

Evaluation of released and pre-released upland rice (*Oryza sativa* L.) for drought tolerance at germination and early seedling stage

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ABSTRACT

In this study, evaluation of the effect of drought on germination and early seedling traits, targeting both released and pre-released upland rice varieties for genotype identification suitable to rainfed conditions in Uganda. Considering the susceptibility of upland rice to water stress at early growing stages, particularly in regions with low and erratic rainfall, this study was also designed to provide more information for breeding programs, as well as policies aimed at promoting robust rice cultivation in these regions. The study which was carried out at Uganda's National Crops Resources Research Institute (NaCRRI) applied a Split Plot arrangement under a Completely Randomized Design evaluating fifteen rice genotypes at 5 levels of simulated drought stress using Polyethylene Glycol 6000 (PEG-6000) concentrations of: 0%, 5%, 10%, 15%, and 20%. Germination metrics (percentage, velocity index, mean germination time, and synchrony) as well as early seedling parameters (shoot length, root length, fresh weight, dry weight, and drought injury scores) were evaluated. All germination and seedling performance indices were significantly reduced by increasing PEG concentrations, with PEG 20 having the most adverse effect on plants. Under extreme stress, the released varieties such as NamChe-5 had higher germination percentages (33.33%), the fastest mean germination time (4.88 days), the highest germination velocity index (3.28), and retained superior seedling dry weight (0.87 mg) and root length (2.47 cm), confirming its strong drought tolerance. However, some of these lines like UP-25(45) which were pre-released compared with released top cultivars demonstrated better performance, indicating their potential for varietal release in near future. While the drought-sensitive control IR64 demonstrated significantly lower responses in all parameters. The results demonstrate that early-generation screening can identify lines that outperform in drought environments, and pre-released genotypes offer potential for reducing yield losses in Uganda's rainfed upland rice production systems. It is, therefore, critical to incorporate early-stage drought tolerance in breeding programs for sustainable rice production under climate variability.

DECLARATION

I, **AYO JOASH JOSHUA**, declare that this Research Dissertation is submitted to the Faculty of Agricultural Sciences of Uganda Christian University Mukono is my original work. Therefore, it has never been submitted, documented, or communicated in any way to any academic institution in exchange for a degree or other educational credentials.

SIGNATURE:
DATE: 15th/04/2025

APPROVAL

This is to endorse that AYO JOASH JOSHUA has finalised his final year research and design project under the supervision of the project supervisor and that the report has been submitted with the approval of the academic supervisor.

Signature:*Adli*..... Date: *15/04/2025*.....

Ms. MILDRED JULIAN NAKANWAGI

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ABBREVIATIONS

IRRI	International Rice Research Institute
NaCRRI	National Crops Resources Research Institute
GVI	Germination Velocity Index
MGT	Mean Germination Time
GS	Germination Synchrony
GP	Germination Percentage
MAS	Marker-assisted selection
SES	Standard Evaluation System
PEG	Polyethylene glycol
QTL	Quantitative Trait Locus
CRD	Complete Randomised Design
NERICA	New Rice for Africa
MT	Metric Tonne
mg	milligram

CHAPTER ONE

1.1 INTRODUCTION

1.2 BACKGROUND

Rice (*Oryza sativa* L.) is a very important food crop worldwide, feeding more than half of the global population (FAO, 2020). It becomes 50%-80% of daily energy intake for around 3 billion people, and an important source of several nutrients/micronutrients-energy, fibre, proteins, vitamins (Ndikuryayo et al., 2023). Estimate data of the world rice production in 2019 are about 756 million metric tons cultivated in 164 million hectares, ranking rice as the third most produced agricultural crop after sugarcane and maize (Ndikuryayo et al., 2023). Africa generated about 37.9 million metric tons from an area of 17.2 million hectares (FAO, 2021).

In the East Africa n region, Tanzania is leading in production and consumption, whereas Kenya and Uganda come in next, and all the other countries have production shortage and inflow of imports (Lamo et al., 2025). There are three main production systems of rice in Uganda, upland (rainfed), rainfed lowland and irrigated wetland; where upland system contributes almost 40% of the national production (Samudin 2023). Domestic production is about 730,000 metric tonnes but consumption is about 1,220,000 metric tonnes. This gap has been compensated by imports, which cost the country approximately USD60 million per year (FAO, 2023). The crop has showed a low yield in the site and, to increase local production of rice, several improved upland rice varieties have been released in the country including NERICA (New Rice for Africa) and “NamChe” varieties (Lamo et al, 2025). These cultivars have traits which make them favourable to farmers, namely substantially greater yields and improved stress tolerance.

Drought stress is an important abiotic factor limiting the production of upland rice in Uganda (Malemba et al., 2017) and the crop has been reported to be highly susceptible to water stress during early seedling development, during the vegetative stage, and at anthesis (reproductive) stage (Roy et al., 2021). Breeding and screening efforts so far

have focused on vegetative and reproductive stages, but erratic rainfall patterns are a reality in many locations, with frequent failures to establish successful crops due to inadequate rainfall (Made et al., 2022). Weakened during germination and the formative stage of the seedling, this vulnerability plays a significant role in the failure of crops (Obura & Lamo, 2024). We know that rice varieties that can withstand early water stress usually have better rooting systems, better osmotic adjustment, better water use efficiency and better activation of stress specific genes, which all of them lead to a vigorous crop establishment and finally high yield (Ndikuryayo et al., 2022). Insufficient water supplied during the seedling period, on the other hand, reduces stand uniformity that will cause serious yield loss (Roy et al. 2021). Thus, identification of these drought tolerant genotypes at these early growth stages is a valuable approach to improving rice production under rainfed conditions significantly (Ndikuryayo et al., 2023)

1.3 PROBLEM STATEMENT

Upland rice varieties grown without irrigation are particularly susceptible to drought stress in the germination and early seedling stages—a sensitivity that could reduce yield by as much as 50% (Langangmeilu et al., 2023). As nearly 40% of Uganda's rice-growing area depends on rainfed agriculture, these areas are highly vulnerable to water deficit (Hong et al., 2021). Climate change has increased climate variability which has led to both severe and extreme drought periods resulting in a drop in rice production (Uganda Bureau of Statistics, 2023) that can indeed threaten food security in 2023. Prior efforts had been undertaken such as releasing drought resistant varieties like NERICA and agronomic improvement (Lamo et al., 2025) Nonetheless, there is a knowledge gap related to the germination and early seedling drought tolerance of released and pre-release upland rice varieties as most previous work has been done on a later growth stage (Roy et al., 2021). Filling this gap will allow for more drought resilient

identification, breeding strategies, and ultimately support sustainable livelihoods for Ugandan rice farmers.

1.4 PURPOSE OF STUDY

Despite the above-described studies, few released, and pre-release upland rice (*Oryza sativa* L.) varieties have been evaluated with respect to their capacity for drought tolerance during the germination and early seedling phase to identify those with superior resistance. It aims also to develop essential information for breeding programs focused on increasing drought resistance in upland rice, which can benefit food security in Uganda in the long run (Domingo et al., 2023).

1.5 MAIN OBJECTIVE

The main research objective was to identify upland rice genotypes with significant drought tolerance that can be incorporated into breeding programs in Uganda.

1.6 SPECIFIC OBJECTIVES

1. To assess germination parameters of released and pre-released upland rice varieties under controlled drought conditions.
2. To evaluate the early seedling growth parameters of the rice varieties under controlled drought conditions.

1.7 HYPOTHESIS

1. There is no significant difference in the germination parameters among released and pre-released upland rice varieties under controlled drought conditions.
2. There is no significant difference in the early seedling growth parameters among released and pre-released rice varieties under controlled drought conditions.

1.8 JUSTIFICATION

The increasing severity and frequency of droughts in Uganda pose a significant threat to rice production and national food security (UBOS, 2023) that makes this research timely and critical. As upland rice is predominantly rainfed, it is particularly vulnerable to erratic rainfall and extended dry periods. Choosing rice varieties tolerant to water deficit leading to crop establishment critical events, i.e. germination and early seedling development, is proposed among these success factors particularly to retain establishment in marginal areas with variable distribution of rainfall (Langangmeilu et al., 2023; Samudin, 2023). Improving tolerance to drought by this time would be a targeted response to impending climate pressures: If this fast-growing and faster early stages would be better at coping with stress later in life, they could cope with what lies ahead better as they grow. The initial growth response to limited water has yet to be fully explored, despite earlier studies that have largely concentrated on later developmental events (Roy et al., 2021). This study aims to bridge this gap by providing critical information on drought resilience in early-generation materials, which can be used to inform breeding and management strategies for upland rice systems exposed to drought.

1.9 SIGNIFICANCE

This research intends to serve several parties. Ugandan rice farmers will receive access to drought - resistant cultivars that improve crop establishment and grain yield under water-limited conditions. Using the discoveries of drought-response traits will enable plant-breeders to use the advanced techniques of breeding programs, he added. The agricultural extension services and policymakers will access evidence-based information for the dissemination of sustainable agricultural practices, as well as the academic and scientific communities through improved methodologies and deeper

understanding of plant stress physiology with potential applications to other crops and regions (Domingo et al., 2023; Shah et al., 2024)

1.10 CONCEPTUAL FRAMEWORK OF THE STUDY

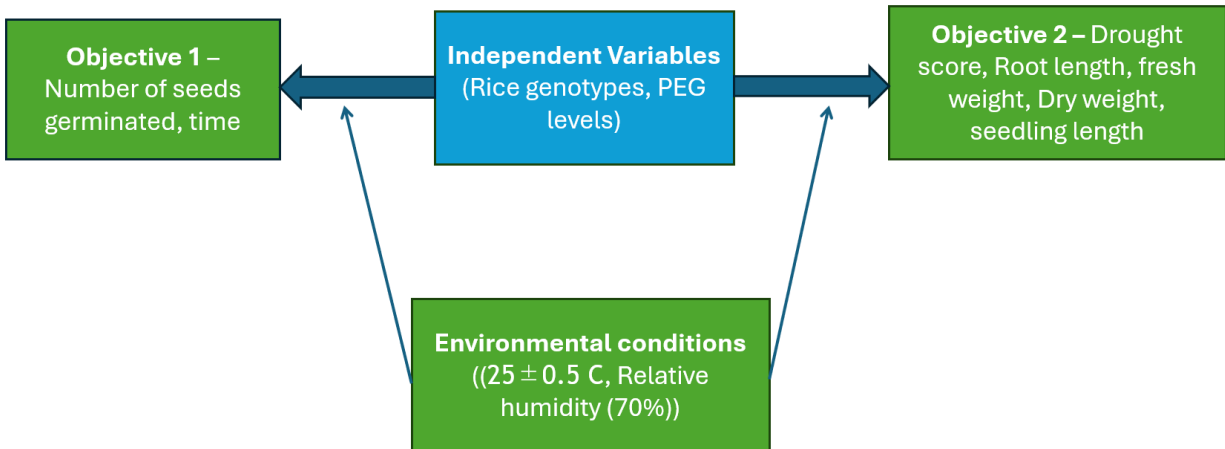


Figure 1 The Conceptual framework of the study

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy, Botany and Agronomic practices of Rice

Rice is a member of the Poaceae family. Taxonomically, it belongs to Kingdom Plantae, Phylum Tracheophyta, Class Liliopsida, Order Poales, Family Poaceae, Genus *Oryza* (Kush 1997). Rice crops are primarily of the *Oryza sativa* (Asia) and *Oryza glaberrima* (West Africa) species (Zhang et al., 2022). 80% of all genetic material in Triticeae are common, however, which indicates that although there may be differences in genetic aspects, environmental adaptation, and a few aspects of agronomy, Triticeae is primarily grown for the grain (Zhang et al., 2022). It is characterized as an annual grass with a fibrous root system and long narrow leaves that are arranged alternately on a hollow stem (Kumar et al., 2020). The flowering structure is a terminal panicle that can be bearing many spikelets and each one contains one to three florets per florets that is self-pollinating. The edible seed vary between cultivars in shape, size, and quality (Samudin, 2023).

Rice is grown in a variety of ecosystems, each necessitating specific management practices. They include irrigated lowland paddies, rainfed lowlands, upland (aerobic) fields and deep-water or flood-prone areas. Successful management of soil fertility, water, pests, and diseases, and better varieties through breeding are also needed for these environments (Shah et al., 2024). In irrigated lowlands, continuous flooding is kept by either continuous water supply or controlled irrigation schedules, whereas for upland rice, no standing water is present, which means that water conservation techniques and drought-resistant varieties are vital (Ndikuryayo et al., 2023).

However, lowland rice production in Uganda and comparable tropical countries is focused on irrigated or wetland areas including valley bottoms or riverbanks, since availability of abundant water places highland rice under high plant cover and ensures high yield. While many cultivars of *Oryza sativa* do well here, high water demands,

costly irrigation infrastructure, soil salinity, and weed infestations in paddies present challenges (Zhang et al., 2023). Moreover, regular flooding can increase how much methane is released, causing environmental problems. Drought stress during critical growth stages may occur in rainfed lowland rice, which depends on the natural rainfall to stay flooded.

Similarly, upland rice—grown on well-drained, non-flooded soils that are often on mountains and plateaus—relies entirely on rainfall and is typically grown by smallholder farmers as a replacement for traditional cereals. In Asian NERICAs (New Rice for Africa), interspecific hybrids between *O. sativa* and *O. glaberrima*, their genetically interrelatedness optimizes upland rice potential, combining high yield potential with durability and drought tolerance. Uganda, with its varied topography, benefits from both available NERICAs and improved upland varieties under rainfed production conditions that lead to high yield (Ndikuryayo et al., 2022).

Perennial rice is a recent breakthrough production in rice cultivation. While traditional annual types have to be planted again every season, perennial rice grows back with the same root system in successive seasons, thereby lowering labour and input costs of land preparation and planting. Some perennial lines, derived through hybridization between annual rice and its wild perennial relatives, have been harvested for 4-8 consecutive seasons with little loss in yield, hypothesized to provide savings of up to 60% in labour and requiring less seed and fertilizer (Zhang et al., 2023). Uganda is testing perennial rice varieties on an experimental basis, which may provide the added benefit of saving labour while also improving the health of the soil by maintaining continuous ground cover. When the climate is changing at an unprecedented pace, the addition of innovations perennially in rice, along with improved agronomic practices, becomes essential to ensure food security and environmental resilience, notwithstanding the threats made by broad seeding, disease resistance, and competitive performance.

Two cultivars of NamChe, developed through national breeding programs with support from AfricaRice, are widely grown in Northern Uganda with yields ranging from 3000-4000 kg/ha under rainfed upland production systems (Hong et al., 2021). They are

appreciated for their drought tolerance, yield potential, and mild aroma suitable for local market demands (Hong et al., 2021). On the other hand, some NERICA varieties have good drought resistance, whereas others are sensitive to stress and susceptible to foliar diseases. The accuracies of both released and emerging cultivars are still carried out for the farmers for high yielding, drought resistant options.

Ongoing challenges to rice production such as water scarcity, irregular rainfall, declining soil fertility, and pest and disease pressures have spurred the development of integrated management and breeding innovations. To mitigate these problems, better water management practices like controlled irrigation and soil moisture conservation, as well as nutrient management implementing the use of organic amendments and targeted fertiliser regimes have been adopted (Shah et al., 2024). Simultaneously, NERICA and NamChe series-focused breeding programs for drought-tolerant and disease-resistant cultivars have substantially improved crop adaptability under harsh conditions (Hong et al., 2021; Ndikuryayo et al., 2022).

2.2 Drought Stress and Its Effects on Upland rice

However, lack of water at germination and early seedling establishment competes for crop establishment and subsequently leads to the full establishment of the yield. Drought impedes key physiological processes ranging from water uptake and cell division to metabolism that lead to morphological differences like delayed or reduced germination, stunted growth in seedlings, and decreased accumulation of biomass (Dong et al., 2025). Such as a deficit of water can negatively impact on seed imbibition, resulting in ineffectiveness germination and inconsistency emergence (Hem et al., 2021). In the seedling phase, the shortage of water reduces cell extension and growth resulting in reduced shoots and root, smaller leaf area and reduced photosynthetic capacity. Also, decreased transpiration in such conditions would negatively impact nutrient uptake, adding another layer of growth limitation (Made et al., 2022). In

severe situations, drought after germination can lead to seedling death, threatening stand establishment.

In upland rice systems where fields are not intentionally flooded maintaining sufficient soil moisture is an ongoing struggle. Even short dry periods can be stressful, with lasting impacts that contribute to incomplete recovery when it rains again. The yield reductions due to drought can differ significantly depending on the severity and duration of the stress and might range from 30% to 80%. Upland rice varieties, such as some of the select NERICAs, or improved lines developed in-country with inherent drought tolerance (Lamo et al., 2025; Ndikuryayo et al., 2023), suffer less from stress than do the sensitive lines—experiencing yield reductions of approximately 20-40% under stress (vs yield reductions > 60% for sensitive lines) (Ndikuryayo et al., 2022). For example, climate studies in East Africa have found that early-season drought stress can reduce yields of moderately tolerant genotypes by 35-50% and of highly susceptible ones by more than 70%.

Rice plants also have mechanisms to resist drought at the cellular level. The main response involved is osmolyte accumulation, for example proline, soluble sugars, glycine betaine, which serves to maintain turgor by reducing osmotic potential and protecting cellular structure (Dong et al., 2025). Also, rice boosts its antioxidative protectors by increasing the levels of the enzyme's superoxide dismutase, catalase, and peroxidases that diminish the harm by the reactive oxygen species produced under stress (Dong et al., 2025; Nagai et al., 2024). Phytohormone alterations, especially elevated levels of abscisic acid (ABA), induce stomatal closure to reduce water loss and stimulate stress-responsive genes. Changes in membrane lipid composition also help stabilize cell membranes under drought stress (Dong et al., 2025).

Morphological adaptations are another important aspect in drought tolerance and it plays a pivotal role. The extensive root system, for instance, allows upland rice genotypes to access moisture from deeper soil layers in dryland environments, where the topsoil dries quickly (Zheng, 2025) rules. Drought tolerance in rice is a complex trait that includes efficient water uptake, conservative water use, osmotic adjustment,

reactive oxygen species detoxification, hormonal regulation, and growth plasticity (Zheng, 2025). It is hence critical to evaluate drought tolerance during germination and early seedling growth, as failure to establish in these life stages can preclude successful establishment of the crop (Dong et al., 2025).

Morpho-physiological indices including germination percentage, Germination Velocity Index (GVI), seedling shoot and root lengths and biomass accumulation, are employed in quantifying drought responses (Nagai et al., 2024). For example, one can compute a drought susceptibility index by comparing germination percentages under varying levels of stress and those under optimal conditions, while a high GVI under drought suggests a rapid and robust germination response to the limitations imposed by water availability (Islam et al., 2018). The plant is incapable of recovering its total number of tillers and biomass after just a short period of drought lasting of 10-14 days after sowing which has been demonstrated to cause large concomitant declines in yield (Ndikuryayo et al., 2022). By contrast, some varieties escape drought damage by entering a quiescent state during early stress, allowing them to mend rapidly in the event of a moisture pulse.

2.3 Polyethylene Glycol Induced Osmotic Stress

Simulating Controlled drought conditions that mimic drought stress allow us to better understand plant responses to abiotic stress in much more detail compared to in-field studies, enable screening of large numbers of genotypes so that genotypes with more drought-tolerant traits can be selected (Kadir et al. 2023). One common approach for this is the induction of osmotic stress via polyethylene glycol (PEG). Poly Ethylene Glycol (PEG) acting as an inert polymer with high molar mass contents when they dissolve in water, the result of this solvent-attached dry state, in fact, declines the solution's water potential, for example, drought stress applied without toxicity reaction since it could not be taken up by the plants due to its high molecular weight such that the PEG entry into plants is significantly lower than the water (Islam et al., 2018; Made et al., 2022).

Peg-induced stress for inducing appearance of autophagy. These can be done easily in petri dishes, germination trays or hydroponic systems, where equal stress conditions can be provided across all samples (Purbajanti et al., 2019). The ability to fine-tune the PEG concentration allows researchers to establish a desired osmotic potential (e.g., -0.5 MPa to replicate moderate drought), something that is difficult to accomplish in field conditions because of soil heterogeneity and weather changes. PEG enables high-throughput screening of many genotypes under uniformly controlled conditions, making it a valuable tool for early-generation selection in breeding programs (Goraguddi et al., 2023).

However, PEG-induced stress does not accurately reflect the complex nature of field drought in which water deficits emerge gradually and are dependent on soil properties and microenvironmental conditions. Although PEG treatments are most effective during early growth stages, PEG is less appropriate for mimicking drought during later developmental periods. In fact, natural drought is a complex of wet periods to dry and re-wet cycles, which only cause relative stress unlike PEG, which applies continuous stress. Nevertheless, data demonstrates that genotypes of rice with good performance in PEG-induced stress also establish better under field conditions that include dry sowing (Purbajanti et al., 2019).

PEG-induced osmotic stress will be applied in this experimentation to simulate drought during germination and early seedling growth. Utilized known concentrations (additive of 15% or 20% PEG-6000) will be used for consistent water stress environment. Seeds will be placed on damp filter paper or onto agar media, saturated the with PEG solution or water (the control), and the differences in germination rates recorded systematically (Goraguddi et al., 2023). Likewise, young seedlings will be grown in nutrient solutions with PEG and measured for root and shoot growth. PEG-mediated osmotic stress, in conjunction with other screening techniques serves as a quick and reproducible method to determine the drought tolerance profiles of genotypes (Gaurana-Nuñez, 2024).

2.4 Visual Scoring

Visual scoring Visual scoring allows a rapid and qualitative measure of plant response to drought through the study of the expressed symptoms. Drought stress symptoms in rice and other crops are normally characterized by rolling, drying, wilting of leaves, and general reduction in vigour and greenness (Islam et al., 2018). The International Rice Research Institute (IRRI) has developed a Standard Evaluation System (SES) that employs a numerical scale (generally from 0 to 9), with lower values reflecting few symptoms and higher numbers indicating more intense stress.

Drought stress is monitored periodically from visual assessments under controlled conditions. For instance, during the seedling stage, leaf rolling may be rated from 1 (fully turgid, no rolling) to 5 (full rolling). Leaf drying usually is expressed either as proportion of affected area or categorical score with respect to degree of dehydration. While wilting is more subjectively scored by the loss of turgor, visual scoring can be particularly advantageous in field trials where complex measurements such as direct water potential are impractical. As it is a qualitative subject, these evaluations require standardization with appropriate training and reference images (Islam et al., 2018).

Today, computer vision and machine learning methods are widely used to complement traditional visual scoring through conversion of digitalised images of tissues into quantitative stress parameters. The shifts in leaf colour, angles and overall plant size can be quantitatively assessed and converted into stress scores. Although moderate responses, such as leaf rolling, can be adaptive, nuanced responses (e.g. severe leaf drying) indicate damage, so visual scoring systems usually give special emphasis to stress-indicating symptoms (Awasthi et al., 2019).

2.5 Drought Tolerance Mechanisms in Upland rice

Upland rice genotypes possess several adaptive mechanisms to cope with the water-limited conditions. Full elucidation of these mechanisms is important for breeding

programs that want to enhance drought tolerance (Ndikuryayo et al., 2023; Shah et al., 2024). For dry conditions, there are broad plant responses that include traits of escape, avoidance, tolerance, and recovery, and the range is primarily focused on avoidance and within-tolerance mechanisms for upland rice.

On the level of morphology, crucial traits in drought avoidance are the development of a deep root system. Drought-tolerant varieties of rice typically develop larger and deeper root systems, enabling access to moisture in subsoil layers—an important trait for crops in upland settings, where soil at the surface can dry out quickly (Zheng, 2025). Stomatal regulation is also important; drought-tolerant plants tend to close stomata earlier or more effectively to minimize water loss at the expense of replication (growth) and photosynthesis (Kadir et al., 2023). Osmotic adjustment, which is driven by osmolyte accumulation, e.g. proline and soluble sugars, assists in retaining water and maintaining turgor in cells at low external water potential (Made et al., 2022).

At both the biochemical and molecular levels, rice activates numerous drought-responsive genes and pathways. Hormonal signals, especially in the case of abscisic acid (ABA), and the involvement of transcription factors like DREB are crucial in controlling these responses. Aquaporins, which facilitate water transport, and protective proteins, such as late embryogenesis abundant (LEA) proteins, also help cells be more resilient. Upregulation of antioxidant enzymes such as superoxide dismutase and catalase also counters oxidative stress (Aggarwal et al., 2025; Dong et al., 2025). New strategies, including seed priming and leaf-application of silicon or silica nanoparticles are effective for inducing drought tolerance by modulating redox signalling and fortifying cell walls (Kang et al., 2025).

Several QTL mappings and genome-wide association studies have demonstrated the first identified regions in the rice genome that contribute to drought tolerance. Importantly, the QTLs like the qDTY loci have been introgressed into popular cultivars, improving drought performance without sacrificing yields under favourable conditions (Srividya et al., 2011). NERICA varieties are a great example of how genetic diversity is put to good use, leading to the combining of advantageous traits of both *O. sativa* and

O. glaberrima leading to early vigour, competitive ability against weeds and superior drought resilience. Another important trait seen in some tolerant genotypes was the ability to recover from drought after plants can grow new leaves once the stress is removed (Kadir et al., 2023).

2.6 Strategies for incorporating drought tolerance into rice breeding programs

Drought tolerance, especially for upland rice systems, is a major target of breeding programs aimed at rainfed ecosystems. Divergent approaches are used to introduce drought-resilient traits into new rice varieties. Dedicated methods, including laboratory germination assays utilizing PEG-induced stress and seedling vigour testing under unirrigated conditions, have been established to identify cultivars that can quickly and robustly take their establishment under early drought conditions (Siddique et al., 2023). These approaches seek to maintain stable yield with minimal trade-offs such as low yield under optimal conditions; for example, the drought susceptibility index (DSI) is an indicator commonly used by breeders when selecting for lines associated with minimal yield loss under stress.

Aspects of these strategies are supported through collaborative work on the international stage, which encompasses organizations such as IRRI and AfricaRice that can promote sharing of promising germplasm and multi-location testing. Such partnerships are vital to developing climate-resilient rice varieties – a requirement for global food security as the threat of water scarcity looms (Ndikuryayo et al., 2023).

Drought tolerance improvement in rice through breeding is complex but still feasible by taking advantage of traditional and novel genomic and phenotyping technologies. Diverse germplasm was evaluated, hybridization strategies, marker-assisted selection and genomic selection were employed, biotechnological advances were made, and participatory breeding approaches were adopted in the development of drought resistant upland rice with yield stability. This work, and its extensive testing in the

field, has far reaching consequences in that it helps separate genetic make-up from the response of a plant to its environment, providing greater assurance that rice, the staple most consumed by hundreds of millions, remains dependable and productive in regions that are likely to experience ever-water-scarcer conditions (Ambikabathiy et al., 2019).

CHAPTER THREE

METHODOLOGY

3.1 Study 1: Effect of drought on germination rates of the upland rice varieties.

3.1.1 Study location

The experiment was done in a seed laboratory of NaCRRRI located at Namulonge, Wakiso District approximately 25 km northwest of Kampala. Namulonge is located within Uganda's central Agro-ecological zone, which is characterized by tropical, equatorial climatic conditions with bimodal rainfall and an annual average precipitation of between 1,000 and 1,500 mm. The region's typical temperature of 20°C to 28°C and environmental context found in the laboratory under a more controlled condition provide the relevant field condition under which upland rice is grown and therefore, the significance and applicability of the experimental results to the agronomic practices found in Uganda.

3.1.2 Planting materials

The study will use fifteen rice genotypes that will be sourced from the rice breeding program at National Crops Resources Research Institute (NaCRRRI). The seeds were harvested in January 2025 and stored at a temperature of 4°C in the Cereals seed bank. The experiment has a **susceptible check** which is IR64 (Cruz et al., 2018). Within the NERICA series, NERICA-4 (released in 2002) is recognised for its early vigor and stable yield under rainfed conditions, establishing it as a benchmark for upland performance (Somado et al., 2008). NERICA-1 and NERICA-10, introduced in 2007, share a number of traits yet display subtle differences in maturation and stress adaptability (Somado et al., 2008). NERICA-6, released in 2014, has attracted attention for its superior grain quality and competitive biomass production under stress conditions. The NARIC series—comprising NARIC-1 and NARIC-2, released in 2002—exhibit intermediate drought tolerance with moderate biomass accumulation. The NamChe group, consisting of five varieties (NamChe-1 through NamChe-5) developed via marker-assisted selection at

NaCRRRI and released in 2013, underscores the diversity of adaptive traits. NamChe-5 exhibits a superior root system under drought stress, while NamChe-1 and NamChe-4 maintain moderate tolerance and consistent seedling vigour (Hong et al., 2021). In contrast, the IR64 variety, representing lowland rice, is a **susceptible check** due to its poor performance under drought stress despite its high-yield potential in irrigated systems (Cruz et al., 2018). The Pre-released from NaCRRRI, 18(18)G, UP-15(45), and UP-25(45) demonstrate promising germination rates, early growth stability, and visual indicators of stress tolerance, with UP-25(45)

3.1.3 Experimental design

The design arrangement of the study was Split Plot arrangement in Completely Randomized. It allows for the two independent variables to be tested within this experiment. The primary independent variable (main plot) was Polyethylene glycol 6000 (PEG-6000) in various concentrations. The adding subplots were the 15 rice varieties stated in 3.1. It had been repeated three times. The PEG-6000 concentrations were prepared as i.e. 0% (control), 5%, 10%, 15%, and 20% by dissolving stipulated amounts of polyethylene glycol 6000 in 100ml of distilled water (Goraguddi et al., 2023).

Rice Seeds were sterilized by soaking in a 70% ethanol solution for 2 minutes followed by distilled water rinse. 10 millilitres of the different PEG-6000 concentrations was added to 10-cm sterile Petri dishes lined with two layers of Whatman paper, and 50 seeds from each genotype were placed in these dishes. The set-up was maintained in a plant growth chamber at a constant temperature of $25\pm0.5^{\circ}\text{C}$ and relative humidity of $80\pm1\%$ for 7 days on Petri dishes (Islam et al., 2018).

3.1.4 Data collection

Seed were considered germinated with radicle protrusion of more than 2 mm, and germination count was done after every 24 hours. Germination percentage, Germination Velocity Index and Mean Germination Time were calculated using the formulas below.

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total numbers of seeds}} \times 100$$

$$\text{Germination Velocity Index} = \sum \frac{N1}{T1} + \frac{N2}{T2} + \dots + \frac{Nn}{Tn} ,$$

Where: N is number of seed germinated on day 1, day 2 and last germination day respectively (Bore et al. 2023).

T1, T2, and Tn are first, second and last day of germination respectively.

$$\text{Mean Germination Time} = MGT = \sum N * T / \sum N$$

Germination Synchrony

$$CUG = \frac{\sum_{i=1}^k N_i}{\sum_{i=1}^k (T - T_i)^2 N_i}$$

Where T is the mean germination time, T_i is the time from the start of the experiment to the *i*th interval, N_i is the number of seeds germinated in the *i*th time interval and *k* is the total number of time intervals (Bore et al. 2023).

3.1.5 Data Analysis

Data analysis was performed using R 4.4.3 and Microsoft Excel 365. Descriptive statistics included mean, standard deviation, and median. Two-way ANOVA was conducted to assess effects of genotype (G), PEG concentration (P), and their interaction (G × P).

LSD test at $p < 0.05$ determined significance. Shapiro-Wilk and Levene's tests verified assumptions of normality and variance homogeneity (Karanjit et al., 2024).

3.2 Study 2: Effect of drought on early seedling stage of the upland rice varieties.

3.2.1 Study location

The experiment was conducted in the seed laboratory at National Crops Resources Research Institute (NaCRRI) in Namulonge, Wakiso district in Uganda. (3.1.1)

3.2.2 Planting materials

The study used fifteen rice genotypes that were sourced from the rice breeding program at National Crops Resources Research Institute (NaCRRI). The same upland rice varieties in 3.1.2 were used materials were used in 3.1.2.

3.2.3 Experimental design

The study used Split Plot design in Completely Randomized design. The main plot was Polyethylene glycol 6000 (PEG-6000) of different concentrations. The subplot was rice varieties stated in 3.1. The layout was replicated three times. Polyethylene glycol 6000 concentrations. Rice Seeds were surface sterilized with a 70% ethanol solution for 2 minutes and rinsed with distilled water. Fifty seeds from each genotype were placed in sterilized Petri dishes lined with two layers of Whatman paper and moistened with 10 ml of the specified concentration of PEG-6000 solution (Sharma, P., & Kumar, S. 2023). Two hundred twenty five Petri dishes were incubated in a growth chamber at a constant temperature of $25 \pm 0.5^{\circ}\text{C}$ and relative humidity of $80 \pm 1\%$ (Islam et al., 2018). The experiment was extended to 14 days to assess seedling responses (Samudin, 2023). The experimental design was the same as that in 3.1.3.

3.2.4 Data collection

On the 14th day, seedling parameters were measured under controlled conditions to evaluate the impact of drought stress. 15 seedlings were sampled from each experimental unit. Seedling length (from root tip to shoot tip) was recorded using a foot ruler. The fresh weight of the seedlings was also taken using an analytical weighing

scale (MODEL SPX222). Biomass accumulation was quantified by drying 15 seedlings from each experimental unit in a laboratory oven (Model EDS-LE-10931) for 48 hours at 70 °C.

Drought score was assessed visually through the Standard Evaluation System (SES) for rice developed by the International Rice Research Institute (IRRI et al., 2021) (IRRI, 2021) (see Appendix Table 12). Drought stress symptoms on seedlings were rated on a scale of 0(no visible symptoms) to 9(complete death of seedlings) according to the SES score. Medium scores correlate with leaf rolling and tip drying. For example, a score of 1 means a little rolling and drying, while a 5 means half the leaves are brown, dry and fully rolled. Seedlings showing total desiccation and death receive the highest score (9). Again, this visual scoring protocol permitted rapid, standardized assessment of physiological drought responses in plants and was well suited to complement quantitative data, such as seedling biomass and elongation metrics collected during the trial.

3.2.5 Data Analysis

Data analysis was similar to that of Study 1. Two-way ANOVA was used to determine main and interaction effects. Data were visualized using Excel 365 charts. LSD ($p < 0.05$) determined differences among means.

Chapter 4

Results

This chapter explains the performance of both established and candidate upland rice cultivars when subjected to varying intensities of PEG-induced drought stress—from a control condition (0% PEG) to severe stress (20% PEG). Nine critical parameters were examined to compare the released cultivars with pre-released lines (18(18)G, UP-15(45), UP-25(45)) and a drought-sensitive check (IR64).

4.1 Mean Germination Time (MGT)

MGT, which indicates the speed of seed germination (with lower values denoting faster germination) as shown in Table 3 below, was similar across all genotypes under control conditions (PEG 0), averaging around 5 days. Minor variations were observed; for example, NamChe-5 recorded the fastest germination at approximately 4.9 days, while NARIC-1 was slower at around 5.5 days. IR64, with an MGT of about 5.3 days, demonstrated moderate germination speed in comparison. Under severe drought (PEG 20), germination was generally delayed, leading to increased MGT values, though the degree of delay varied. Some released and pre-released lines, such as UP-25(45) which shifted marginally from 5.26 to 5.31 days and NERICA-10 from 5.33 to 5.31 days, maintained nearly consistent MGT values under stress.

Table 1 Mean Germination Time under different drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
18(18)G	5.25 de	5.27 abc	5.16 abc	5.18 def	5.36 cde
IR64	5.27 d	5.30 ab	5.13 abc	5.31 abcd	4.91 f
NARIC-1	5.46 a	5.27 abcd	5.21 a	5.30 abcde	5.25 e

NARIC-2	5.15 f	5.26 abcd	5.07 bc	5.19 def	5.23 e
NamChe-1	5.33 c	5.32 a	5.18 ab	5.41 a	5.63 a
NamChe-2	5.34 c	5.32 a	5.08 bc	5.27 bcde	5.41 abcde
NamChe-3	5.28 d	5.19 d	5.07 bc	5.10 f	5.25 e
NamChe-4	5.04 g	5.32 a	5.17 ab	5.36 abc	5.63 ab
NamChe-5	4.88 h	5.23 bcd	5.15 abc	5.17 ef	5.40 cde
NERICA-1	5.39 b	5.29 ab	5.12 abc	5.36 abc	5.47 abcd
NERICA-4	5.33 c	5.31 a	5.18 ab	5.38 ab	5.55 abc
NERICA-6	5.31 c	5.31 a	5.18ab	5.36 abc	5.51 abcd
NERICA-10	5.33c	5.30 ab	5.14 abc	5.29 abcde	5.31 de
UP-15(45)	5.23e	5.21 cd	5.03 c	5.25 bcde	5.41 bcde
UP-25(45)	5.26de	5.23 bcd	5.09 abc	5.24 cde	5.31 de
CV%	2.7	1.1	1.6	2	3.9
LSD (0.05)	0.06	0.024	0.034	0.046	0.088
Pvalue	$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$
(Var/PEG/)	16	16	16	16	16

4.2 Germination Percentage (GP)

According to the results in Table 4 below, Germination Percentage, which quantifies the proportion of seeds that successfully germinate, was high under control conditions (PEG 0) for all genotypes, ranging from approximately 74% to 86%. NamChe-5 achieved the

highest GP at 86%, while IR64 recorded the lowest at 74%. Most released upland varieties including the NamChe, NERICA, and NARIC series surpassed 80% GP under optimal conditions, and the pre-released lines were comparably around 79-81%, indicating high seed viability generally. Under severe drought (PEG 20), all genotypes experienced significant declines in GP. IR64 was most affected, reduced to 12.7% implying that nearly nine out of ten seeds failed to germinate under stress. While the other varieties maintained GP values between 20% and 33%.

Table 2 Germination Percentage under varied levels of PEG

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
18(18)G	80.00 e	76.67 ab	50.00 cd	40.67 bcd	28.00 defg
IR64	74.00 g	67.33 b	48.00 d	28.00 f	12.67 h
NARIC-1	78.00 f	79.33 ab	53.33 b	40.67 bcd	30.00 bcd
NARIC-2	80.00 e	75.33 ab	52.67 bc	36.67 e	26.00 g
NamChe-1	80.00 e	78.67 ab	54.00 b	42.67 bc	28.67 cdef
NamChe-2	82.00 cd	78.00 ab	54.00 b	39.3 cde	27.33 efg
NamChe-3	80.00 e	76.00 ab	52.67 bc	39.33 cde	26.67 fg
NamChe-4	82.00 cd	78.67 ab	54.67 b	42.00 bc	28.67 cdef
NamChe-5	86.00 a	84.00 a	64.67 a	50.66 a	33.33 a
NERICA-1	82.00 cd	77.33 ab	54.67 b	43.33 b	30.00 bcd
NERICA-4	84.00 b	79.33 ab	54.67 b	40.67 bcd	30.67 bc
NERICA-6	83.33 bc	80.00 ab	54.67 b	43.33 b	31.33 ab

NERICA-10	80.00 e	77.33 ab	54.00 b	37.33 de	28.67 cdef
UP-15(45)	80.66 de	76.67 ab	53.33 b	40.00 bcde	29.33 bcde
UP-25(45)	79.33 ef	76.67 ab	49.33 d	41.33 bc	28.00 defg
CV%	3.5	11.3	7.2	12.2	16.8
LSD					
(0.05)	1.192	3.702	1.643	2.094	1.981
Pvalue	1.81e ⁻¹⁴ / ^{<2e}		^{<1.81e}	^{<1.81e}	^{<1.81e}
(Var/PEG) ¹⁶		^{<1.81e⁻¹⁴/^{<2e⁻¹⁶}}	^{14/^{<2e⁻¹⁶}}	^{14/^{<2e⁻¹⁶}}	^{14/^{<2e⁻¹⁶}}

4.3 Germination Velocity Index (GVI)

GVI combines germination speed and uniformity, reflecting early vigour as shown in Table 5. NamChe-5 dominated across all stress levels—from 9.90 under PEG 0 to 3.28 at PEG 20. NERICA-6 and NERICA-4 also showed robust values under low to moderate stress, maintaining GVI above 5.5 up to PEG 10. IR64's GVI fell drastically to 1.39 at PEG 20%, confirming its sluggish and inconsistent germination. The slow decline of GVI in UP-15(45), NamChe-2, and NARIC-1 suggests their seeds maintain metabolic activity longer under dehydration stress. While PEG 0 levels showed a clustered performance, drought stress clearly separated the high-performing upland genotypes from the vulnerable IR64.

Table 3 Germination Velocity Index of rice varieties under varied drought stress

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
NamChe-5	9.90 a	8.72 a	6.77 a	5.27a	3.28 a
NamChe-4	9.02 b	8.07 ab	5.69 b	4.21 b	2.72 cde
NERICA-4	8.60 c	8.15 ab	5.68 b	4.09 bc	2.97 bc

NERICA-6	8.55 cd	8.21 ab	5.68 b	4.34 b	3.05 ab
NARIC-2	8.49 cd	7.74 ab	5.56 b	3.73 c	2.64 e
NamChe-2	8.40 de	7.98 ab	5.75 b	3.99 bc	2.69 de
UP-15(45)	8.40 de	7.99 ab	5.67 b	4.09 bc	2.88 bcde
18(18)G	8.29 ef	7.89 ab	5.18 c	4.21 b	2.79 cde
NamChe-3	8.27 ef	7.92 ab	5.56 b	4.09 bc	2.70 de
NERICA-1	8.27 ef	7.99 ab	5.78 b	4.34 b	2.95 bcd
NERICA-10	8.24 ef	7.94 ab	5.65 b	3.80 c	2.88 bcde
NamChe-1	8.19 f	8.05 ab	5.60 b	4.23 b	2.73 cde
UP-25(45)	8.19 f	7.96 ab	5.21 c	4.22 b	2.82 bcde
NARIC-1	7.74 g	8.25 ab	5.51 b	4.11 bc	2.71 cde
IR64	7.67 g	6.91 b	5.04 c	2.81 d	1.39 f
CV%	6.4	11.3	7.2	12.7	15.4
LSD (0.05)	0.228	0.382	0.173	0.221	0.18
Pvalue	<2e ⁻¹⁶ / ⁻ <2e ⁻				
(Var/PEG)	<2e ⁻¹⁶ / ⁻ <2e ⁻¹⁶	<2e ⁻¹⁶ / ⁻ <2e ⁻¹⁶	<2e ⁻¹⁶ / ⁻ <2e ⁻¹⁶	<2e ⁻¹⁶ / ⁻ <2e ⁻¹⁶	<2e ⁻¹⁶ / ⁻ <2e ⁻¹⁶

4.4 Germination Synchrony

Synchrony reflects how uniform germination is within a population; values close to 0.18 signify tight clustering of emergence. From Table 6 below, at PEG 0%, NamChe-1 had the highest synchrony (0.180), indicating highly synchronized germination. Under stress, some varieties like NARIC-2 saw increased synchrony (0.207 at PEG 15), possibly due to fewer but more coordinated germination events. In contrast, IR64 dropped significantly to 0.067 at PEG 20%, suggesting disorganized, staggered germination. NamChe-5 and

NamChe-3 consistently sustained higher synchrony under stress, which is desirable for uniform crop establishment.

Table 4 Germination Synchrony of Rice varieties under varied drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
18(18)G	0.173 c	0.170 bcd	0.187 a	0.173 bc	0.153 a
IR64	0.172 c	0.163 d	0.170 c	0.150 d	0.067 b
NARIC-1	0.172 c	0.167 cd	0.173 bc	0.173 bc	0.157 a
NARIC-2	0.171 c	0.183 a	0.183 ab	0.207 a	0.170 a
NamChe-1	0.180 a	0.173 bc	0.177 abc	0.160 cd	0.143 a
NamChe-2	0.162 d	0.167 cd	0.177 abc	0.170 bcd	0.147 a
NamChe-3	0.173 c	0.173 bc	0.183 ab	0.187 ab	0.170 a
NamChe-4	0.160 d	0.167 cd	0.177 abc	0.163 cd	0.150 a
NamChe-5	0.176 ab	0.177 ab	0.173 bc	0.177 bc	0.173 a
NERICA-1	0.170 c	0.173 bc	0.173 bc	0.157 cd	0.150 a
NERICA-4	0.172 c	0.170 bcd	0.173 bc	0.150 d	0.143 a
NERICA-6	0.173 bc	0.170 bcd	0.173 bc	0.157 cd	0.143 a
NERICA-10	0.173 c	0.170 bcd	0.173 bc	0.163 cd	0.163 a
UP-15(45)	0.170 c	0.170 bcd	0.187 a	0.163 cd	0.167 a
UP-25(45)	0.173bc	0.170 bcd	0.173 bc	0.173 bc	0.153 a
CV%	3.2	3.9	4.5	9.6	15.3
LSD (0.05)	0.002	0.003	0.003	0.008	0.014
Pvalue		<1.81e ⁻	<1.81e ⁻		<1.81e ⁻¹⁴ / ⁻
(Var/PEG)		¹⁴ / ⁻ <2e ⁻¹⁶	¹⁴ / ⁻ <2e ⁻¹⁶	<1.81e ⁻¹⁴ / ⁻ <2e ⁻¹⁶	¹⁶

4.5 Seedling Drought Stress Score

This score quantifies visible drought symptoms, with higher values indicating greater injury as shown in Table 7 below. IR64 had the worst scores across all PEG levels, rising from 1.33 at PEG 0 to 8.04 at PEG 20—nearly complete damage. Conversely, upland varieties had low scores under control (e.g., NERICA-4 at 0.20) and moderate increases under stress. Notably, UP-15(45), UP-25(45), and NamChe-5 all scored below 5.5 at PEG 15, and under 7.3 at PEG 20, outperforming IR64. This confirms visual tolerance and health under limited water.

Table 5 Seedling Drought Score of Rice varieties under varied drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
18(18)G	0.29 de	1.31 bcdef	3.13 bcde	5.04 cd	7.22 b
IR64	1.33 a	2.27 a	3.67 a	6.00 a	8.04 a
NARIC-1	0.49 bcde	1.02 ef	2.80 de	4.80 de	6.82 de
NARIC-2	0.42 bcde	1.38 bcd	2.89 cde	4.80 de	6.87 cde
NamChe-1	0.65 b	1.49 b	3.13 bcde	5.20 bc	7.13 bcd
NamChe-2	0.62 bc	1.24 bcdef	2.89 de	5.18 bc	7.25 b
NamChe-3	0.46 bcde	1.35 bcde	3.09 bcde	4.91 cde	6.96 bcde
NamChe-4	0.29 de	1.04 def	2.87 de	4.98 cd	6.85 de
NamChe-5	0.49 bcde	1.47 bc	3.16 bcd	5.22 bc	7.00 bcde
NERICA-1	0.27 de	1.02 ef	2.82 de	4.58 e	6.89 cde
NERICA-4	0.20 e	1.00 f	2.93 cde	5.07 cd	6.78 e
NERICA-6	0.33 cde	1.25 bcdef	2.78 e	5.22 bc	7.04 bcde
NERICA-10	0.53 bcd	1.13 cdef	2.91 cde	5.11 bcd	6.85 de
UP-15(45)	0.69 b	1.31 bcdef	3.42 ab	5.47 b	7.18 bc
UP-25(45)	0.71 b	1.44 bc	3.27 bc	5.22 bc	7.24 b
CV%	60.2	26.6	10.2	7.2	4.9

LSD (0.05)	0.133	0.149	0.132	0.156	0.147
P-value				$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$
(Var/PEG)	$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$	16	16

4.6 Seedling Fresh Weight

Seedling fresh weight as shown in Table 8, was measured before drying. Under control conditions (PEG 0), upland cultivars accumulated substantial biomass—several lines recorded values between 54 and 57 mg per seedling like NamChe-4 at 56.7 mg, NARIC-2 at 55.9 mg, and NamChe-1 at 55.1 mg whereas IR64 had a lower fresh weight of approximately 45.7 mg. Under severe drought (PEG 20), fresh weight declined dramatically for all genotypes; seedlings that typically weighed between 50 and 56 mg under optimal conditions were reduced to about 1-2 mg, corresponding to a reduction of roughly 96-98%.

Table 6 Seedling Fresh Weight of Rice varieties under varied drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
NamChe-4	56.70 a	34.74 c	21.64 a	7.84 a	1.66 a
NARIC-2	55.90 a	35.95 bc	20.78 a	7.35 a	1.66 a
NamChe-1	55.14 a	36.89 abc	22.07 a	7.99 a	1.90 a
UP-25(45)	55.11 a	35.25 bc	21.26 a	7.93 a	1.79 a
NERICA-4	55.09 a	39.82 a	20.56 a	7.79 a	1.68 a
NamChe-3	54.75 a	39.82 a	20.86 a	7.70 a	1.66 a
NamChe-5	54.67 a	36.61 bc	20.82 a	7.74 a	1.92 a
UP-15(45)	54.06 a	37.48 abc	20.90 a	7.81 a	1.89 a
NARIC-1	53.93 a	36.88 abc	20.33 a	8.02 a	1.90 a

NERICA-10	53.92 a	36.66 bc	20.08 a	8.18 a	1.82 a
18(18)G	53.87 a	36.64 bc	20.43 a	7.88 a	1.75 a
NERICA-6	53.45 a	37.09 abc	20.26 a	8.08 a	1.70 a
NERICA-1	53.39 a	37.87 ab	20.34 a	7.85 a	1.75 a
NamChe-2	53.37 a	37.09 abc	20.17 a	7.49 a	1.83 a
IR64	45.73 b	30.47 d	17.09 b	6.36 b	1.15 b
CV%	5.8	7.1	7.8	7.8	16
LSD (0.05)	1.325	1.104	0.679	0.257	0.118
P-value		<2.6e ⁻¹³ / <2e ⁻	<2.6e ⁻	<2.6e ⁻	<2.6e ⁻
(Var/PEG)	<2.6e ⁻¹³ / <2e ⁻¹⁶	16	13 / <2e ⁻¹⁶	13 / <2e ⁻¹⁶	13 / <2e ⁻¹⁶

4.7 Seedling Dry Weight

Seedling dry weight as shown in Table 9, representing the biomass after drying (and thus the accumulated solids), reflected the trends observed for fresh weight. Under control conditions (PEG 0), upland varieties produced approximately 10-11 mg of dry matter per seedling, in contrast to IR64, which produced about 8.8 mg. Under 20% PEG, dry weight nearly collapsed for all genotypes. The coefficient of variation was less than 10% which showed that the data was reliable. Upland varieties retained between 0.8 and 0.95 mg of dry weight, while IR64's seedlings dropped to around 0.54 mg. Consequently, IR64 experienced a biomass reduction of roughly 94%, compared to a 90-93% reduction in the upland lines. Statistically, IR64's dry weight under stress was significantly lower than that of the upland cultivars.

Table 7 Seedling Dry Weight of Rice varieties under varied drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
---------	-------	-------	--------	--------	--------

NamChe-4	11.34 a	8.69 c	6.19 a	2.61 ab	0.86 a
NARIC-2	11.18 a	8.99 bc	5.94 a	2.46 b	0.83 a
NamChe-1	11.03 a	9.22 abc	6.30 a	2.66 ab	0.92 a
UP-25(45)	11.02 a	8.82 bc	6.07 a	2.64 ab	0.90 a
NERICA-4	10.84 a	9.96 a	5.87 a	2.60 ab	0.84 a
NamChe-3	10.95 a	9.95 a	5.96 a	2.50 b	0.83 a
NamChe-5	11.16 a	9.15 bc	5.95 a	2.58 ab	0.87 a
UP-15(45)	10.81 a	9.37 abc	5.97 a	2.61 ab	0.95 a
NARIC-1	10.79 a	9.22 abc	5.81 a	2.67 ab	0.85 a
NERICA-10	10.79 a	9.16 bc	5.74 a	2.73 ab	0.86 a
18(18)G	10.90 a	9.16 bc	5.93 a	2.87 a	0.94 a
NERICA-6	10.82 a	9.27 abc	5.79 a	2.69 ab	0.85 a
NERICA-1	10.68 a	9.47 ab	5.81 a	2.62 ab	0.87 a
NamChe-2	10.49 a	9.27 abc	5.76 a	2.50 b	0.92 a
IR64	8.82 b	6.91 d	4.44 b	1.74 c	0.54 b
CV%	6.7	8.5	9.1	11.2	13.6
LSD (0.05)	0.307	0.33	0.226	0.122	0.05
P-value	<2e ⁻¹⁶ / _{<2e⁻}		<2e ⁻		<2e ⁻¹⁶ / _{<2e⁻}
(Var/PEG)	16	<2e ⁻¹⁶ / _{<2e⁻¹⁶}		16/ _{<2e⁻¹⁶}	<2e ⁻¹⁶ / _{<2e⁻¹⁶} 16

4.8 Seedling Root Length

Root length is a vital indicator of drought tolerance, as longer roots may access moisture from deeper soil layers. As shown in Table 10, under control conditions (PEG 0), upland cultivars developed substantial root systems, with lengths typically ranging from 8.0 to 8.3 cm. For instance, NamChe-5 exhibited roots of approximately 8.31 cm, while several other upland varieties (e.g., NamChe-2, UP-15(45), NARIC-1) ranged from 8.1 to 8.3 cm, and NamChe-1 slightly less at 7.97 cm. IR64, however, developed significantly shorter roots (around 7.03 cm), placing it in a lower statistical category.

Under severe drought (PEG 20), root elongation was considerably hindered across all genotypes; while upland cultivars maintained a relative advantage, with most recording root lengths between 2.3 and 2.5 cm (NamChe-5 at 2.47 cm and UP-15(45) at 2.40 cm). In contrast, IR64's roots averaged only about 1.53 cm, roughly half the length of those in drought-tolerant lines.

Table 8 Seedling Root Length of Rice varieties under varied drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
NamChe-5	8.31 a	7.20 a	5.53 a	3.83 abc	2.47 a
NamChe-2	8.27 ab	6.97 ab	5.53 a	3.87 abc	2.27 a
UP-15(45)	8.27 ab	7.03 a	5.47 a	3.93 a	2.40 a
NARIC-1	8.20 abc	6.93 ab	5.50 a	3.73 abc	2.33 a
NERICA-1	8.17 abc	7.07 a	5.43 a	3.83 abc	2.33 a
18(18)G	8.13 abc	7.00 ab	5.50 a	3.83 abc	2.47 a
NamChe-4	8.13 abc	6.97 ab	5.43 a	3.80 abc	2.37 a
NERICA-10	8.13 abc	7.20 a	5.27 a	3.87 abc	2.27 a
NERICA-4	8.13 abc	6.93 ab	5.40 a	3.90 ab	2.40 a
NERICA-6	8.10 abc	6.73 b	5.40 a	3.80 abc	2.40 a
NARIC-2	8.07 abc	7.00 ab	5.47 a	3.80 abc	2.30 a
UP-25(45)	8.07 abc	6.97 ab	5.33 a	3.83 abc	2.40 a
NamChe-3	8.0 bc	7.13 a	5.43 a	3.67 c	2.33 a
NamChe-1	7.97 c	7.03 a	5.33 a	3.70 bc	2.33 a
IR64	7.03 d	5.77 c	4.37 b	2.90 d	1.53 b
CV%	4	5.2	5.8	7.1	10.8
LSD (0.05)	0.137	0.153	0.132	0.113	0.105
P-value	<2e ⁻¹⁶ / ¹⁶ <2e ⁻¹⁶		<2e ⁻¹⁶	<2e ⁻¹⁶ / ¹⁶ <2e ⁻¹⁶	
(Var/PEG)	16	<2e ⁻¹⁶ / ¹⁶ <2e ⁻¹⁶		16	16/ ¹⁶ <2e ⁻¹⁶

4.9 Total Seedling Length (Shoot + Root Length)

Table 11 that shows Total seedling length which combines shoot and root lengths, provides measure of early seedling development. Under control conditions (PEG 0), upland varieties exhibited total seedling lengths between approximately 11.6 and 12.4 cm, reflecting growth. At PEG 0, NamChe-5 and NERICA-6 again topped performance (>12 cm), while IR64 was consistently the shortest. Under PEG 20, NamChe and UP lines managed ~4.5-5 cm, whereas IR64 was reduced to barely 3 cm. For example, UP-15 achieved a total length of 12.43 cm, and 18(18)G measured 12.37 cm, while other varieties such as NERICA-10, NERICA-1, UP-25(45), NamChe-5, NamChe-4, and NERICA-4 clustered around 12.0-12.2 cm. IR64, consistently underperformed in this parameter. These differences indicate stronger initial establishment potential among upland varieties.

Table 9 Total Seedling Length of Rice varieties under varied drought stress levels

Variety	PEG 0	PEG 5	PEG 10	PEG 15	PEG 20
UP-15(45)	12.43 a	10.50 a	9.57 a	7.83 a	5.37 a
18(18)G	12.37 ab	10.37 a	9.40 a	7.60 ab	5.50 a
NERICA-10	12.23 ab	10.30 a	9.33 a	7.80 a	5.30 a
NERICA-1	12.17 ab	10.27 a	9.57 a	7.63 ab	5.40 a
UP-25(45)	12.13 ab	10.40 a	9.17 a	7.63 ab	5.40 a
NamChe-5	12.10 ab	10.67 a	9.47 a	7.70 ab	5.50 a
NamChe-4	12.03 ab	10.43 a	9.20 a	7.53 abc	5.40 a
NERICA-4	12.03 ab	10.40 a	9.33 a	7.77 ab	5.27 a
NamChe-1	11.97 ab	10.43 a	9.37 a	7.63 ab	5.23 a
NARIC-2	11.90 ab	10.53 a	9.33 a	7.60 ab	5.23 a

NamChe-3	11.83 ab	10.47 a	9.30 a	7.57 ab	5.27 a
NERICA-6	11.70 ab	10.27 a	9.17 a	7.20 c	5.37 a
NamChe-2	11.67 b	10.13 a	9.67 a	7.83 a	5.40 a
NARIC-1	11.63 b	9.90 ab	9.57 a	7.43 bc	5.27 a
IR64	10.17 c	9.17 b	7.83 b	6.23 d	4.40 b
CV%	5.4	5.1	5.5	5.6	5.6
LSD (0.05)	0.273	0.224	0.218	0.181	0.125
P-value	$<2e^{-16}$	$<2e^{-16}$	$<2e^{-16}$	$<2e^{-16}$	$<2e^{-16}$
(Var/PEG)	10^{-16}	$10^{-16}/<2e^{-16}$	$10^{-16}/<2e^{-16}$	$<2e^{-16}/<2e^{-16}$	$10^{-16}/<2e^{-16}$

Chapter 5

The discussion is structured along important parameters, comparing the performance of released and unreleased upland varieties and situating the results in relation to other research papers.

5.1 Discussion for objective 1 - To assess germination parameters of released and pre-released upland rice varieties under controlled drought conditions.

One of the most sensitive parameters to this stress is the final germination percentage (GP) across rice genotypes, indicating the effects of drought stress on germination characteristics by polyethylene glycol (PEG) treatment. Under the same well-watered conditions, GP is generally greater than 80% in GP-susceptible genotypes, yet exposure to 20% PEG caused GP to drop significantly to 25-30% in drought-tolerant hotspots and even to 12-13% in the sensitive IR64 genotype, in agreement with findings of Islam et al. (2018) and (Siddique et al., 2023), who highlighted rice's susceptibility during germination. This trend is consistent with (Sagar et al., 2020), who reported sudden collapses in GP with increasing PEG-induced osmotic stress (-0.9 MPa). Among 210 rice accessions evaluated by (Fontonah et al., 2023) PEG stress, GP under PEG stress range was wide, with some lines maintaining approximately 50% GP while others showed near complete failure (Siddique et al., 2024). This variability emphasizes the genetic diversity in response to drought stress as illustrated by comparing the performance gap in NamChe-5 ($\approx 33\%$ GP) and IR64 ($\approx 12-13\%$ GP) and further accentuates the degree of susceptibility of IR64.

Although not specified in some of the datasets, germination vigour and germination vigour index (GVI) demonstrated similar trends; PEG stress significantly reduced GVI especially in susceptible genotypes by reducing the rate and synchronicity with which germination occurred. Kawaguchi et al. (2023) provide evidence that drought-tolerant lines have higher GVI under drought-induced stress, indicating that rapid early

metabolic activation occurs in a water deficit condition and is therefore plastic. Finally, germination uniformity, often the silent villain in literature, is paramount for field establishment. Germination dynamics were highly erratic for IR64 at under 20% PEG, which was in marked contrast to the more synchronized emergence in upland varieties. The consistent responses of the drought tolerant lines may harmonize due to similar regulation of hormonal signalling pathways, for example, abscisic acid signalling (as demonstrated by (Evamoni et al, 2023), emphasizing the future exploration of genetic and physiologic basis of uniformity under similar stress conditions.

5.2 Discussion for objective 2 - To evaluate the early seedling growth parameters of the rice varieties under controlled drought conditions.

As shown in the PEG-induced drought visual scoring of other genotypes (Pope et al., 2024; Samudin, 2023), the stress response upon drought showed highly significant genotypic variation, where sensitive cultivars such as IR64 infected consistently scored above 8 levels (IRRI $\sim \leq 8$ levels aligned with near-lethal stress), whereas moderately resilient genotypes including NERICA-4 and NamChe-4 scored much less (around stress scores of 6.8). PEG stress significantly inhibited biomass accumulation, with reductions in fresh and dry seedling weight of up to 95% in some genotypes. This correlates (Sobahan et al., 2022), who explained the biomass decline under 20% PEG by impaired cell expansion and photosynthesis because of limited water uptake. Root analysis also exposed significant differences; NamChe-5/PEG, a drought-tolerant line under PEG stress maintained longer root systems than IR64 which reflects higher capacity of water foraging ability. This observation is in contrast to the findings of (Sathyabharathi et al., 2022), rooted in that landraces can maintain root elongation under this osmotic stress due to their innate tolerance to drought. In contrast, shoot length decreased across all genotypes, highlighting the strong effect that drought imposed on aboveground growth.

There were differences in germination metrics, biomass traits, or morphological resilience when comparing released and pre-released genotypes. The promising pre-released lines (UP-15(45) and UP-25(45)) out yielded in terms of average performance elite lines, NERICA-4 and NamChe-5 (56.02-37.52%) suggest that drought-resisting alleles has been introgressed into newly bred materials. That development reflects (Pavithran et al., 2024), demonstrating that backcrossed inbred lines enriched for drought QTLs had strong early-stage drought tolerance. In addition, the consistency of NERICA-4 performance across various African upland environments (Wade et al., 2019), confirming its reliability, and the capabilities of UP-15(45) and related lines to serve as next generation, farmer-preferred, drought-adapted cultivars with enhanced agronomic traits.

Chapter 6

6.1 Conclusion

The results showed that drought significantly reduced germination and seedling performance across all varieties, but with notable differences in tolerance. NamChe-5 consistently outperformed all others, demonstrating the highest germination percentage (64.67% at PEG 10%, 33.33% at PEG 20%), fastest germination speed (MGT of 4.88 days), highest GVI (9.90 at PEG 0), and superior biomass retention under stress. Pre-released lines like UP-25(45) and UP-15(45) also showed strong performance, comparable to and sometimes better than some released varieties, especially in maintaining root length, seedling mass, and visual drought resilience under severe stress levels.

The statistical analysis confirmed these trends, leading to the rejection of Hypothesis H1, as significant differences were observed among genotypes in germination performance under drought. Similarly, Hypothesis H2 was rejected, showing that early seedling growth responses also significantly varied between released and pre-released lines. These findings highlight the existence of considerable genetic variability that can be exploited in breeding programs. The drought-susceptible check IR64 performed poorest, validating its use as a sensitive control. The study identifies NamChe-5 and UP-25(45) as strong candidates for varietal promotion and breeding, offering a pathway to improve resilience in Uganda's rainfed upland rice production systems.

6.2 Recommendations

These findings highlight the promise of both released and pre-released upland rice varieties for sustainable crop establishment in water-limited environments, offering a viable strategy for reducing drought-induced yield losses and improving food security in drought-affected areas. And suggest more studies are carried out in-field for germination and early seedling. I suggest repeating the research from germination and

early seedlings under drought stress conditions to maturity and yield to determine the impact early drought stress had on yield.

APPENDIX



Figure 2 Confirmation letter from NaCCRI

Table 10 The Released and Pre-released rice varieties

NO	Variety	Year of release	Ecology
1	NERICA-4	2002	Upland
2	NARIC-1 (ITA 257)	2002	Upland
3	NARIC-2 (ITA 325)	2002	Upland
4	NERICA-1	2007	Upland
5	NERICA-10	2007	Upland
6	NamChe-1 (ARICA-5) (WAB 95-B-B-40-HB)	2013	Upland
7	NamChe-2 (NM7-8-2-B-P-11-6)	2013	Upland
8	NamChe-3 (NM7-29-4- B-P-80-8)	2013	Upland
9	NamChe-4 (ARICA-4) (ART3-11L1P1-B-B-2)	2013	Upland
10	NamChe-5 (NM7-27-1- B-P-77-6)	2013	Upland
11	IR 64		lowland
12	NERICA-6	2014	Upland
13	18(18)G	Pre-released	upland
14	UP-15(45)	Pre-released	upland
15	UP-25(45)	Pre-released	upland

Table 11 Standard Evaluation System (SES) for rice (IRRI, 2021)

Description	Visual Score
No drought stress symptoms	0
Slight leaf rolling and tip drying	1
Leaf rolling and tip drying persists to 1/4 length in 25% of all leaves (normally the older leaves)	2
Leaf rolling and tip drying extended to 1/4 length or more in less than 50% of all leaves	3
Leaf tip drying and rolling reached 1/4 length or greater in 50% of total leaves, 25% of leaves rolled fully dried	4
50% of all brown and dry leaves rolled	5
> 50% but < 70% of all leaves fully rolled and dried	6
Seventy percent of all leaves fully rolled and dried	7
Over 70% of entire leaves tightly rolled and dried	8
All are dead	9

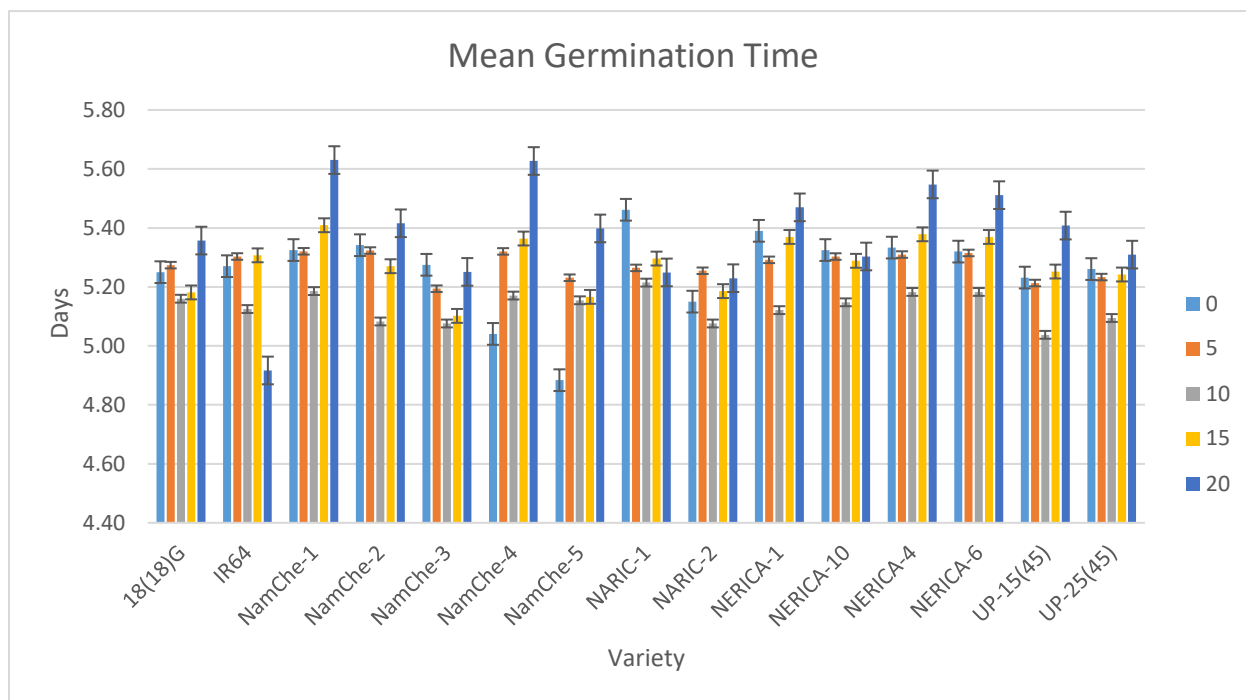


Figure 3 Mean Germination Time under different drought stress levels

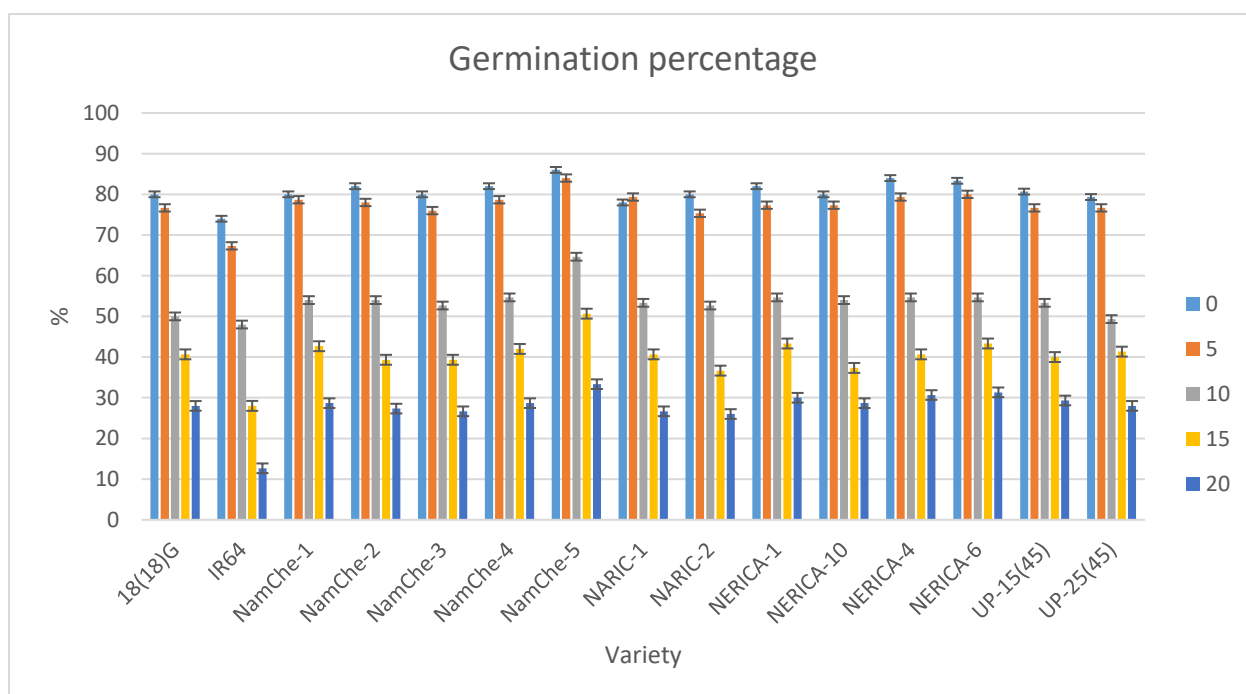


Figure 4 Germination Percentage under varied levels of PEG

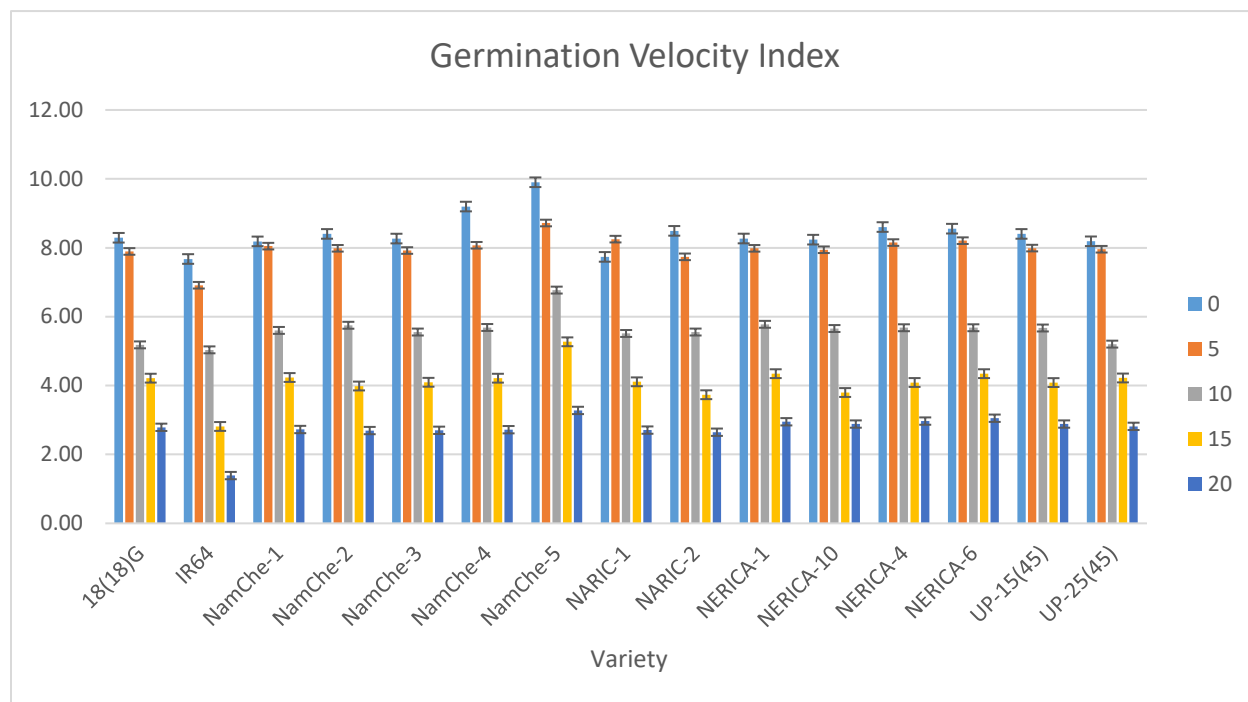


Figure 5 Germination Velocity Index of rice varieties under varied drought stress

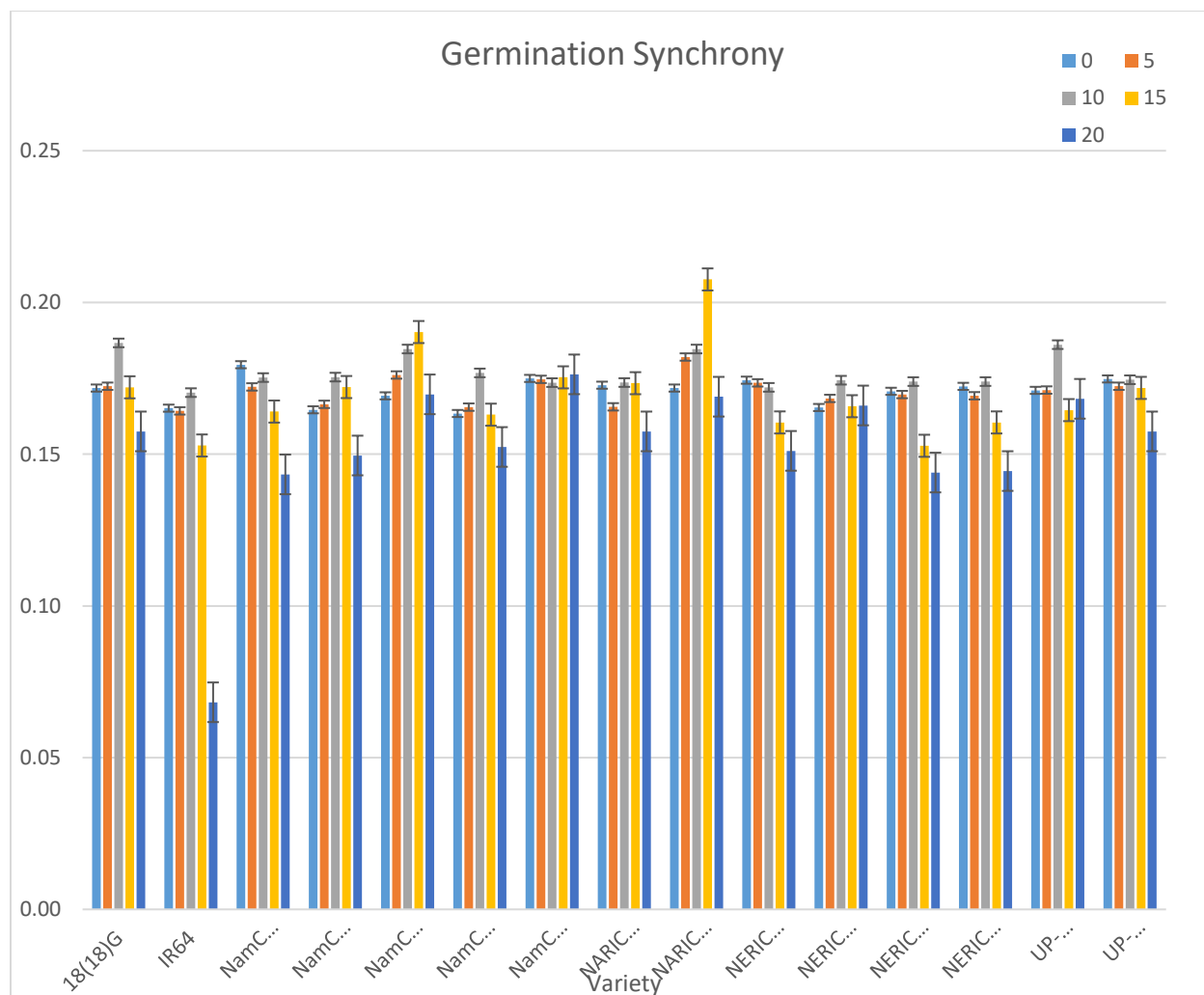


Figure 6 Germination Synchrony of Rice varieties under varied drought stress levels

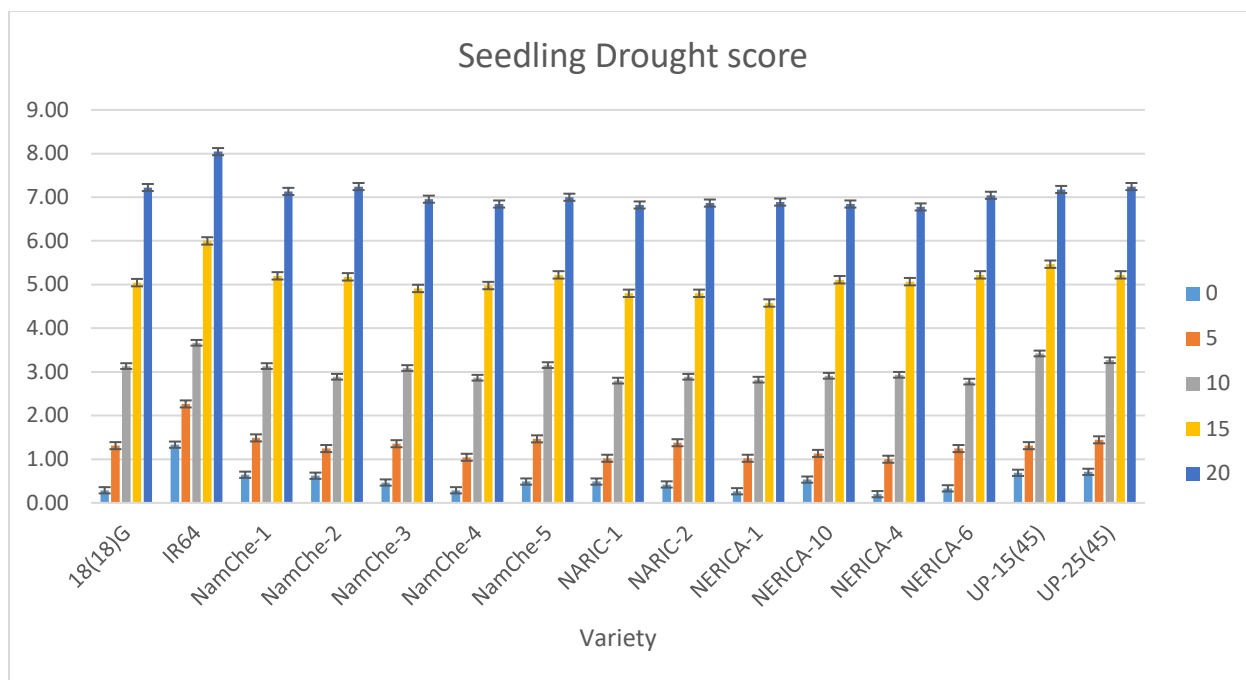


Figure 7 Seedling Drought Score of Rice varieties under varied drought stress levels

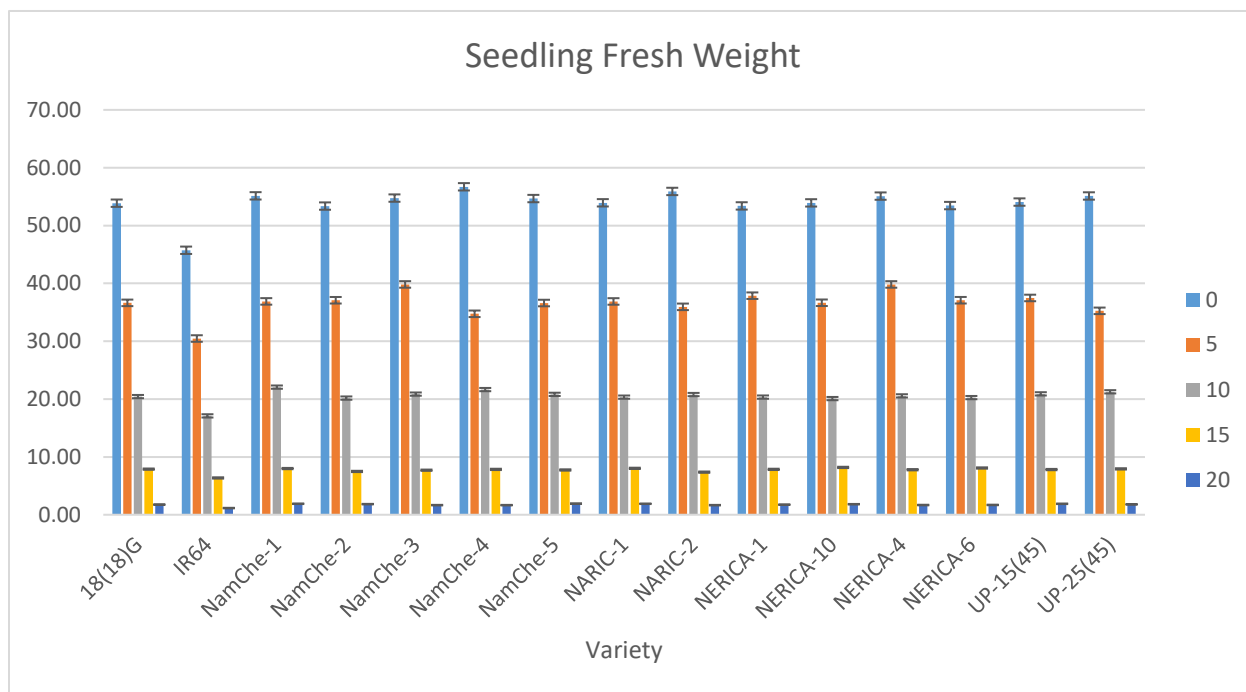


Figure 8 Seedling Fresh Weight of Rice varieties under varied drought stress levels

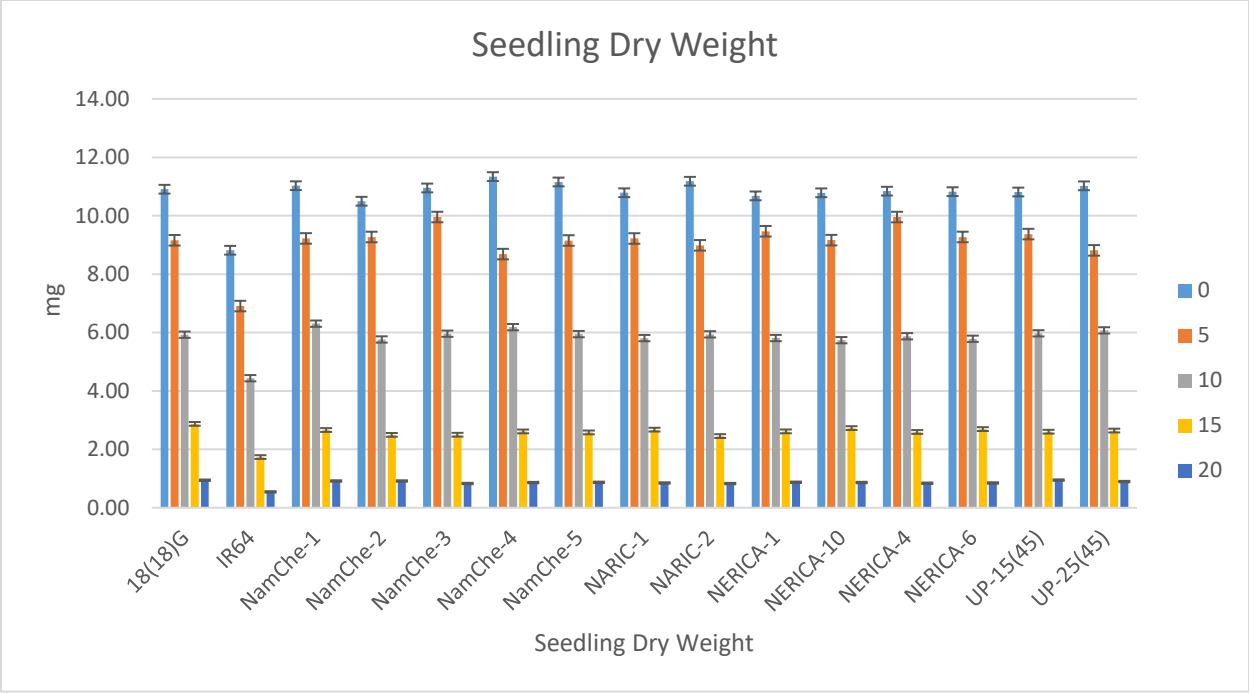


Figure 9 Seedling Dry Weight of Rice varieties under varied drought stress levels

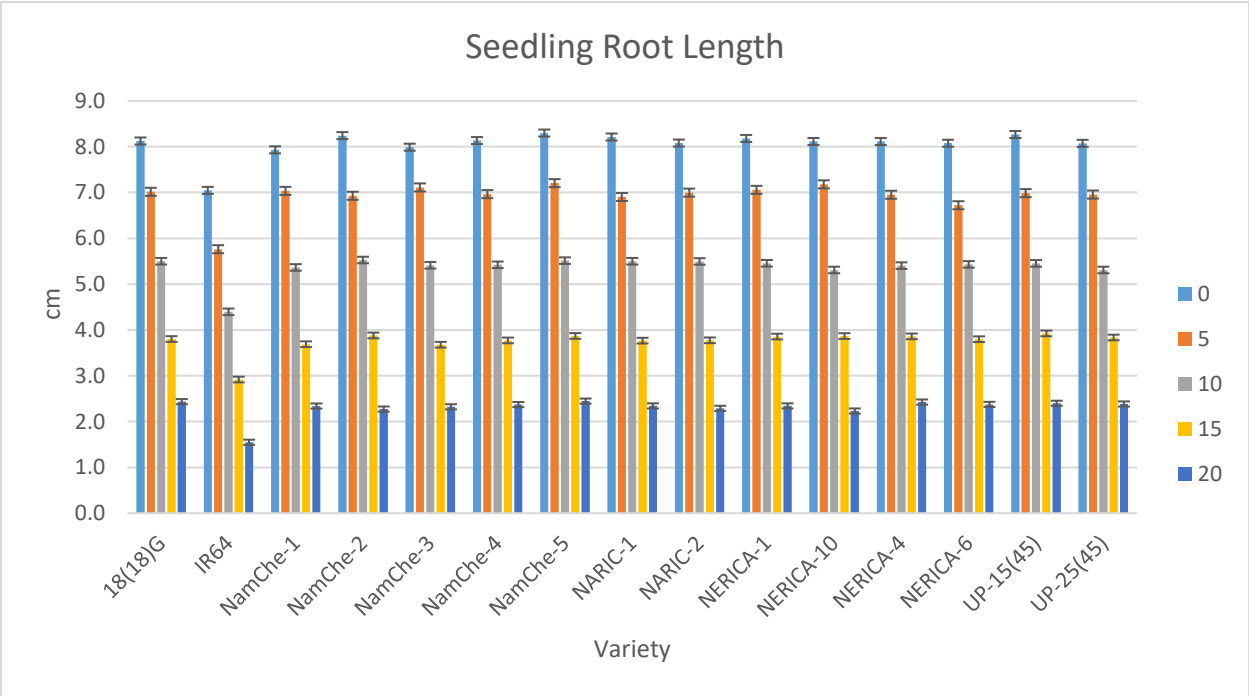


Figure 10 Seedling Root Length of Rice varieties under varied drought stress levels

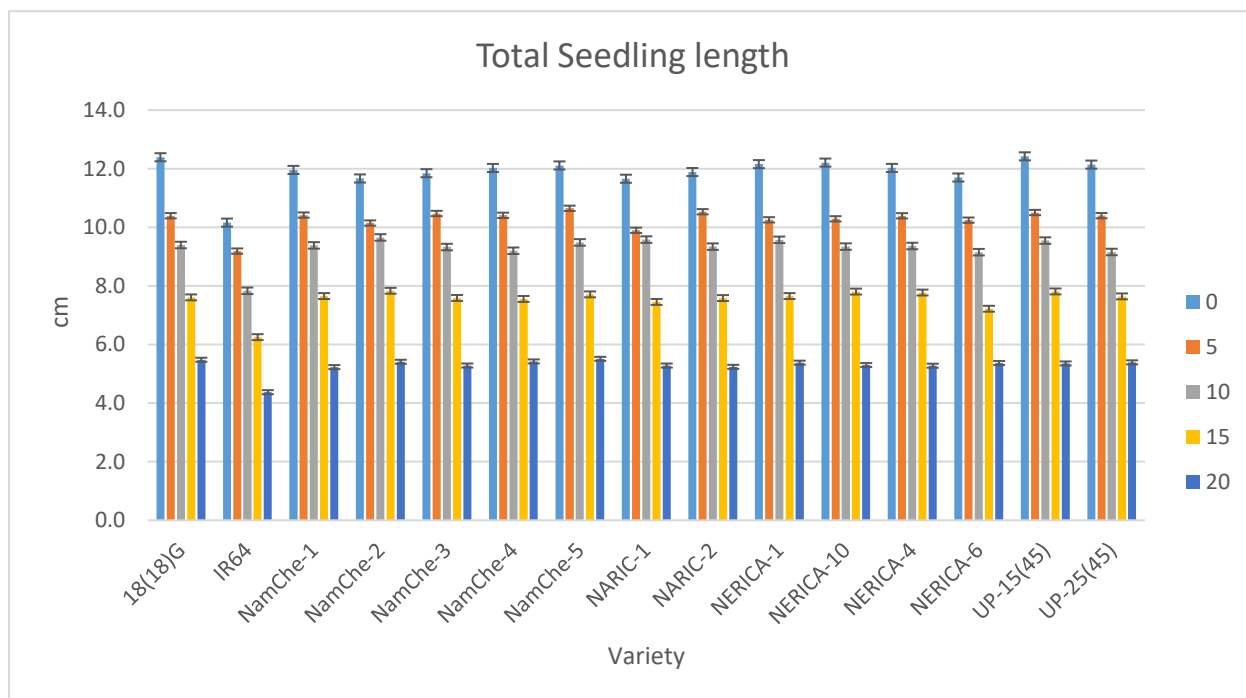


Figure 11 Total Seedling Length of Rice varieties under varied drought stress levels



Figure 12 Model of the Plant Growth chamber



Figure 13 Polyethylene Glycol 6000



Figure 14 Counting seeds in the Growth Chamber



Figure 15 Working in a Biosafety Cabinet in the Lab



Figure 16 Weighing the PEG for dissolution



Figure 17 Seedling in a Petri dish

Experimental layout

Replication 1

0% PEG	10	2	6	5	9	1	15	3	14	11	13	12	7	4	8
5% PEG	7	9	14	5	13	12	4	1	6	10	15	8	11	2	3
10% PEG	5	15	13	1	9	2	7	6	10	8	3	14	4	12	11
15% PEG	12	5	7	8	10	2	1	6	9	13	4	3	14	15	11
20% PEG	3	6	14	12	10	8	5	13	1	11	9	2	7	15	4

Replication 2

0% PEG	14	6	9	8	12	3	13	10	5	15	2	7	4	1	11
5% PEG	14	15	3	5	1	8	11	7	9	4	10	12	2	13	6
10% PEG	15	3	11	9	7	2	6	14	12	4	5	1	13	10	8
15% PEG	9	4	14	15	6	5	13	1	3	8	2	7	10	12	11
20% PEG	5	15	11	2	6	8	1	4	3	12	7	9	14	13	10

Replication 3

0% PEG	8	14	9	2	6	11	5	15	12	3	1	10	7	13	4
5% PEG	8	6	11	15	7	2	13	3	10	14	4	12	9	1	5
10% PEG	4	3	14	6	9	2	1	12	15	8	10	7	5	11	13
15% PEG	14	10	2	8	6	13	1	12	9	4	5	11	7	15	3
20% PEG	9	2	7	11	12	8	5	4	1	14	15	10	6	3	13

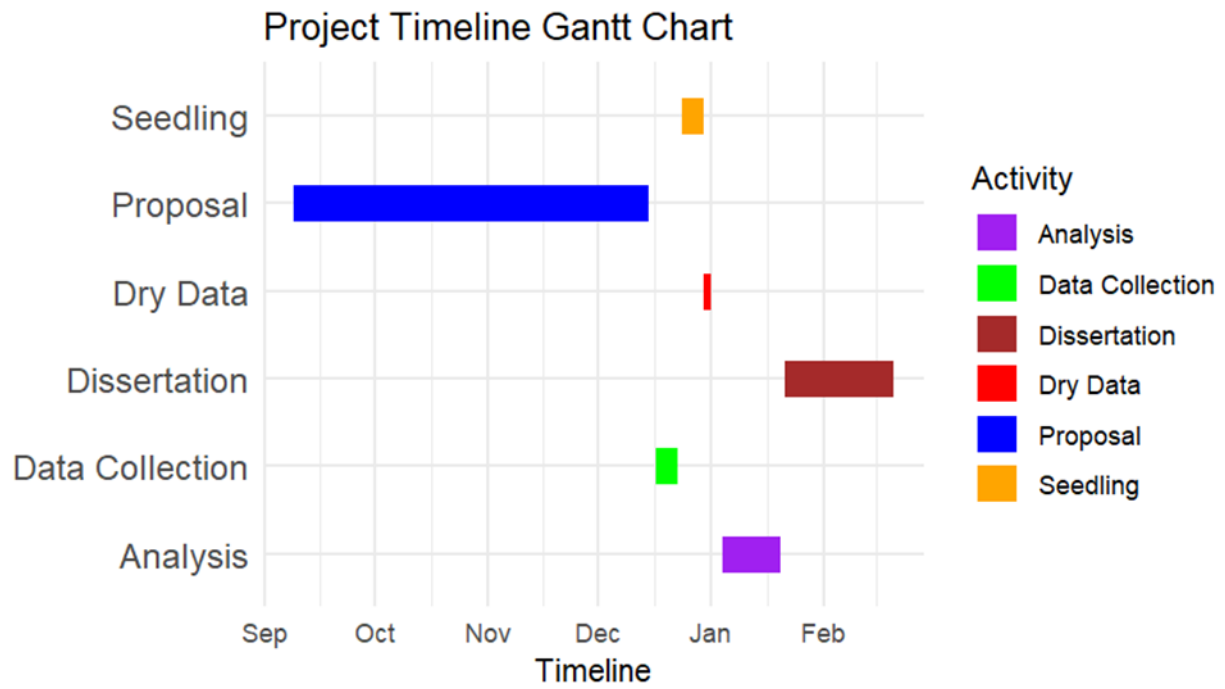


Figure 18 Project timeline

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