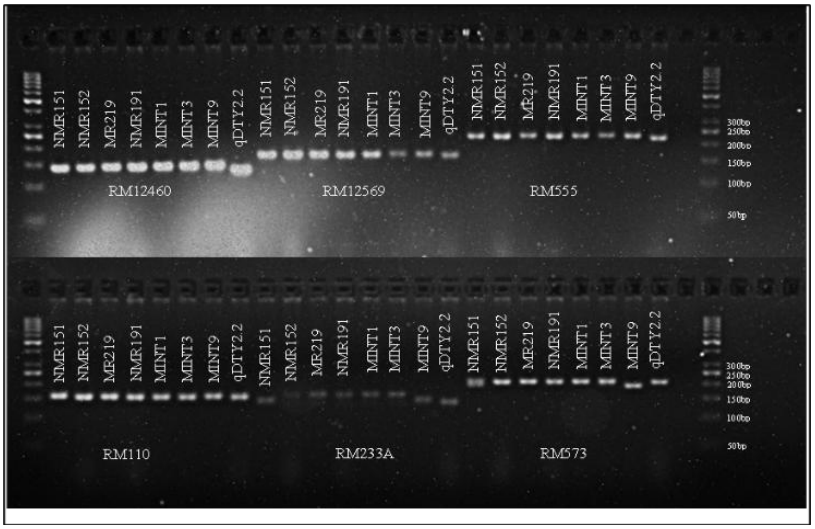

Plate 4.1 Amplification of DNA by RM12460, RM12569, RM555, RM110, RM233A and RM573 marker for qDTY2.2.

Next marker used in this study is RM110. Amplification of DNA using RM110 shows the tolerant band at approximately 146 bp while the susceptible band is approximately at ~150 bp. From plate 4.1, NMR152, NMR191, MINT1, and MINT3 amplify similar allele size to the positive check control, while only MINT9 amplify similarly with the negative check control. The indicates the presence of qDTY2.2 in the NMR152, NMR191, MINT1 and MINT3 and absent in NMR151 and MINT9.

By contrast, some markers provided more promising evidence of qDTY2.2 introgression. RM233A differentiated tolerant (133 bp) from susceptible (156 bp) alleles, though in this study MINT1 and MINT3 amplified bands similar to MR219, suggesting a low probability of carrying qDTY2.2. In previous study by Noraziyah et al. (2016b), RM233A has been used as peak marker for foreground genotyping of qDTY2.2 for cross between MRQ74 and IR77278.

Next marker used for detecting qDTY2.2 is RM573. In plate 4.1, the tolerant band can be seen at ~200bp, while the susceptible band can be observed at ~205bp. By using the software AlphaEaseFCTM, only NMR151 can be seen amplify similar to the drought tolerant variety at 200 bp, while NMR152, NMR191, MINT1 and MINT3 shows amplification similar to the drought susceptible variety at 205 bp.

Next marker used to identify qDTY2.2 in the advanced mutant lines is RM109. The observed base pairs for tolerant and susceptible lines are 83 bp and 77 bp respectively (Plate 4.2). None of the advance mutant lines identified amplifying similar allele size to the positive check control, while NMR151, NMR152, NMR191, and MINT1 are identified at approximately 77 bp, together with the negative check control (MR219), indicating the absence of gene qDTY2.2 in these genotypes.

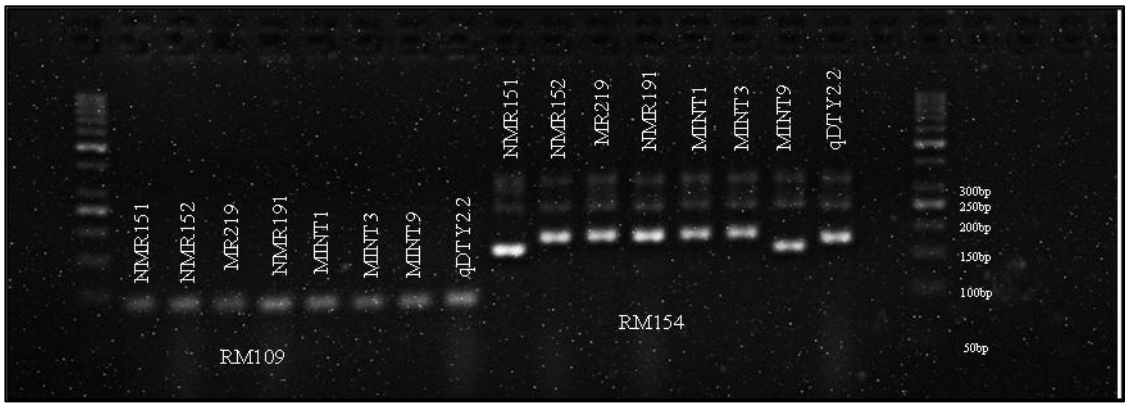

Plate 4.2 Amplification of DNA by RM109 and RM154 marker for qDTY2.2.

Next marker used in this study is RM154. In this study, the positive check amplifies at ~171 bp, while the negative check amplifies at ~177 bp. In the current study, only NMR152 multiply similar to the drought tolerant variety, suggesting the presence of qDTY2.2 in NMR152; while none of other the advance mutant lines amplify similar to the negative check. RM154 is a widely used SSR marker for foreground selection of the drought grain-yield QTL qDTY2.2 on chromosome 2. In marker-assisted breeding pipelines, qDTY2.2 is commonly tracked using RM154 together with additional markers (e.g., RM279, RM555 and/or flanking/peak markers in the same region) to reduce false positives caused by recombination within the QTL interval (Ramayya et al., 2021). Previous study by Noraziyah et al. (2016b) has included RM154 among the foreground SSR markers for the qDTY2.2 region during the MRQ74 qDTY pyramiding program.

The next marker used is RM236. The amplification of DNA marker reveals that the tolerant band is located at ~207 bp, while the susceptible band amplify at ~215 bp. Based on Plate 4.3, NMR191 mutant lines are detected at 215bp, together with the susceptible variety, MR219; while none of the tested lines amplify similar to the drought tolerant variety. These findings illustrated the absence of qDTY2.2 in NMR191.

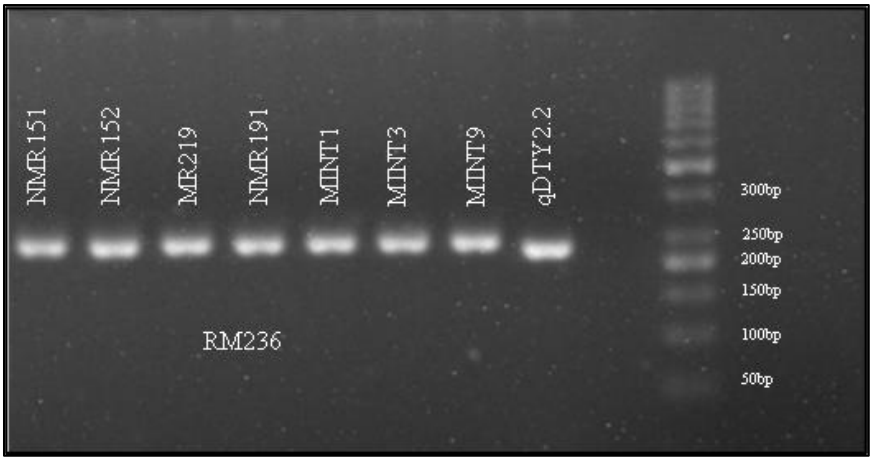

Plate 4.3 Amplification of DNA by RM236 marker for qDTY2.2.

Taken together, the molecular analysis revealed that while several markers failed to detect the tolerant allele in the tested mutant lines, RM110, RM573 and RM154 successfully identified qDTY2.2 in the advanced mutant lines, making the three markers the most reliable markers in this study. The contrasting results across markers highlight both the complexity of drought tolerance as a quantitative trait and the challenges

inherent in relying solely on SSR markers. These findings emphasize the necessity of using multiple markers and complementary phenotypic evaluations to validate QTL presence and effect. Ultimately, the detection of qDTY2.2 in selected mutant lines provides promising material for further breeding and genetic improvement under drought-prone environments.

4.2.2.2 qDTY3.1

The qDTY3.1 is a major-effect drought-yield QTL under severe drought stress conditions (Grondin et al., 2018). qDTY3.1 is a major-effect drought-yield QTL that was identified in a rice mapping population of 361 BC2F3:4 lines developed from the cross between Moroberekan and Swarna (recurrent parent) by Dixit et al. (2014).

P. Dwivedi et al. (2021) reported the introgression of two QTLs, qDTY2.1 and qDTY3.1 in a set of thirty-five near isogenic lines (NILs) developed in the background of the popular rice cultivar, Pusa 44 introgressed. Venuprasad et al. (2009) identified major QTLs, qDTY3.1, on chromosome 3, from the population of Apo/2*Swarna, that explained 31% of the genetic variances. The subsequent use of qDTY3.1, has been demonstrated to impart improved drought tolerance among several genetic backgrounds, such as F3 populations obtained from the crossing between Improved White Ponni and Apo (Shamini et al., 2019), MR219 (Noraziyah et al., 2016a), Samba Mahsuri (Sandhu et al., 2018), IR64 and Vandana (Kumar et al., 2014). However, in contrary, Shamini et al. (2019) remarked that qDTY3.1 did not exhibit any association with days to flowering under normal condition in a population developed involving Swarna and Apo.

In this project, four SSR markers were used for the identification qDTY3.1 namely RM520, RM416, RM16030, and RM15935 (Plate 4.4). All those markers were often used together or individually in many identification projects of qDTY3.1 in rice including Anjali, Swarna and Samba Mahsuri (Badri et al., 2022; Kumar et al., 2014; Noraziyah et al., 2016a; Rai et al., 2023; Sandhu & Kumar, 2017). The markers were also recommended by Noraziyah et al. (2016b) and Mohd Ikmal et al. (2021) for the identification of qDTY3.1 in local MRQ74 and rice variety respectively.

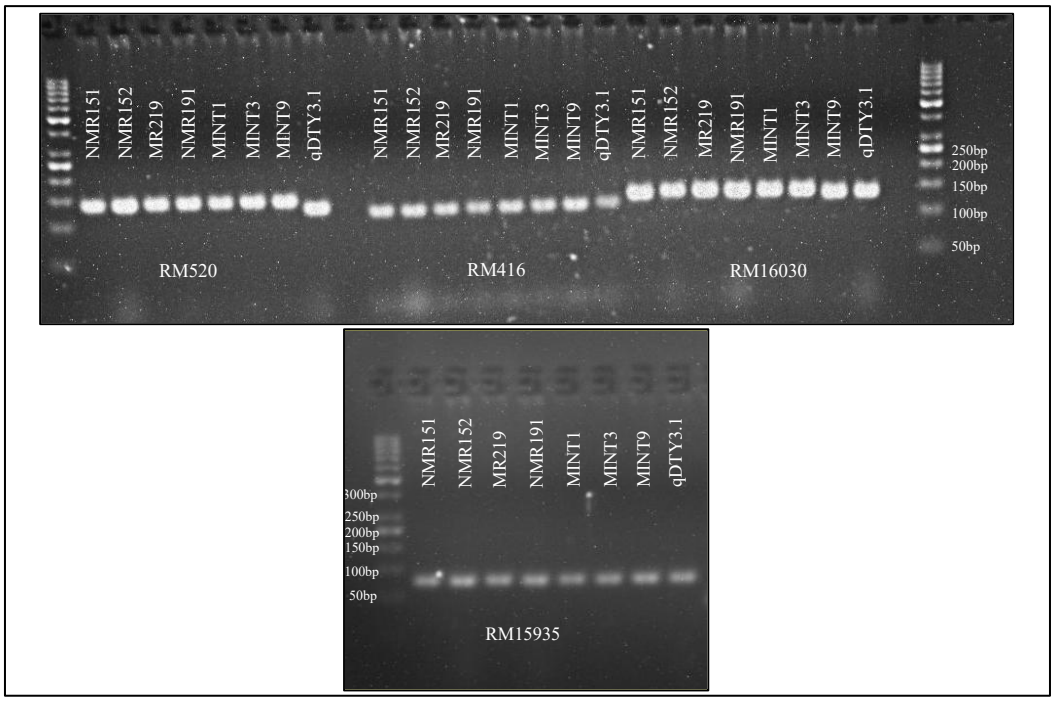

Plate 4.4 Amplification of DNA by RM520, RM416, RM16030, and RM15935 marker for qDTY3.1.

RM520 was used for foreground selection of qDTY3.1 similar to the study conducted by Noraziyah et al. (2016a). The expected base pairs for tolerant and susceptible lines are approximately 96 bp and 104 bp respectively. Based on Plate 4.4, none of the tested mutant lines amplify at the same locus similar to IR81896-B-B-195 (positive-check control) while NMR191 amplify similarly marker RM520 with MR219 (negative-check control), indicating the possible absent of qDTY3.1 in NMR191.

The next marker used is RM416. The amplification of DNA by this marker reveals the tolerant band at approximately 107 bp and the susceptible band at 94 bp. As illustrated in Plate 4.4, at RM416, none of the tested lines amplify similarly neither to the positive check control nor the drought susceptible. Nevertheless, all of the advanced mutant lines exhibited superior drought performance relative to MR219 in the phenotyping assay. Because RM416 is a linked SSR rather than the causal variant, identical RM416 allele sizes do not preclude drought tolerance. The data suggest that the advanced mutant lines tolerance likely derives from loci outside of qDTY3.1 or from mutational changes not tagged by RM416.

The amplification of DNA by markers RM16030 reveal the tolerant band at approximately 129bp and the susceptible band at 131bp. As illustrated in Plate 4.4, NMR151 and MINT9 advanced mutant lines are identified at 129 bp together with

IR81896-B-B-195 (positive control). It strongly stipulates the existence of qDTY3.1 in the tolerant lines while MINT3 shows similar amplification of allele are identified at 131 bp similar to MR219, suggesting the absence of qDTY3.1.

For marker RM15935, the amplification of DNA marker reveals both drought tolerant and the drought susceptible band amplify similarly, indicating that this marker to be monomorphic, thus cannot be used to distinguish between drought-tolerant from susceptible genotypes in this study. Although this marker is considered monomorphic in this study, study from Noraziyah et al. (2016b) successfully use RM15935 as one of the markers set in the MRQ74/MR219 breeding programs.

4.2.2.3 qDTY12.1

Drought tolerance in rice is governed by multiple quantitative trait loci (QTLs), of which qDTY12.1 on chromosome 12 is one of the most consistently reported across environments. Bernier et al. (2009) first demonstrated its large and stable contribution to grain yield under reproductive-stage drought stress, particularly in upland ecosystems. Subsequent studies reinforced its significance, with qDTY12.1 explaining up to 42% of phenotypic variation in drought-affected grain yield with an additive effect of 172 kg ha^{-1} and no significant yield penalty under non-stress conditions (Mishra et al., 2013; Venuprasad et al., 2009). Importantly, its introgression into breeding lines has produced highly drought-resilient materials. For example, Mukherjee et al. (2018) reported a 45.3% additive effect in backcross inbred lines (BILs) derived from IR74371-46-1-1 × Sabitri, while Dixit et al. (2017) successfully pyramided qDTY12.1 with qDTY3.2 to develop drought-tolerant near-isogenic lines of Savitri (CR1009). These findings establish qDTY12.1 as a robust QTL that improves yield stability under drought and remains a primary target in marker-assisted selection (MAS) programs.

In the present study, nine SSR markers linked to qDTY12.1 (RM28166, RM28048, RM28089, RM28099, RM28130, RM511, RM28172, RM28076 and RM1261) were used to screen advanced mutant rice lines, with IR 84984-83-15-18-B as the positive control and MR219 as the negative control. These markers have been widely validated in diverse rice genetic backgrounds including Anjali, Kalinga, IR64, Vandana, and Sabitri (Roy et al., 2021; Sandhu & Kumar, 2017), and have also been recommended for use in Malaysian varieties such as MRQ74 (Mohd Ikmal et al., 2020).

Plate 4.5 shows the PCR product for marker RM28166, RM28048, RM28089 and RM28099. The first marker used to detect the presence of qDTY12.1 is RM28166. Amplification of DNA by using marker RM28166 reveals that the tolerant band and the susceptible band are located at ~205 bp and ~200 bp respectively.

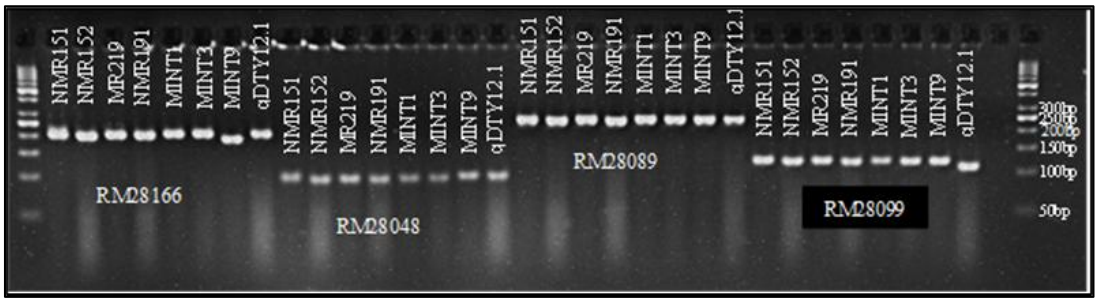

Plate 4.5 Amplification of DNA by RM28166, RM28048, RM28089, RM28099 marker for qDTY12.1.

From plate 4.5, NMR151, MINT1 and MINT3 amplify similarly to drought tolerant variety, while NMR191 amplify similar to the drought susceptible variety. RM28166 is a chromosome-12 SSR that has been reported as the peak (diagnostic) marker during the original mapping and validation of qDTY12.1, and tolerant donors in that work carried the same RM28166 allele, underscoring its utility for foreground detection of the QTL region. Previous study by Mishra et al. (2013) shows that drought tolerant varieties Way Rarem and IR74371-46-1-1 carry the same allele at RM28166 indicating the presence of qDTY12.1 in those varieties.

The amplification of DNA marker RM28048 reveal the tolerant band at approximately 101 bp and the susceptible band at 95 bp. As illustrated in Plate 4.5, NMR151 and MINT9 shows amplification of allele size similar to the positive check control suggesting the presence of qDTY12.1, while NMR152 shows similarities in allele size of the negative check control suggesting the absence of qDTY12.1. RM28048 is a validated chromosome-12 SSR marker that tags the qDTY12.1 interval and has been repeatedly used to track the donor haplotype in mapping and marker-assisted breeding (Mishra et al., 2013). Noraziyah et al. (2016a) in a previous study report the use of this marker as a flank to identify the presence of qDTY12.1. The presence of this marker stipulates the presence of qDTY12.1.

The next marker used is RM28089. Amplification of DNA by this marker shows the tolerant band at ~265 bp, while the susceptible band at 260 bp. In plate 4.5, none of

the tested line amplify similar to the positive check control, while NMR151, and the MINT lines amplify similarly with MR219 at 260 bp. RM28089 lies within the core qDTY12.1 region and was one of the diagnostic loci used during mapping. Previous study by Mishra et al. (2013) had use this marker to detect the drought tolerant donors between Way Rarem and IR74371-46-1-1, where drought tolerant donors share the same allele at RM28089.

The next marker used to assay qDTY12.1 is RM28099. The amplification of DNA by this marker reveals the tolerant band at approximately 106 bp and the susceptible band at 120 bp. In this study, none of the tested genotypes amplify similar allele size to the positive check control, but NMR151 amplify similarly to the negative check control suggesting absence of qDTY12.1. RM28099 is one of the standard SSRs screened to detect the donor haplotype when introgressing qDTY12.1 into elite varieties, for example Pusa 44. Previous study as reported by Anyaoha et al. (2019) used the recombination selection with marker RM28099 in a marker assisted selection to introgress qDTY12.1 with qDTY2.3 to fix the donor segment, which results in lines showing higher yield under drought stress.

The next marker used to assay qDTY12.1 is RM28130. The amplification of DNA by this marker reveals the tolerant band at approximately 170 bp and the susceptible band at 163 bp (Plate 4.6). None of the tested genotype amplify the same allele size similar to the positive check control, while only NMR151 amplify the same allele size with MR219 suggesting the absence of the qDTY12.1.

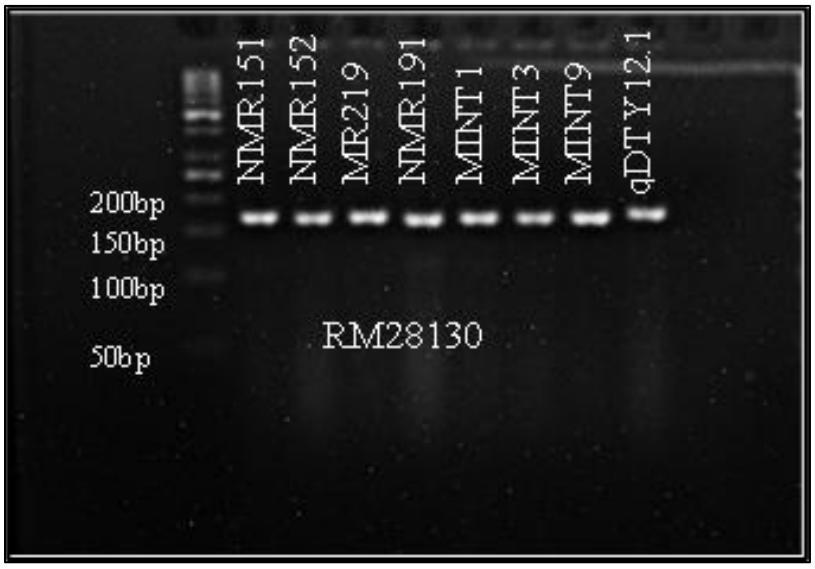

Plate 4.6 Amplification of DNA by RM28130 for qDTY12.1.

The next marker used is RM511. The amplification of DNA by this marker reveals the tolerant band at approximately 135 bp and the susceptible band at 130 bp. From plate 4.7, only MINT1 amplify the same allele size as the positive check control, and none of the tested lines amplify the same allele size as MR219 indicating only MINT1 are present with qDTY12.1. RM511 is a widely used peak/diagnostic marker for qDTY12.1.

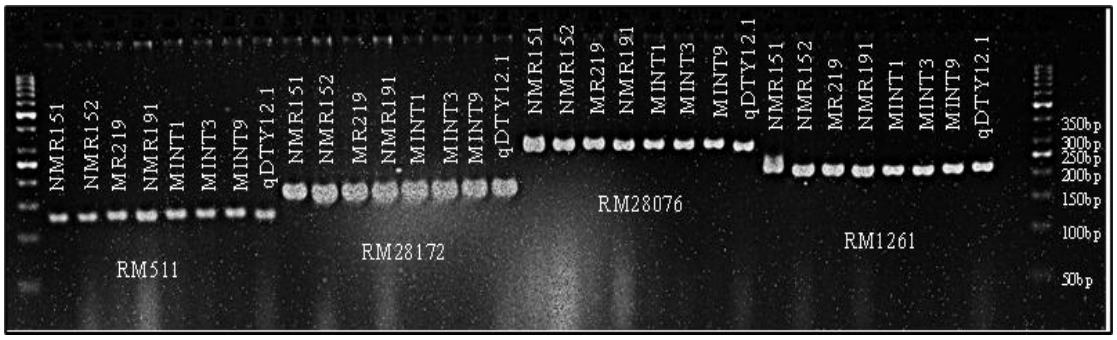

Plate 4.7 Amplification of DNA by RM511, RM28172, RM28076 and RM1261 marker for qDTY12.1.

Next marker used in RM28172. The amplification of DNA by this marker reveals the tolerant band at approximately 184 bp and the susceptible band at 176 bp. In plate 4.7, MINT3 and MINT9 amplify similar allele size with the positive check control, while NMR151 and NMR191 amplify similarly to the negative check control, suggesting the absence of qDTY12.1.

Next marker used in RM28076. The amplification of DNA by this marker reveals the tolerant band at approximately 303 bp and the susceptible band at 316 bp. In plate 4.7, none of the tested genotypes amplify similar allele size with the positive check control, while NMR151 and advanced mutant lines developed through ion-beam irradiation amplify similarly to the negative check control, suggesting the absence of qDTY12.1.

Next marker used in RM1261. The amplification of DNA by this marker reveals the tolerant band at approximately 246bp and the susceptible band at 236 bp. In plate 4.7, only NMR151 amplify similar allele size with the positive check control, while NMR152, MINT 1 and MINT3 amplify similarly to the negative check control, suggesting the absence of qDTY12.1.

From a breeding perspective, the confirmation of qDTY12.1 in NMR151, MINT1, MINT3 and MINT9 provides a valuable resource for future marker-assisted

selection and pyramiding with other major QTLs (e.g., qDTY2.2 and qDTY3.1). Its presence suggests the potential for yield stability under drought conditions without compromising performance in irrigated environments, as reported by Venuprasad et al. (2009) and Dixit et al. (2017). Importantly, integrating these molecular findings with the phenotypic evaluation of the same lines will enable a more rigorous assessment of their true drought resilience and suitability for varietal release. Further cluster analysis was run to analyse the combination of qDTY2.2, qDTY3.1 and qDTY12.1 in providing greater tolerant against drought.

4.2.3 UPGMA-Based Cluster Analysis of Genetic Divergence in Advanced Mutant Rice Lines

All six advanced mutant lines (including NMR151 and NMR152) were previously selected for molecular identification of qDTY as they displayed high tolerance rate against drought based on measurement of leaf rolling and leaf drying (Score 1 according to IRRI, 2019). Table 4.14 summarizes the response of all 6 advanced mutant lines toward drought as well as qDTY carried by each of them. Only one advanced mutant line namely NMR151 consist of 3 qDTYs. Another two advanced mutant lines (MINT1 and MINT3) recorded only 2 qDTYs (presence of qDTY2.2 and qDTY12.1). Based on the SSR marker analysis, a dendrogram was constructed using the SAHN/UPGMA method from a Dice similarity matrix computed on binary band data.

Table 4.17
Summary of Phenotypic and Genotypic Screening for Drought Tolerance in Six Advanced Mutant Lines Compared to Check Varieties.

Rice lines	QTLs			No. of carried qDTY
	qDTY2.2	qDTY3.1	qDTY12.1	
Tolerant line, IR 77298-14-1-2-10	+	-	-	1
Tolerant line, IR 81896-B-B-195	-	+	-	1
Tolerant line, IR 84984-83-15-18-B	-	-	+	1
Susceptible control (MR219)	-	-	-	
NMR151	+	+	+	3
NMR152	+	-	-	1
NMR191	+	-	-	1
MINT1	+	-	+	2
MINT3	+	-	+	2

MINT9	-	+	-	1

Note: + = present, - = absent.

As shown in Figure 4.2, the six advanced mutant lines together with tolerant checks (IR 77298-14-1-2-10, IR 81896-B-B-195, IR 84984-83-15-18-B) and the susceptible check MR219 were partitioned into seven groups at a 0.50 Dice similarity threshold. Lower inter-cluster Dice similarity (i.e., higher Dice distance = 1 − similarity) reflects a wider divergence in the composition of qDTY-associated alleles and the number of SSR loci amplifying the desirable (tolerant) bands. According to Ultsch & Lotsch (2022), a higher Euclidian distance between clusters indicates wider spectrum of performances between them in qDTY carried by rice lines and number of SSR markers that amplified desirable bands (tolerant).

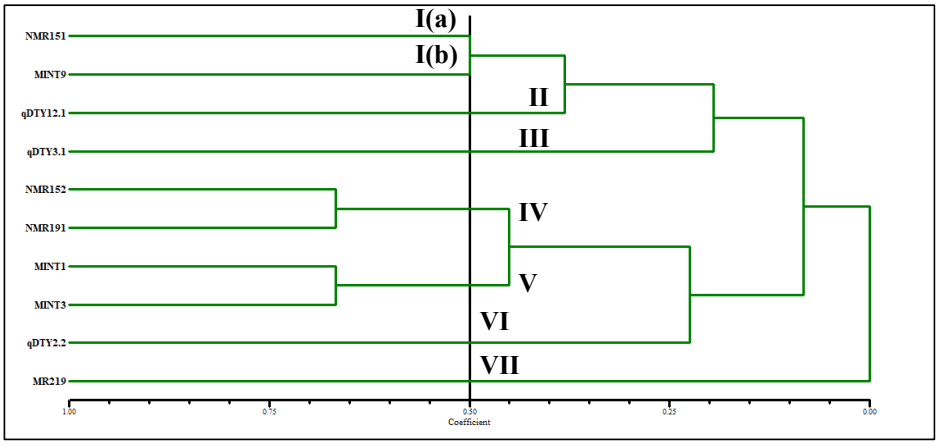

Figure 4.2 Clustering of Six Advanced Mutant Lines and Control Varieties Based on SSR Marker Analysis.

Based on Figure 4.2, both Cluster I, IV and V had two advanced rice lines each, followed by Cluster II, III, VI, VII consist of one rice line each which is positive control or negative control. Cluster III records the highest number of advanced rice lines with 4 mutant lines. If taking consideration stricter < 0.5 similarity, Cluster 1 can be further divided into two clusters (I (a) and I (b)). NMR151 and MINT9 are grouped in Cluster I as it possessed one qDTY (3.1) as identified by RM16030 marker. As for Cluster IV and V, two rice lines are grouped together in each cluster (NMR152 with NMR191; MINT1 with MINT3). The last cluster generated is Cluster VII which contains MR219 (negative control). MR219 is alone in this cluster as it carries no qDTY as identified through all twenty-three SSR markers.

Overall, the rice lines in Cluster V, consists of MINT1 and MINT3, are recommended to be further tested in Multi Locations Trial (MLT) and local verification trial (LVT) before commercialization. These lines contain two qDTY (2.2 and 12.1) and illustrate high tolerant against drought based on agronomic screening. Further crossing with lines from Cluster 1 (NMR151 and MINT9) can further be carried out in the future in hope to introgress qDTY3.1 into the MINT1 and MINT3 lines. These rice lines in Cluster I and V which carries qDTY could be further selected for crossbreeding purposes with other rice variety that carried biotic genes. Multiple projects already reach the target of developing a drought tolerant variety through combination of qDTY with biotic genes (Chukwu et al., 2019; Oladosu et al., 2019).

4.3 Assessment of Antioxidant Properties Under Drought Stress.

Antioxidant enzymes are central to protecting plants from oxidative damage by scavenging reactive oxygen species (ROS) and maintaining redox homeostasis. Key components of this defence network include catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX). CAT rapidly converts hydrogen peroxide (H_2O_2) into water and oxygen, preventing the accumulation of this harmful ROS. GPX and APX likewise detoxify H_2O_2, protecting cellular structures from oxidative injury. Proline, which is a type of amino acid functioning as an effective osmolyte, typically accumulates under drought stress; it helps maintain cell turgor, limits electrolyte leakage, and supports ROS homeostasis, thereby reducing the risk of oxidative bursts (Hayat et al., 2012).

Enhancing drought tolerance in rice can be facilitated by examining genetic mutants with altered antioxidant capacity, which provide insight into the mechanisms of stress resilience. Modulating antioxidant activity in mutant lines can reveal the genetic and biochemical bases of drought tolerance and inform the development of climate-resilient cultivars.

One of the objectives in this study is to evaluate antioxidant responses in drought-stressed rice mutants, focusing on catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and proline. Assessing these biochemical markers elucidates the relationship between antioxidant defences and drought tolerance and informs breeding strategies for cultivars with enhanced resilience to environmental stress.

Table 4.18 showed that the drought treatment has a significant effect on the antioxidant activity involving four enzymes studied. Significant main effects of treatment (T), genotype (G), and their interaction (T×G) were detected for all variables, which are proline, APX, GPX, and CAT ($p < 0.05$). Significant G effects confirm inherent differences among genotypes, and significant T×G effects indicate that genotypic rankings and response magnitudes varied between well-watered and drought conditions.

Table 4.18
ANOVA for Antioxidant Capacity of Selected Rice Lines.

	df	Mean Square			
		Proline	APX	GPX	CAT
R	3	0.0138	0.0047	0.0052	0.0045
T	1	2.2560*	58.5420*	71.0326*	74.6361*
G	6	0.0378*	0.5042*	0.3180*	0.2367*
T x G	6	0.0157*	0.1020*	0.1740*	0.1708*
Error	36	0.0027	0.0031	0.0128	0.0052

Note: * shows significant differences at $p < 0.05$.

Table 4.19 summarises the mean comparison between the genotypes used in this study using HSD test. NMR151 and NMR152 act as positive control (tolerant drought varieties) and MR219 act as negative control (susceptible to drought).

Table 4.19
Mean Comparison Between Genotypes for Each Biochemical Test Using HSD Test.

Genotypes	Mean							
	Proline		APX		GPX		CAT	
	C	D	C	D	C	D	C	D
NMR151	0.53 ab	0.97 a	1.92 b	4.21 a	2.24 ab	4.96 a	0.55 a	2.91 a
NMR152	0.48 ab	0.94 a	1.67 cd	3.84 b	2.09 ab	4.55 bc	0.52 ab	2.67 b
MR219	0.41 b	0.66 c	1.61 de	3.19 d	2.00 b	3.98 e	0.39 b	2.10 c
NMR191	0.52 ab	0.94 a	1.78 c	3.73 bc	2.27 b	4.22 de	0.48 ab	2.94 a
MINT1	0.50 ab	0.80 b	1.55 e	3.69 c	2.15 ab	4.14 de	0.41 ab	2.90 a
MINT3	0.48 ab	0.93 a	2.06 a	4.11 a	2.23 ab	4.63 b	0.54 ab	3.05 a
MINT9	0.51 ab	0.87 ab	1.55 de	3.64 c	2.07 ab	4.32 cd	0.46 ab	2.93 a

Note: Mean with similar letters indicates no significant differences at $p < 0.05$.

The results of the statistical analysis revealed significant difference in antioxidant enzyme activities between the advanced mutant rice lines and drought susceptible variety under drought stress. This enhanced antioxidant activity suggests that the mutant has a more robust defence mechanism against oxidative stress caused by drought. The higher catalase activity in the mutant is particularly important, as catalase plays a crucial role in breaking down hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS) that can accumulate under stress and cause cellular damage. Similarly, increased GPX activity further supports the mutant's improved ability to detoxify ROS, contributing to better cellular protection. These findings imply that the rice mutant's superior antioxidant response may be a key factor in its enhanced drought tolerance, potentially allowing it to maintain cellular integrity and reduce oxidative damage in drought conditions. This suggests that the mutant could serve as a valuable candidate for developing drought-resistant rice varieties, with improved stress resilience and yield stability.

4.3.1 Catalase Activity

Significance differences ($p < 0.05$) can be observed for T, G, and T × G for catalase (CAT) activity. Under well-water condition, only NMR151 and MR219 differ significantly while NMR152 and the advanced mutant lines were placed in the intermediate. Under drought stress environment, the drought tolerant varieties and the advanced mutant lines differ significantly to MR219, noted by the different letter groups (Figure 4.3). drought tolerant varieties exhibit higher CAT activity compared to MR219 (2.10 U mg^{-1} protein) at 2.91 U mg^{-1} protein (NMR151) and 2.67 U mg^{-1} protein (NMR152). The advanced mutant lines, have higher CAT activity compared to drought tolerant genotypes used in this study, ranging from 2.93 U mg^{-1} protein (MINT9) to 3.05 U mg^{-1} protein (MINT3), indicating better drought tolerance compared to drought tolerance and drought susceptible varieties.

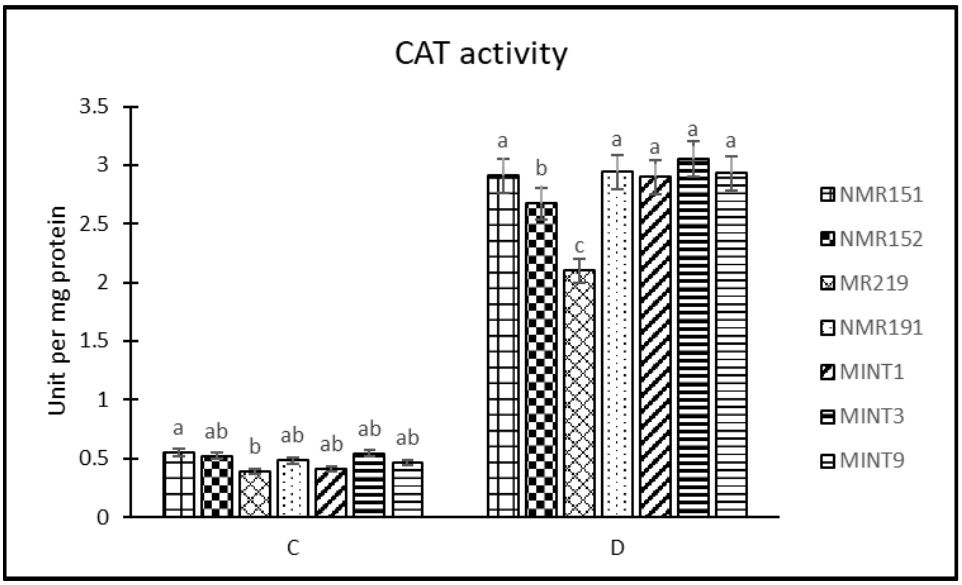

Figure 4.3 Differences in Catalase Activity Between Genotypes and Two Different Treatments.

Note: Similar letter indicates no significant differences between the mean of tested genotypes in the same treatment using LSD test at $p < 0.05$; bars sharing the same letter are not significantly different; C = Control, D = Drought stress.

Under drought conditions, CAT activity in leaf tissues of rice genotypes rises significantly (Figure 4.3). This elevated activity serves as a protective mechanism to neutralize photorespiratory hydrogen peroxide (H_2O_2) generated during water-deficient states. These findings align with previous studies that reported increased antioxidant enzyme activity under drought stress, reinforcing the idea that such conditions stimulate the plant's antioxidant defence system (Mondal et al., 2024; Tavu & Redillas, 2025; Zayed et al., 2023; Zhang et al., 2024). Furthermore, increased catalase (CAT) activity is widely recognized as an adaptive response across abiotic stresses, contributing to ROS homeostasis by lowering harmful H_2O_2 levels (Mishra et al., 2023; Raza et al., 2025).

Drought stress triggers an increase in catalase (CAT) activity, highlighting its role in oxidative stress response. The rice genotype MR219 exhibits significantly lower CAT activity, indicating a weaker oxidative defence mechanism. In contrast, control varieties and mutant lines display higher CAT activity, suggesting enhanced tolerance to oxidative stress.

In this study, the mutant line MINT3 exhibited the highest CAT activity under drought stress, followed by NMR191, MINT9, and MINT1. This increased activity may result from stress-induced changes in CAT isozyme abundance/expression and/or post-translational modulation of CAT activity (Raza et al., 2025), and according to Sandalio

et al. (2021) may also be influenced by peroxisome quality-control processes, particularly ROS-regulated pexophagy, in which oxidized/ubiquitinated catalase is implicated and which is modulated by the peroxisomal protease LON2. These findings underscore the crucial role of CAT in mitigating oxidative damage and enhancing drought tolerance in rice genotypes.

4.3.2 Guaiacol Peroxidase Activity

Significant differences ($p < 0.05$) can be observed between T, G, and T × G for guaiacol peroxidase (GPX) activity. Under control environment, NMR191 exhibits the highest GPX activity at 2.27 U mg^{-1} protein by MR219 at 2.00 U mg^{-1} protein (Figure 4.4). Only NMR191 differ significantly with MR219, while the other genotypes not significantly different to the prior genotypes (Figure 4.4). Under drought stress, the highest GPX activity was observed in NMR151 at 4.97 U mg^{-1}protein, followed by MINT3 at 4.63 U mg^{-1} protein and NMR152 (4.55 U mg^{-1} protein). All of the genotypes, aside from NMR191 and MINT1, differ significantly from MR219.

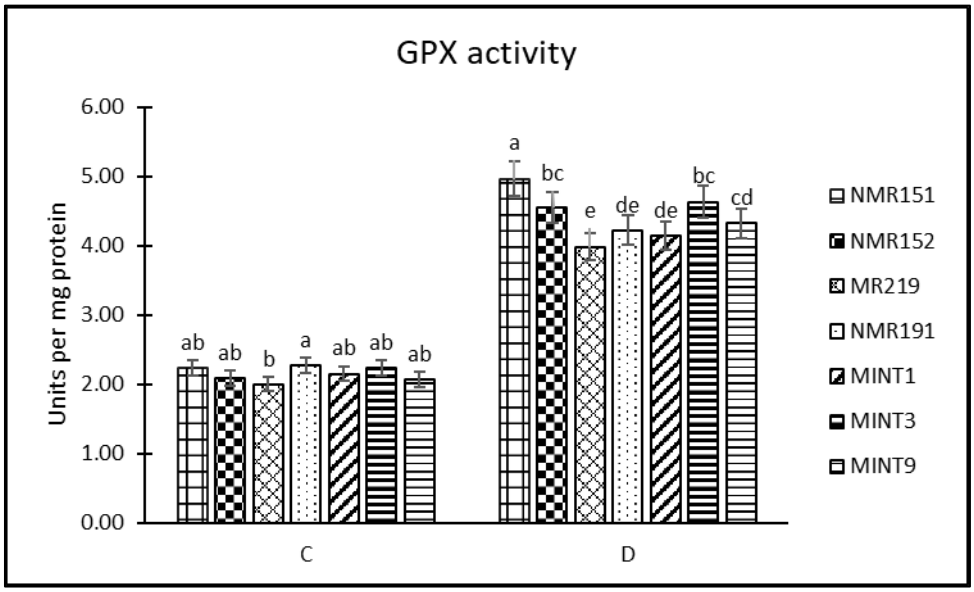

Figure 4.4 Mean Comparison for GPX Activity Between Two Different Treatments.

Note: Similar letter indicates no significant differences between the mean of tested genotypes in the same treatment using LSD test at $p < 0.05$; bars sharing the same letter are not significantly different.

Glutathione peroxidase (GPX) plays a vital role in protecting rice plants from oxidative damage caused by drought. Under drought stress, plants generate high levels of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide

(H_2O_2), which can damage cellular components. According to Bela et al. (2022) and do Carmo Santos et al. (2025), to mitigate oxidative injury, antioxidant enzymes such as glutathione peroxidases (GPXs) reduce H_2O_2 and lipid/organic hydroperoxides, thereby limiting lipid peroxidation and helping preserve cell membrane integrity.

Results from Zarifth Shafika et al. (2018) indicates that GPX activity increases more significantly in drought-tolerant rice cultivars compared to drought-sensitive ones. This supported the result from this study where drought tolerant varieties and the advanced mutant lines have higher GPX activity compared to MR219. This enhanced activity strengthens the plant's ability to detoxify ROS, reducing oxidative stress and maintaining physiological functions. In contrast, drought-susceptible genotypes often exhibit lower GPX activity or weaker GPX induction, which can reduce peroxide detoxification capacity and contribute to greater oxidative damage (e.g., lipid peroxidation) under drought stress (Ardıç et al., 2025; Zarifth Shafika et al., 2020).

GPX does not function in isolation but works alongside other antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD). In drought-tolerant cultivars, according to Rao et al. (2025), these enzymes can operate synergistically to efficiently scavenge ROS and maintain redox homeostasis. Conversely, an imbalance in antioxidant activity in drought-sensitive plants can exacerbate oxidative stress, ultimately impairing growth and productivity (Tavu & Redillas, 2025).

Additionally, studies by Mohagheghian et al. (2025) and Mohammadi Nodehi et al. (2025) remark GPX (and other peroxidases) compensates for reduced CAT activity during severe drought by taking over H_2O_2 breakdown. This adaptive mechanism ensures continuous metabolic activity and helps maintain cellular homeostasis. Understanding GPX's role in drought tolerance is crucial for plant breeding and genetic engineering efforts aimed at developing stress-resilient rice cultivars. By enhancing GPX expression, either through selective breeding or biotechnological approaches, a promising strategy for improving drought resistance in rice can be developed.

4.3.3 Ascorbate Peroxidase Activity

Figure 4.5 shows the mean differences for ascorbate peroxidase (APX) activity among studied genotypes between two treatments. Significant differences can be

observed where under well-watered where only NMR151, MINT1, and MINT3 significantly different from each other.

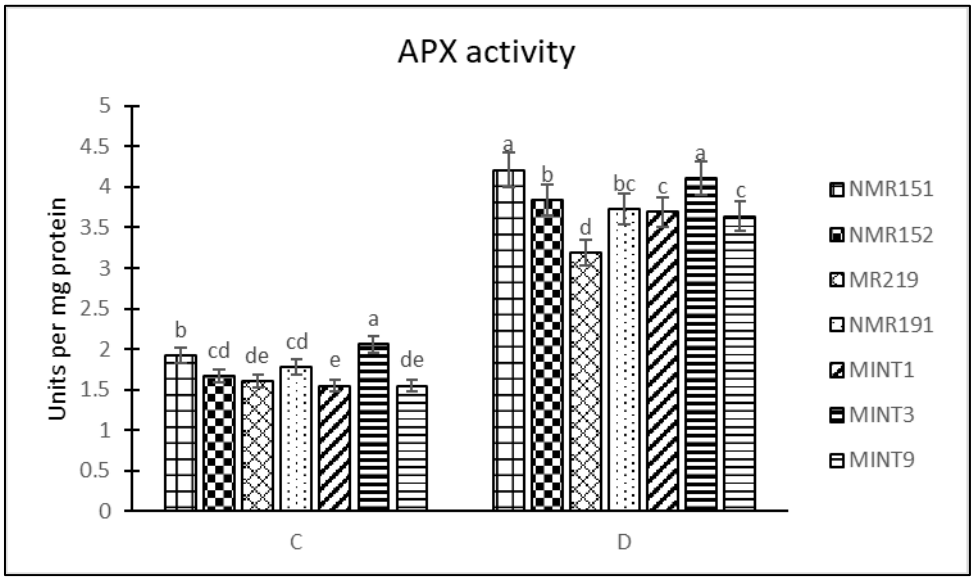

Figure 4.5 Mean of APX Under Well-watered (C) and Drought-stress (D) Treatment conditions.

Note: Similar letter indicates no significant differences between the mean of tested genotypes in the same treatment using LSD test at $p < 0.05$; bars sharing the same letter are not significantly different.

Among the genotypes tested, MINT3 exhibits the highest APX activity at an average of 2.06 U mg^{-1} protein followed by NMR151 at 1.92 U mg^{-1} protein. MINT1 exhibits the lowest APX activity at 1.54 U mg^{-1} protein compared to other genotypes studied. Under drought stress treatment, NMR151 exhibits the highest APX activity followed by MINT3 at 4.21 U mg^{-1} protein and 4.11 U mg^{-1} protein respectively. Although the other three advanced mutant lines shows lower APX activity compared to the drought tolerant varieties, the APX activity is still higher than MR219 (3.19 U mg^{-1} protein) at 3.73 U mg^{-1} protein (NMR191), 3.69 U mg^{-1} protein (MINT1) and 3.63 U mg^{-1} protein. From this study, all of the advanced mutant lines exhibit higher level of drought tolerance compared to drought susceptible variety. This result was consistent with finding from Zarifth Shafika et al. (2018) where genotypes with higher APX activity compared to MR219 were considered as drought tolerant variety.

Zarifth Shafika et al. (2018) discussed that increases in APX activities may be resulted from the excess energy intercepted by photosynthetic pigments although the increase in activity can create a protection mechanism in plant under drought stress. Increase in APX activity under drought stress may also be caused by abundance of

chloroplast APX isoform (Jardim-Messeder et al., 2022). The elevated APX activities observed in the drought-tolerant lines, particularly MINT3 and NMR151, may be associated with enhanced chloroplastic APX capacity/induction (e.g., increased contribution of chloroplast APX isoforms), rather than definitively 'abundance', unless isoform-specific expression/protein abundance was measured. Stromal (OsAPX7) and thylakoid (OsAPX8) isoforms rapidly reduce H_2O_2 within chloroplasts, a compartment lacking catalase, thereby limiting photodamage and sustaining electron transport during drought (Wang et al., 2025). The comparatively low APX activity in MR219 suggests weaker induction or abundance of these isoforms and a more vulnerable chloroplastic redox balance.

4.3.4 Proline Content

Figure 4.6 shows the mean differences of proline content between different tested genotypes and treatments. Significant differences ($p < 0.05$) can be observed among G, T and T × G. Under well-watered condition, only NMR151 and MR219 differ significantly while NMR152 and the advanced mutant lines were grouped within the same intermediate group (ab). Under drought stress treatment, the NMR191 and MINT3 were grouped with the drought tolerant varieties (NMR151 and NMR152), indicating higher tolerant to drought compared to MR219. Proline content of MR219 under drought treatment shows the lowest amount of proline content at an average of 0.66 mg g^{-1} FW while for NMR151 is at the highest at 0.96 mg g^{-1} FW followed by NMR152 at 0.94 mg g^{-1} FW. For the advanced mutant lines, NMR191 shows the highest amount of proline content per gram of fresh weight at an average of 0.94 mg g^{-1} FW, followed by MINT3 with 0.93 mg g^{-1} FW.

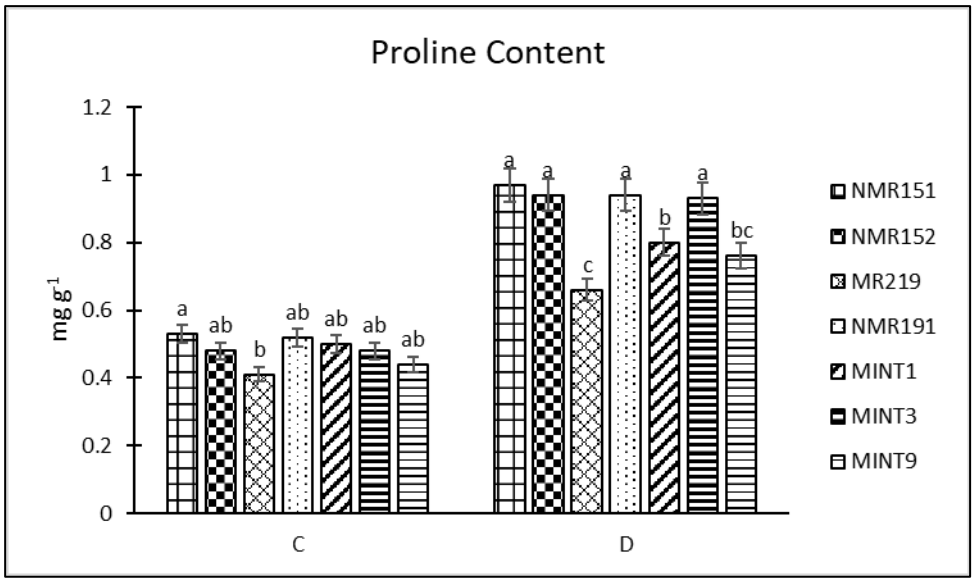

Figure 4.6 Proline Mean for Different Genotypes Under Well-watered (C) and Drought (D) Conditions.

Note: Similar letter indicates no significant differences between the mean of tested genotypes in the same treatment using LSD test at $p < 0.05$; bars sharing the same letter are not significantly different.

Proline is frequently used as a key indicator to observe drought tolerance in plants. According to Arteaga et al. (2020), proline accumulates significantly under drought stress and its concentration can be used for screening genotypes for drought and salinity tolerance. An increase in proline content is observed to be higher in plants under drought stress (Abid et al., 2018; Ghania & Benlaribi, 2006; Karataş et al., 2014). Moreover, according to Urmi et al. (2023), proline contain antioxidant activity which protect the redox balance by reducing lipid peroxidation and cell homeostasis promotion

In this study, the advanced mutant line shows higher proline content compared to drought tolerant varieties indicating the advance mutant lines have higher abilities to maintain cell turgor and water uptake which enables survival under dehydration (Chun et al., 2018). Current study agrees with the result from Zarifth Shafika et al. (2018) where drought tolerant genotypes (MR219-4; also known as NMR151 and MR219-9; also known as NMR151) have higher accumulation of proline under drought stress thus attributing to their high tolerance efficiency to drought stress.

CHAPTER 5
CONCLUSION

5.1 Introduction

In this study, we successfully evaluated the morpho-physiological, molecular characteristics and biochemical parameters of advanced mutant rice lines (*Oryza sativa* L.) developed through ion-beam irradiation, with the aim of assessing their drought tolerance. Field evaluations revealed that the advanced mutant lines generally outperformed the drought-susceptible variety MR219, particularly in key morpho-physiological evaluation of the mutant lines under drought stress revealed significant variations in key traits such as plant height, number of tillers, number of panicles, length of panicle, number of filled and unfilled grain, spikelet fertility and chlorophyll content. These results highlight the potential adaptability to water-limited environment.

Molecular characterization using SSR markers further confirmed the presence of major drought-yield QTLs. NMR151 was discovered to carry three qDTY loci, affirming its strong genetic potential for drought tolerance, while MINT1 and MINT3 each carried two qDTYs loci (qDTY 2.2 and qDTY12.1). NMR152, NMR191 and MINT9 carried one qDTY with NMR152 and NMR191 carried qDTY2.2 and MINT9 carried qDTY3.1 loci. This genetic evidence provides valuable confirmation of their resilience under drought stress.

In parallel, biochemical assays demonstrated significant increases in catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and proline accumulation across the advanced mutant lines under stress, performing better than the drought susceptible variety MR219. These enhanced antioxidant defences and osmoprotectant accumulation suggest a stronger physiological capacity to mitigate oxidative stress, supporting the field performance data.

Overall, the integration of morpho-physiological, molecular and biochemical analyses provides a comprehensive understanding of drought tolerance in these advanced mutant rice lines. The identified advanced mutant lines, particularly NMR191 and MINT3 and also NMR151 represent promising candidates for incorporation into future breeding programs targeting the development of drought-resilient rice varieties. These findings not only advance knowledge of the genetic and physiological basis of

drought adaptation but also contribute directly to ongoing efforts to secure rice production and food security in Malaysia under changing climate conditions.

5.2 Recommendations for Future Study

Based on the findings of this study, the following recommendations are proposed:

1. Expanded Multi-Location and Multi-Season Trials: While this study evaluated performance under controlled drought stress across two seasons, testing the advanced mutant lines in diverse agro-ecological zones and across multiple years would validate their stability and adaptability. This would also capture genotype × environment interactions under natural field conditions.
2. Yield Trials at Pre-Breeding and On-Farm Levels: The mutant lines that showed promising tolerance (e.g., NMR151, NMR152, MINT1 and MINT3) should be advanced to larger-scale yield trials and farmer participatory evaluations. This will confirm their agronomic performance and farmer acceptance before official release.
3. Fine-Mapping and Validation of QTLs: Although this study confirmed the presence of qDTY loci using SSR markers, further fine-mapping with high-resolution markers (e.g., SNP-based markers, KASP assays, or GWAS approaches) would strengthen the genetic evidence. Candidate gene identification and expression profiling under drought could clarify the molecular mechanisms.
4. Transcriptomics and Proteomics Approaches: RNA sequencing and proteomic profiling of tolerant vs. susceptible lines under stress would identify key drought-responsive genes, regulatory pathways, and proteins. Integrating omics data will provide a holistic view of drought tolerance mechanisms in the mutants.
5. Physiological and Root System Characterization: Since drought tolerance is strongly influenced by root architecture and water-use efficiency, future work should assess root traits (e.g., rooting depth, root density) and physiological responses such as stomatal conductance, transpiration efficiency, and photosynthetic rates.

6. Biochemical and Metabolomic Profiling: The antioxidant enzyme assays provided insights, but a broader metabolomic analysis (osmolytes, secondary metabolites, ABA levels, etc.) would deepen the understanding of biochemical strategies employed by tolerant lines.
7. Molecular Breeding and Pyramiding Strategies: The identified drought-tolerant mutant lines carrying qDTY loci could be integrated into marker-assisted backcross breeding (MABB) programs to introgress multiple QTLs into elite Malaysian rice cultivars. Pyramiding drought tolerance with resistance to major diseases (blast, bacterial blight) could produce multi-stress resilient varieties.

By implementing these recommendations, it will be possible to enhance the drought tolerance of rice, contributing to global food security, especially in regions affected by water scarcity.

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APPENDICES

APPENDIX 1

Suggested Rate and Timing for Fertilizing for Transplanting Method
Jadual: Kadar dan Masa Pembajaan bagi Kaedah Mencedung

Peringkat Pembajaan	Tempoh Matang 95–105 (HLT)	Tempoh Matang 105–115 (HLT)	Tempoh Matang >115 (HLT)	Peringkat Tumbesaran	Jenis Baja	Pengesyoran Sebenar (kg/ha)	Bantuan Kerajaan (kg/ha)	Keperluan Tambahan Petani (kg/ha)
Pembajaan Pertama	5–7	5–7	5–7	Vegetatif (3 helai daun)	Baja Sebatian* (17:5:15:5:10 atau 17:20:10)	140	140	0
Pembajaan Pertama	5–7	5–7	5–7	Vegetatif (3 helai daun)	Triple Super Phosphate (TSP – 46% P_2O_5)	57	0	57
Pembajaan Pertama	5–7	5–7	5–7	Vegetatif (3 helai daun)	Muriate of Potash (MOP – 60% K_2O)	42	0	42
Pembajaan ke-2	20–25	25–30	25–30	Beranak aktif	Urea (46% N)	80	80	0
Pembajaan ke-3	40–45	45–50	55–60	Pembentukan tangkai	Baja Sebatian* (17:5:15:5:10 atau 17:20:10)	107	100	7
Pembajaan ke-3	40–45	45–50	55–60	Pembentukan tangkai	Baja Sebatian Tambahan* (17:3:25 + 2 MgO)	100	100	0
Pembajaan ke-3	40–45	45–50	55–60	Pembentukan tangkai	Urea (46% N)	12	0	12
Pembajaan ke-4	60–65	65–70	75–80	Terbit dan berbunga	Baja Sebatian Tambahan* (17:3:25 + 2 MgO)	50	50	0
Pembajaan ke-4	60–65	65–70	75–80	Terbit dan berbunga	Urea (46% N)	20	0	20

Note: *"Baja Sebatian" merujuk kepada baja NPK berformulasi seperti yang dinyatakan. Semua kadar dinyatakan dalam kg/ha.

APPENDIX 2

Records of Daily Rainfall Amount from January 2021 until December 2021 in Sekinchan, Selangor

JABATAN METEOROLOGI MALAYSIA
Records of Daily Rainfall Amount

Station: MARDI TANJONG KARANG **Unit:** millimetre
Latitude: 3° 27' 17" N **Year:** 2021
Longitude: 101° 09' 23" E
Elevation: 2.4 m

Date	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	0.0	10.5	0.0	0.0	0.2	0.0	4.4	0.0	0.0	0.3	49.5	0.5
2	21.2	0.0	0.0	0.0	16.4	0.0	0.0	0.0	2.9	0.0	0.0	0.6
3	13.2	0.0	29.9	11.1	14.5	0.0	0.0	32.2	0.0	0.0	0.0	1.5
4	0.0	0.0	0.0	0.1	5.7	0.0	2.2	6.6	0.0	0.0	0.0	0.0
5	0.0	0.0	0.2	0.0	32.6	4.8	0.0	0.0	0.0	0.0	0.0	3.0
6	0.2	0.0	0.2	0.5	1.2	0.0	0.0	0.0	0.0	1.6	0.0	0.0
7	0.2	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	5.5	1.8	0.0
8	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.3	0.0	0.0	0.0
9	0.2	0.0	0.0	0.0	0.0	0.0	5.8	1.5	9.1	0.0	0.0	0.0
10	0.0	37.8	32.8	0.0	0.0	0.0	18.6	0.2	0.0	0.0	4.8	34.2
11	0.0	0.0	0.0	0.0	0.0	0.0	0.4	17.2	0.0	0.0	31.8	36.1
12	0.0	0.0	6.0	0.0	0.0	0.0	5.2	0.0	47.8	0.0	0.0	26.0
13	0.7	0.5	0.0	0.3	34.2	0.0	0.0	0.0	1.5	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.2	0.0	0.0	0.0	0.0	18.8	0.0	0.2	3.0	0.0	2.2	0.0
16	0.3	0.0	7.5	51.3	1.1	3.5	0.0	2.5	0.0	0.0	0.5	3.8
17	0.0	0.0	0.0	0.0	14.2	1.5	0.0	24.2	10.8	115.3	32.6	116.8
18	0.3	0.0	0.0	0.0	0.0	15.5	0.0	15.0	2.5	0.5	2.2	56.0
19	0.0	0.0	0.0	24.0	3.2	0.5	0.0	155.8	58.2	13.5	0.0	11.4
20	0.0	0.0	0.0	0.0	5.6	0.0	0.0	0.8	0.0	101.2	0.8	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.7	0.0	3.0	0.0
22	0.2	0.0	36.6	1.5	0.0	0.0	3.4	14.5	0.0	0.0	4.5	0.0
23	1.8	0.0	1.9	0.3	0.0	0.0	0.0	47.8	0.0	0.0	0.4	0.0
24	27.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	6.5	1.8	0.0
25	4.5	0.0	16.6	0.0	0.0	0.0	0.0	0.0	0.0	0.2	17.2	0.0
26	0.3	0.0	1.7	1.5	34.2	0.0	0.0	0.0	32.5	0.0	0.5	0.0
27	0.3	0.0	0.3	0.0	13.6	0.0	0.0	1.2	0.6	0.0	0.0	0.0
28	0.0	0.0	3.8	0.0	0.0	0.0	5.0	12.1	0.2	0.0	7.4	0.0
29	0.0		0.3	0.0	0.0	59.3	0.0	0.5	0.3	9.2	9.8	0.0
30	0.0		0.4	0.0	0.0	0.0	19.2	0.2	0.0	22.4	0.0	31.1
31	10.2		0.0		0.0		42.8	29.3		0.5		15.2
Total	81.6	48.8	138.5	90.6	180.5	103.9	107.0	363.6	180.4	276.7	170.8	336.2
No. of Raindays	17	3	15	9	15	7	10	19	14	12	17	13

Definition : MST - Malaysian Standard Time
Trace - Rainfall amount less than 0.1 millimetre
N.A. - Not Available, Def. - Defective Value
} - Accumulated Value

b/p Ketua Pengarah
Jabatan Meteorologi,
Malaysia

AUTHOR'S PROFILE

Muhamad Adib Najmi bin Ja'afar obtained Bachelor of Science in Biology (Hons.) in 2020 from Universiti Teknologi MARA. His BSc thesis titled "Risk Assessment and Antibiotic Resistance of E. coli in Freshwater Fish at Retail Levels" explore the risk assessment of E. coli contamination in catfish (Pangasius sp.) at different retail level and their antibiotic resistance. Currently as in 2025, he is involved with national level project under MOSTI's SRF-APP fund entitled "Development and Commercialization of Climate-Resilient Rice Varieties to Increase National Yield Production" which focused on drought-resilient varieties that have desirable traits such as early maturity, high-yielding, and resistant towards pest and disease.

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