THESIS

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ABIOTIC STRESS TOLERANCE OF DH RICE LINES FOR THE DEVELOPMENT OF AEROBIC RICE PRODUCTION SYSTEMS

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List of Abbreviations

AI	Active Tillering
ANOVA	Analysis of Variance
AWD...	Alternate Wetting and Drying technology
CCI...	Chlorophyll Content Index
CNDVI..	Chlorophyll Normalized Difference Vegetative Index
CRD	Completely Randomized Design
CRI...	Carotenoid Reflectance Index
CRISPR.......................................	Clustered Regularly Interspaced Short Palindromic Repeats
DCNI...	Double Peak Canopy Nitrogen Index
DH..	Double Haploid
DSR...	Direct Seeded Rice
FAOSTAT...................................	Food and Agriculture Organization Statistics
FAO...	the Food and Agriculture Organization
FGP	Final germination percentage
HSD...	Honestly Significant Difference test
IAD	Absorbance Difference Index
IBM	International Business Machines Corporation
IPCC..	Intergovernmental Panel on Climate Change
PEG	Polyethylene Glycol
PI...	Panicle Initiation
PRI	Photochemical Reflectance Index
PTR	Paddy Transplanted Rice
RCBD..	Randomized Complete Block Design
RGA	Rapid Generation Advance
SPSS..	Statistical Package for the Social Sciences
SRID	Statistics Research and Information Directorate
MoFA..	Ministry of Food and Agriculture
SSD	Single Seed Descent
T50 /MGT	Median Germination Time
USA	United States of Agriculture

1. INTRODUCTION

Rice (*Oryza sativa L.*) is a major staple for more than fifty percent of the world's population (Fuagawa & Ziska*,* 2019) and its consumption rate continues to increase (Statista, 2023). It is mainly a lowland cultivated crop usually grown in flooded areas. About one third of our freshwater resources is used in the production of flooded paddy rice (Bouman *et al*., 2007; Surendran *et al*., 2021**)**. This production method however, has raised some concerns in our day especially on its sustainability (Jayakumar *et al*., 2004; Singh & Dadse, 2021). This has been due to periodic news on the world's depleting freshwater resources (Tuong *et al*, 2005), water scarcity due to unpredictable timing and quantum of rains and contributions of flooded paddies to methane greenhouse gas emissions (Wang *et al*., 2017). As the frequency and intensity of hydrology variations become unpredictable with climate change, drought stress will pose a great threat to agriculture, especially in developing countries (Turral *et al*., 2011). Drought stress-constrained rice production will causes enormous economic losses and huge food security issues. With this growing concern, drought stress tolerance in rice is gaining a wider appeal (Panda *et al*., 2021) and many have called for more sustainable water saving rice production technologies (Mandal *et al*., 2019).

Some upland rice varieties exist that are cultivated in areas with little water. This water saving production system is the aerobic rice production system (Meena *et al*., 2019). In this production system, Direct Seeded Rice (DSR) is often used. The constraint of this system is that there are inadequate rice cultivars that can be grown under these water- scarce conditions (Çolak, 2021). DSR, conversely, provides avenues for efficient water and nitrogen use, and a reduction of both greenhouse gas emissions and labor demand (Shekhawat *et al*., 2020). The downside of DSR technique is that the semi-aquatic botany of rice usually leads to fairly less yields when compared with yield from Paddy Transplanted Rice (PTR). Although there may be a 20–30% yield reduction, aerobic rice production can save 60–90% more water than traditional flooded paddy rice (Tuong and Bouman,2003; Zhang *et al*., 2009; Mostafa & Fujimoto, 2014; Çolak, 2021).

From the fifth assessment report of the intergovernmental panel on climate change (IPCC ARS), Pauchari & Meyer (2014) reported that the average global surface temperature had risen by

0.85°C from 1880-2012, and over the past ten years, the figure continues to be on the ascendency. Saud *et al.* (2022) emphasized that the ecosystem of rice production was one of the most sensitive to climate change. Drought stress could contribute to about 50% loss in crop yield (Ashraf *et al*., 2008) and over the years, rice breeders have faced numerous challenges in their attempt to breed rice cultivars (i.e. rice lines) that meets the pace of these fast-changing stress conditions (Hernández-Soto *et al*., 2021). Rice to combat drought stress over the years has evolved mechanisms within it; morphologically, physiologically, biochemically or even molecularly by adapting specific biosynthetic pathway in its daily functions (Panda *et al*., 2021). It may develop deeper and stronger roots (Zombori *et al.*, 2008), greatly reduce its photosynthetic activity through leaf hairs, thickened cuticula, hidden stomata, bent leaves and stomata closure (Zhu *et al*.,2020), increase its osmolyte accumulation such as proline (Mishra *et al*., 2018) adapts an "escaper" strategy by producing seeds early (generative phase) (Zombori *et al.*, 2008).

Massive breeding efforts in the past years especially after the green revolution were able to double rice productivity by holding high yield as their main breeding goal whilst increasing the use of high input agriculture conditions (Pingali, 2012). Notwithstanding, by 2023, world rice production needs to be increased to meet the increasing world population demand (Foley *et al*., 2011). Other studies have indicated that by 2030, production must increase by about 40% to meet this increasing demand (Khush, 2005). Much of this increase will need to come from rice cultivars that have been improved; high- yielding robust rice cultivars that are tolerant to drought stresses. The double haploid technique (a tissue culture technique), from literature (Hernández-Soto *et al*., 2021), seem to have been acclaimed as one of the transformation approaches that could significantly speed up the rice breeding process and aid to select faster, drought tolerant cultivars, in aerobic rice production Systems. Fixed or homozygous crop lines could be achieved in a short time (Gomez-Pando *et al*., 2009; Fazaa *et al*.,2016) and also screen for useful traits which we could not attained with normal conventional methods (Pauk *et al*.,2009).

To safeguard food security in the wake of heightened unpredictable rainfall patterns, increasing drought stress conditions and the prevalence of few drought tolerant cultivars, in concert with the increasing consumer demand or rice consumer demand for rice, there is a need to screen more drought-stress tolerant rice cultivars that can thrive in aerobic rice production systems. However, due to the complex nature of drought stress trait, screening for drought tolerance in rice will have to take on a holistic point of view. Seed germination and seedling growth (vigour and height), leaf traits (such as pigment content and water band index), root traits, photosynthetic capacities (carotenoid content)), biochemical markers (such as increased proline content) are all important factors that will need to be considered by breeders in the emergence of drought tolerant cultivars.

In this thesis, methods used to produce new rice cultivars are briefly discussed together with the whole system of rice production and some highlights on constraints to rice production in Hungary and in Ghana are given. The thesis then zooms in on the procedure followed to screen (assess) twenty double haploid (DH) rice lines for drought tolerance and ends with some results of promising DH rice lines that can be grown in aerobic rice production systems.

This present study has an overall aim to screen for drought tolerance in twenty double haploid rice lines. The sub-objective is to:

I. Find the on-field drought stress indices, yield (height and milled weight) parameters of 20 DH rice Lines.
II. Find the limiting PEG concentration to use for the screening of the germination parameters of the twenty rice lines in vitro.
III. Screen the germination parameters of 20 lines under the limiting PEG concentration in vitro.

2. LITERATURE REVIEW

2.1 General description of Rice

Rice (*Oryza sativa* L) is a major staple for more than fifty percent of the world's population (Fuagawa & Ziska, 2019). The three topmost producers of rice are China, India and Indonesia (FAOSTAT, 2019) (Fig. 1). Two main species of rice are usually grown; *Oryza sativa* and *Oryza glaberrima*. *O. Sativa* has two ecotypes: *indica*; adapted to tropical climates, and *japonica*; adapted to the temperate regions and tropical highlands. In West Africa, *Oryza glaberrima* is usually grown however, some indica types exist. The agronomy of rice takes into consideration the whole system of rice production; from soil requirements, seedbed preparation, water management, planting type, planting material all the way to fertilization. Rice thrives in fertile, well-drained soils with a pH range of about 6.0 to 7.5 that have been well prepared to ensure proper seed growth. Nitrogen is its main nutrient requirement for proper growth and development. Yield in rice is measured as rough rice (rice with husk) at a moisture content of 14%. Upland rice yields typically range from 1 to 2 tonnes per hectare, whereas aerobic rice can produce a double of that amount per hectare with an application of fertilizer (Huaqi *et al*., 2002). Also rain-fed rice usually yield twice as much yields than that recorded in aerobic rice production system nonetheless, the aerobic rice saved water and reduces greenhouse methane emissions than in flooded rice systems.

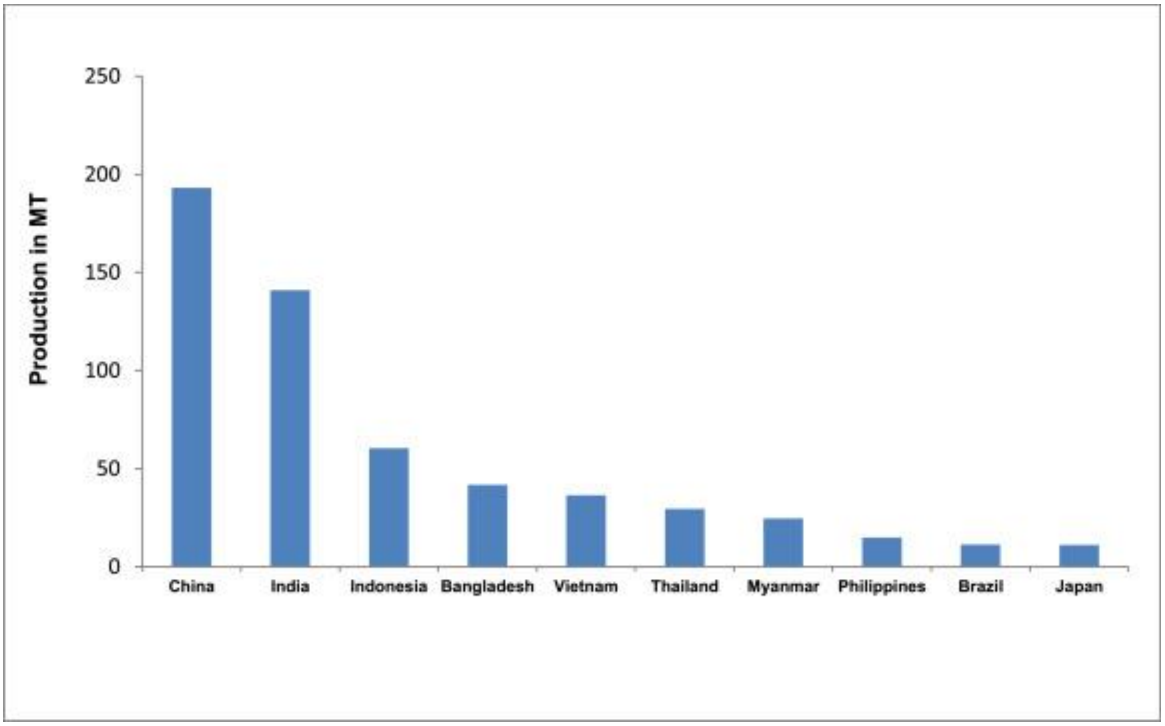

Fig. 1: Top ten rice producing countries in the world (Source: FAOSTAT - FAO, 2019).

2.2 Systems of Production

Rice is grown usually under two main agronomical systems; direct-seeded-rice (DSR) and paddy transplanted-rice (PTR). PTR, the conventional method, thrives mostly on water-flooding. Review from literature reveals that, all over the world, about one third of our freshwater resources is used in the production of flooded paddy rice (Bouman *et al*., 2007; Surendran *et al*., 2021**)**. This production method however, has raised some concerns in our day especially on its sustainability (Jayakumar *et al*., 2004; Singh & Dadse, 2021). This has been due to periodic news on the world's depleting freshwater resources (Tuong *et al*, 2005), water scarcity due to unpredictable timing and quantum of rains and contributions of flooded paddies to methane greenhouse gas emissions (Wang *et al*., 2017). Many have called for more sustainable water saving rice production technologies (Mandal *et al*., 2019). DSR, conversely, provides avenues for efficient water and nitrogen use, and a reduction of both greenhouse gas emissions and labor demand (Shekhawat *et al*., 2020). The downside of DSR technique is that the semi-aquatic botany of rice usually leads to fairly less yields when compared with yield from PTR. Although there may be a 20–30% yield reduction, aerobic rice production can save 60–90% more water than traditional flooded paddy rice (Tuong and Bouman,2003; Zhang *et al*., 2009; Mostafa & Fujimoto, 2014; Çolak, 2021).

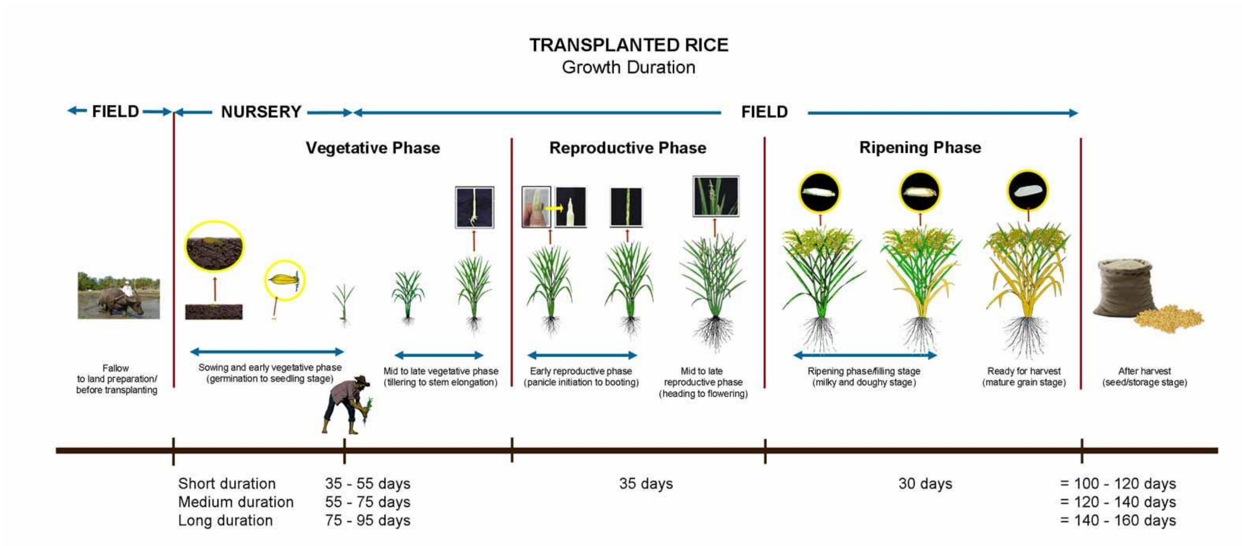

Fig. 2: Growth duration and phases in transplanted rice (Source: knowledgebank.irri.org)

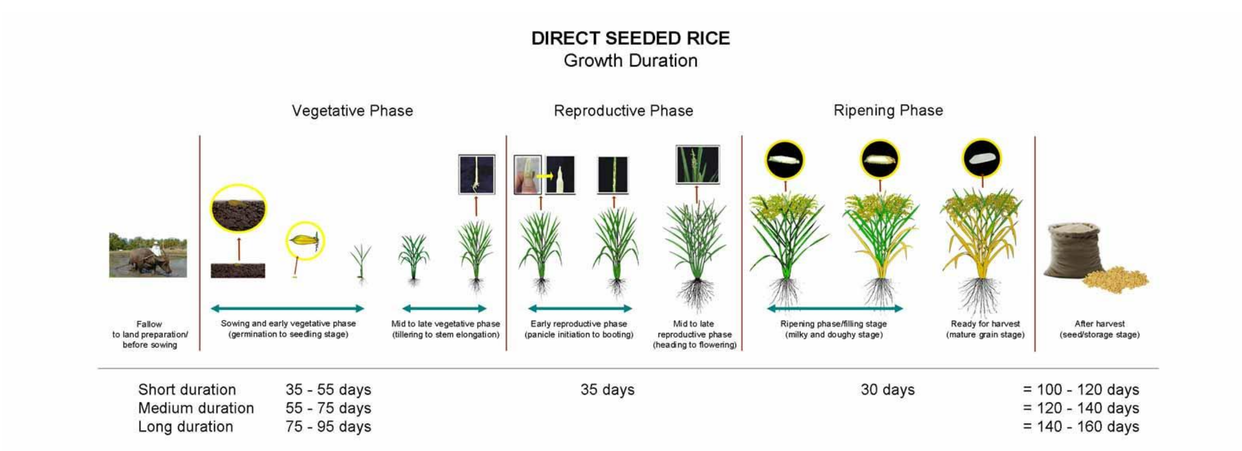

Fig. 3: Growth duration and phases in Direct seeded rice (Source: knowledgebank.irri.org)

2.2.1 Water requirement of rice

Rice due to its semi-aquatic ancestry, has a shallow root system and hence, is quite sensitive to water deprivation. It needs water at almost all stages of its development however the most critical stages are Active tillering (AT), panicle initiation (PI) and grain filling stage (Surendran *et al*.,2021). The amount of water used in land preparation, crop evapotranspiration, losses due to seepage and percolation, and actual water used in the agro ecosystem during crop growth are all accounted for in the amount of water use in the cropping of rice. The water required by rice is nonetheless influenced by environmental conditions, type and length of growing season, weather parameters, soil type, and other hydrology parameters; 1000–2000 mm ± 350 mm has been the typical value stated in many literature (Tuong & Bouman, 2003; Datta *et al*., 2017; Surendran *et al*., 2021). Lowland rice uses more water than upland rice because it is flooded. Overall, rice production is responsible for the withdrawal of about 24 to 30% of the world's freshwater resources and globally, 34 to 43% of the irrigation water used (Surendan *et al*., 2021).

2.3 Drought Stress and Tolerance of Rice

Drought, amongst the other abiotic elements, is a severe constraint to rice production (Nelson *et al*., 2014; Pandey & Shukla, 2015; Panda *et al*., 2021). This is because plant root is the main organ for absorbing water hence the ability of a plant to withstand osmotic and drought stressors greatly depends on it (Zombori *et al.*, 2008). The original root system of rice however, is very short making it one of the most drought stress-sensitive crops. Drought stress in rice can be seen in the highly reduced seed germination and seedling growth (Vibhuti *et al*., 2015), reduced leaf growth Zhu *et al*.,2020), Leaf rolling and early death (Anjum *et al*., 2011), reduction in leaf size and stomata (Rollins *et al*.,2013), reduced photosynthesis (Papp *et al*., 2004; Zhu *et al*., 2020), falling Chlorophyll and carotenoinds (Ashraf & Harris 2013; Zhu *et al*.,2020), and increased accumulation of osmolytes such as Proline (Anjum *et al*., 2017).

As such, rice, to combat drought stress over the years has evolved mechanisms within it; morphologically, physiologically, biochemically and molecularly by adapting specific biosynthetic pathway to use (Panda *et al*., 2021). It may develops deeper and stronger roots (Zombori *et al.*, 2008), greatly reduce its photosynthetic activity through leaf hairs, thickened cuticula, hidden stomata, bent leaves and stomata closure (Zhu *et al*.,2020), increase its osmolyte accumulation such as proline (Mishra *et al*., 2018) adapts an "escaper" strategy by producing seeds early (generative phase) (Zombori *et al.*, 2008). Wu & Cosgrove (2000) have argued that greater root development by rice in the long term was a better long term strategy. However, with ground water table further receding through climate change and other human-caused factors, there is the need for a holistic consideration of the several factors that will culminate in selecting better drought tolerant cultivars.

Abiotic stress particularly drought stress is a complex trait in screening for drought tolerance in rice. Seed germination and seedling growth (vigour and height), leaf traits (such as pigment content and water band index), root traits, photosynthetic capacities (carotenoid content)), biochemical markers (such as increased proline content) are all important factors that are considered by breeders.

2.4 Importance of Rice improvement in the wake of Climate change

Massive breeding efforts in the past years especially after the green revolution were able to double rice productivity by holding high yield as their main breeding goal whilst increasing the use of high input agriculture conditions (Pingali, 2012). Notwithstanding, by 2023, world rice production needs to be increased to meet the increasing world population demand (Foley *et al*., 2011). Much of this increase will need to come from rice cultivars that have been improved. Considering rice's contribution to over fifty percent of the world's population as a staple (Fuagawa & Ziska, 2019) and its increasing consumption rate (Fig 4 and 5), numerous studies have indicated that by 2030, production must increase by about 40% to meet this increasing demand (Khush, 2005). This increase in production must take place within the current happenings of our day which include worsening climatic conditions and its attendant consequences of harsher biotic and abiotic stresses to crop production. More so, as the frequency and intensity of hydrology variations become unpredictable with Climate change, drought stress will pose a great threat to agriculture, especially in developing countries (Turral *et al*., 2011). Drought stress- constrained rice production will causes enormous economic losses and huge food security issues. With this growing concern, drought stress tolerance in rice is gaining a wider appeal (Panda *et al*., 2021). Rice varieties that yield high and are tolerant to biotic and abiotic stresses, need to be produced to help meet increasing rice demand (Dar *et al*., 2021).

Panda et al. (2021) further argued that the growing demands for increased food productivity due to this climate change made it imperative for future genetic improvement efforts to be geared towards developing drought tolerant rice cultivars. Their claims are supported by Surendran *et al*. (2021). Panda et al. (2021) however emphasized that due to the multivariate nature and multi-genic traits that govern drought tolerance, breeding for it will be quite challenging.

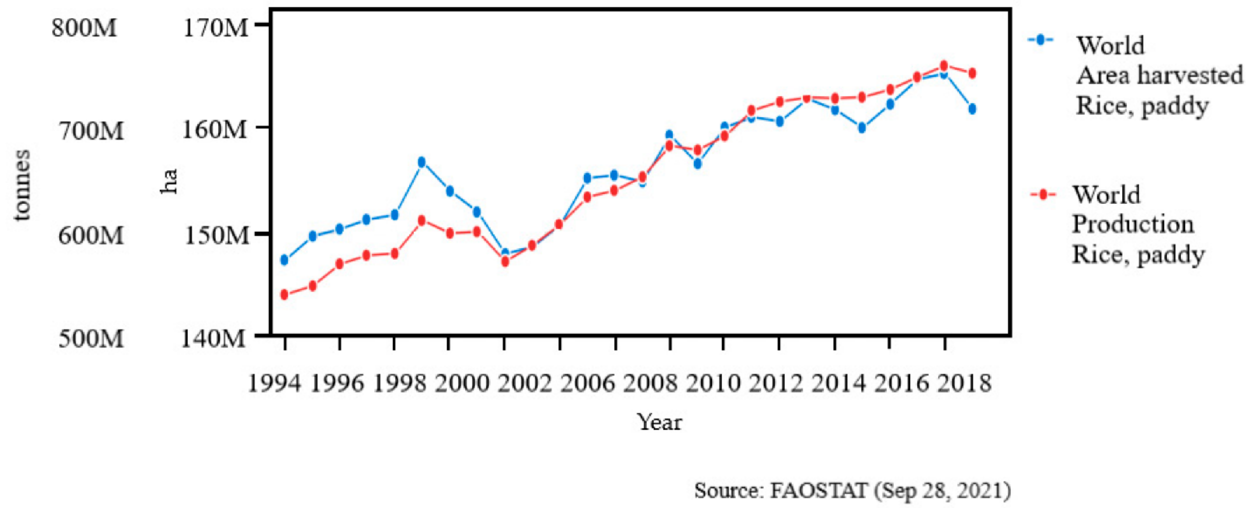

Fig. 4: World Production/yield quantities of rice paddy in the world (1994–2019)

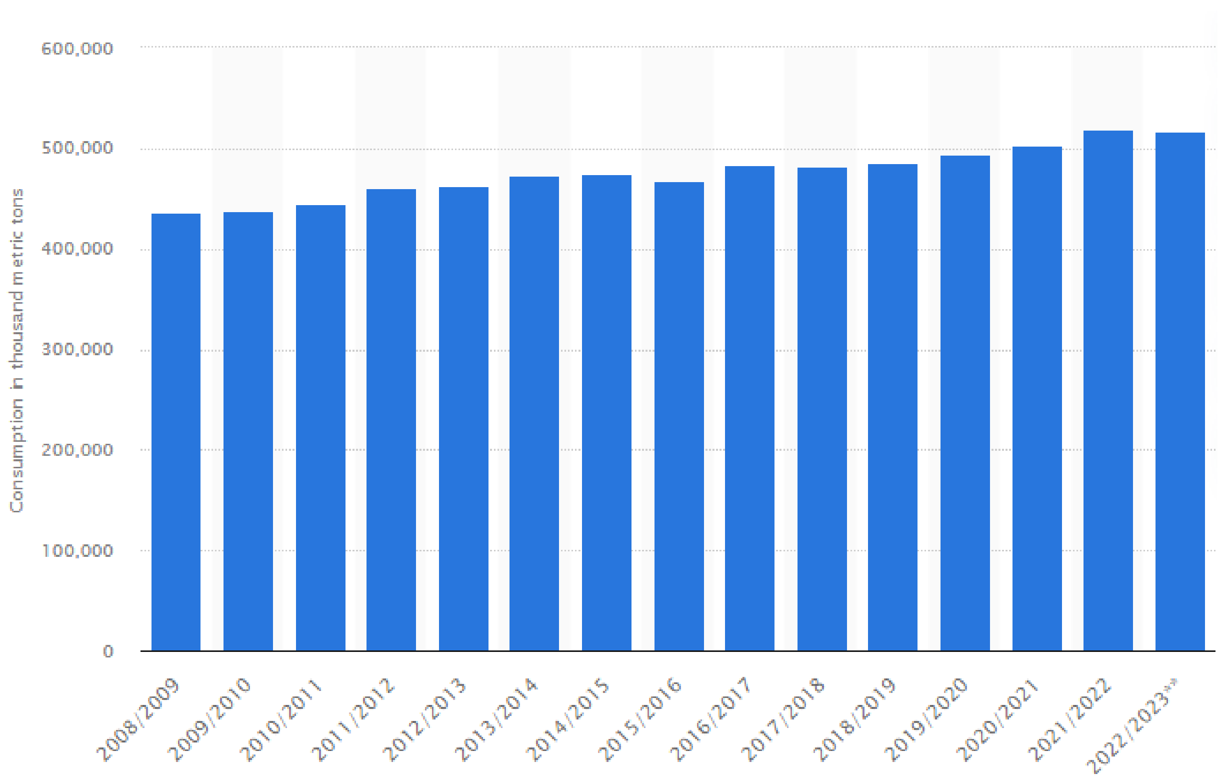

Fig. 5: Total rice consumption worldwide from 2008/2009 to 2022/2023 (in 1,000 metric tons) (Source: Statista, 2023)

2.4.1 Rice breeding under Abiotic constraints

Abiotic stress has been identified as one of the main causes of crop losses all over the world, and crop production losses could drop to about 50% due to abiotic stress (Ashraf *et al*., 2008; Hernández-Soto *et al*., 2021). Breeders face challenges in finding numerous rice varieties resistant to biotic and abiotic stresses (Herna *et al*., 2021). Li *et al*. (2013) noted that modern semi-dwarf rice cultivars have hardly achieved their yield potentials in farmers' fields due to many abiotic and biotic stresses. Also, advent of droughts, increased temperature extremes, storms, floods, increasing population and urbanization, as well as the increasing frequency of temperature extremes, continue to be major blockades to sustaining food security (Qian *et al*., 2016; Zeng *et al*., 2017). These shift us to the inclination that future rice breeding would need breeders to improve many traits and not just high yield potential and desirable quality (Li *et al*, 2013). Developing high-yielding, climate-resilient, and high-quality rice varieties is therefore central. (Zeng *et al*., 2019).

Breeding strategies in times past have included selection, hybridization, soma-clonal variation and, mutation induction with either chemical or physical agents. In recent times, the advent of genome editing tools, genome sequences, efficient tissue culture, and transformation approaches has been recommended as one that could ease the rice breeding process (Hernández-Soto *et al*., 2021). With natural or induced mutagenesis, several techniques exist for rice breeding; mutation breeding, tissue culture, and new breeding methods (CRISPR mutagenesis, base editing, and prime editing (Hernández-Soto *et al*., 2021).

2.4.2 Methods of Rice breeding

Rice is a self-pollinated crop and there exist numerous breeding methods employed to develop new varieties. Namely among them are the pedigree, bulk, modified bulk, single seed descent (SSD)/ Rapid Generation Advance (RGA) and doubled haploid (DH) technique. The most famous method employed in breeding programmes is the Pedigree method (Lenaerts *et al.*, 2019) followed by the bulk breeding method (Collard *et al*., 2017) and then after the RGA method (Lenaerts *et al.*, 2019). Details on the use of rice breeding methods have been scantily reported and this may be due to the fact that breeders hardly publish results from their breeding programmes (Bertrand *et al*., 2017). The cost of breeding programmes is usually high and sometimes complex, hence varied methods may be picked over the other (Collard *et al*., 2017). Lenaerts *et al.* (2019) in a meta-analysis of recent rice breeding impact assessments concluded that shorter breeding cycles helped to immensely save cost in a breeding programme. Hernández-Soto *et al*. (2021) noted that efficacious tissue culture and transformation approaches could significantly speed up the rice breeding process. One of such transformation approaches is the use of the Double Haploid technique particularly anther culture which can be used to produce fixed or homozygous crop lines in a short time (Gomez-Pando *et al*., 2009; Fazaa *et al*.,2016) and also screen for useful traits which we could not attained with normal conventional methods (Pauk *et al*.,2009).

2.5 Rice production constraints in Ghana and Hungary

2.5.1 Constraints in Ghana

Rice is amongst the most consumed staples in Ghana. Though Ghana has a lot of potential for producing rice for domestic use, the average amount produced only covers around half of the needs of the nation (Bissah *et al*., 2022). The average annual consumption growth rate is 8.1% (SRID, 2015) and due to increasing population and urbanization the consumption rate of rice is tipped to continue rising. Although demand for rice is growing, the country's rice output is not able to keep up (Oteng, 2017; SRID-MoFA, 2015) and there have been several reasons attributed to this phenomenon (See Table 1).

Table 1: Rice production constraints faced by farmers in Ghana (Source: Asante *et al*., 2013).

Production constraint	**Rank**
Lack of credit	1
Lack of market for local rice	2
High cost of inputs, fertilizers, agrochemicals	3
Lack of varieties that compete with imported rice	4
Poor yielding variety	5
Lack of farm machinery, plough, power tillers, harvesters	6
High cost of labour	7
Diseases-RYMV, Blast	8
Abiotic stress (fertility, flooding, iron toxicity)	9
Pests including weeds and birds	10
Low profitability	11
Land tenure problems	12

Ghana is a net importer of rice (SRID, 2015) as only 57.14% of the rice consumed in Ghana is produced in Ghana. Some of the reasons for this observation has been captured by Asante *et al*., (2013). Although poor yielding varieties and lack of varieties that compete with foreign varieties may play a key role, in Ghana, credit for upscaling production to the standard stands as the number one reason upon which other issues stem from.

In Ghana, rice production is mainly rain-fed though there exist irrigated production systems. The irrigated system is less popular due to limited access to water resources and infrastructure for such production. Very recently, due to the unpredictable rainfall pattern being experienced all over the world today, there have been efforts to promote and expand irrigated rice production in Ghana (for instance the Alternate Wetting and Drying (AWD) technology as a climate-smart production technique (Obido, 2023).

2.5.2 Production Constraints in Hungary

In Hungary, rice is not a major crop farmed as more production is geared towards the cultivation of wheat, maize and sunflower. Simon-Kiss (2001) mentioned that the reduction in rice area and decreased financial support to rice research served as a deterrent to improved research and increased production. Also, the continental climate location of Hungary (giving rise to cold and blast damages) initially hampered profitable production (Gombos & Simon-Kiss, 2008).

FAO (2004) report on rice production in Europe tabled the major constraints facing rice production in Europe as low temperature, water scarcity, biotic stresses, grain quality, high production cost and environmental concerns. Jancsó (2011) noted that, drought tolerance was an important rice breeding trait especially in rain-fed systems since plants needed to manage with water scarcity once in a while. This trait he mentioned was also needed in the temperate zone such as Hungary where aerobic rice growing system was being practiced and success in this growing system depended on varieties that were drought tolerant, provided uniform stands, able to manage weeds and irrigation set to the crop water needs in the midst of reducing available resources to agriculture water (Jancsó, 2011).

Courtois et al. (2012) and Sulmon et al. (2015), added that, the temperate climate especially hindered isolating and selecting for drought tolerance. Jancsó et al. (2017) opined that the yield of rice could dwindle even with the least sensing of stress environment. They added that the temperate location of Hungary made it difficult to adapt to tropical aerobic rice varieties due to sensitivity in photoperiod, duration and sensitivity to cold (Jancsó *et al*.,2017). Also, considering the geographical location of Europe, fruitful rice cultivation required right seed sowing time, tolerance to cold and satisfying these conditions was still a major production constraint. Simon-Kiss (2001) mentioned that more improved rice genotypes and testing of breeding lines from abroad were needed to uphold rice production in the temperate region.

2.6 Correlations in studied Parameters

Since selecting for drought tolerant rice lines was a very complex process, there is the need to sometimes see if there exist correlation between studied parameters that lead us in our final Selection.

2.6.1 Seed priming on germination and Growth Performance traits

Seed-priming has been considered as one of the methods that boost germination Parameters. Polyethylene Glycol (PEG) solution is a chemical solution that simulates drought and environmental stress condition in plants as would have existed on the field. Polyethyelene Glycol (PEG) treatment has been used as an osmo-prime to select for homozygous DH rice lines for production (Gosal & Wani, 2018). In the DSR technique, osmopriming has been found to have a positive correlation with field germination stand number (Farooq *et al*., 2006). In some cases there has been a positive correlation between primed seeds and shoot and root length average (Raees, *et al*., 2022).

Research by Sagar *et al*.(2020) highlighted that PEG- induced drought stress of five rice genotypes at early seedling stage revealed a steady decrease in growth and growth related parameters values as concentration increased from 0%-15% Polyethylene glycol 6000 (PEG-6000). From Literature, several limits of PEG had been stated to drastically affect germination of rice from 15% mM to 25% mM. There had been no clear point PEG concentration stated. Germination in Nembo variety from previous research had been linked to be hindered in PEG concentration from 15% mM to 25% mM but the exact concentration was unknown.

2.6.2 Germination Quality Parameters; Median germination time and final Germination Time

Median germination time (T50) and Final germination percentage (FGP) are parameters used to evaluate seed germination quality and vigour. T50 is the average amount of time it takes for 50%

of the potential seeds that would have germinated for that seed lot to germinate, whilst FGP is the proportion of seeds that eventually germinate after a given period of time.

There could exist an association between T50 and FGP, but it is not always direct. Generally, seeds with a shorter T50 tend to have a higher FGP (Fayaz *et al*., 2022), especially when osmoprimed (Mamun *et al*., 2018) as they are able to germinate rapidly and thus have a better chance of growing into healthy plants. Nevertheless, other factors such as seed quality, environment are potential factors could affect FGP.

2.6.3 Plant height and yield

One of rice's complicated parameter is yield. It is affected by both direct and indirect traits (Huang *et al*., 2013) and plant height is one of the indirect traits that influence yield. Li *et al* (2019) also found the influence of height on yield to be dependent on the ecotype of rice' in some cases giving a positive correlation yet in others, the converse was true.

3. MATERIAL AND METHODS

3.1. Experimental Site

The Research experimental site took place at two main places of the Research Center for Irrigation and Water Management (ÖVKI), Institute of Environmental Sciences (IES), Hungarian University of Agriculture and Life Sciences (MATE). There was an on-field research at the MATE ÖVKI Lysimeter Research Station in Szarvas (46,8629104, 20,5268078) followed by a lab experimental setup at the MATE ÖVKI Galambos Rice Research Station (46,8710571, 20,5271307). The exact location for the research are shown with their coordinates by the digital map (See Fig. 6)

In the on-field research, regeneration and multiplication of DH lines took place together with the measurement of some stress indices and other agronomic data. In the lab experimental setup, the screening of samples from the on field regenerated DH lines took place (See Fig 7).

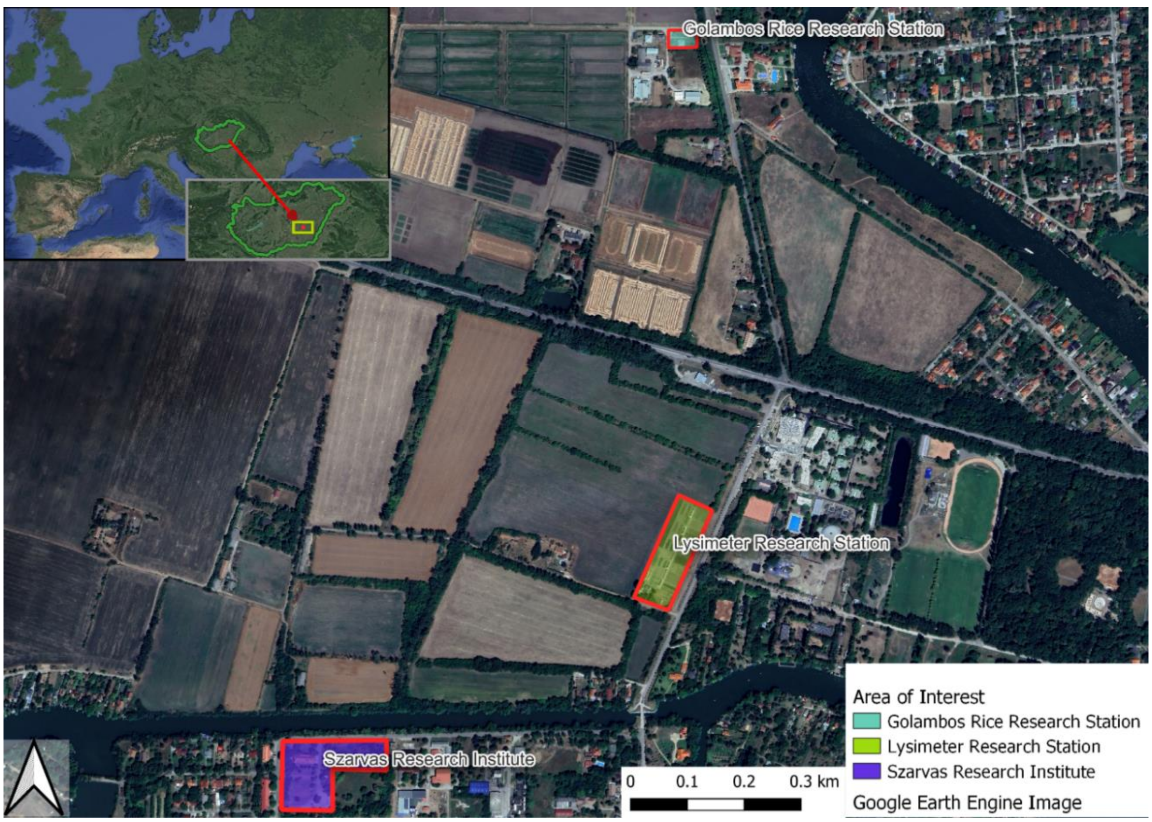

Fig. 6: A digital map showing the experimental sites for the rice research.

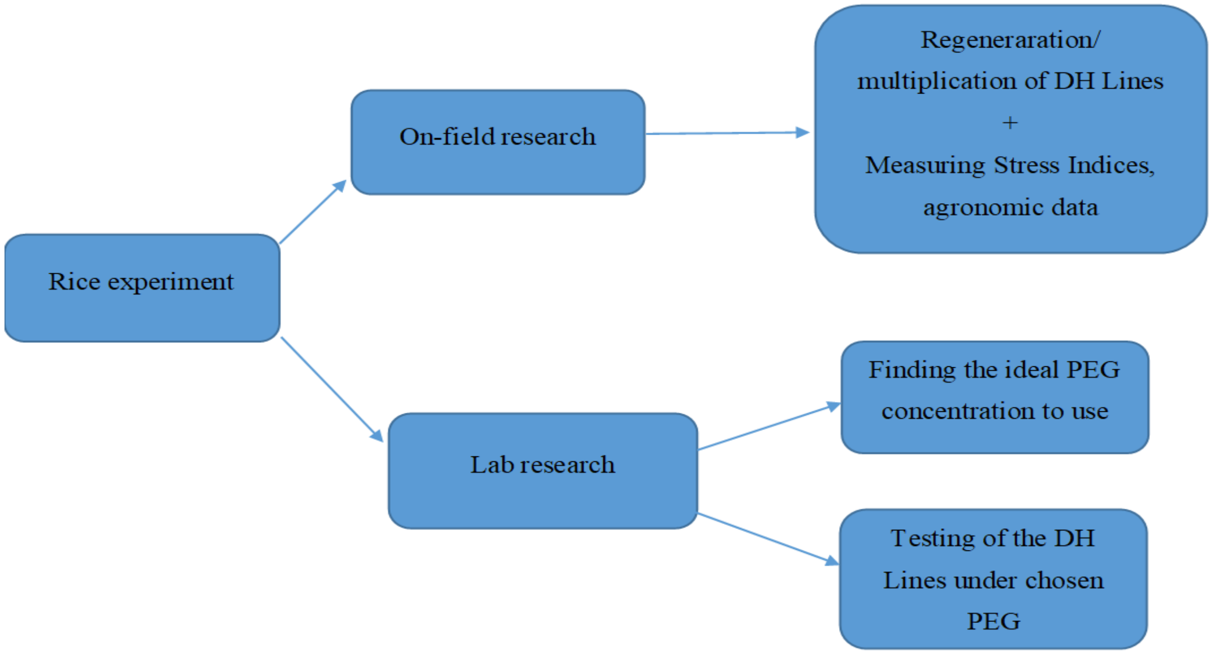

Fig. 7: Diagrammatic representation of present study framework

3.2 Rice seeds used for the experiment

The rice seeds used for the experimentation were Double haploid (DH) seeds obtained from the Cereal Research Non-profit Company in Szeged through a cross between Da'ma (a Hungarian rice variety referred also as HSC1) and Irat 109 (a rice variety from Ivory Coast). These DH lines were generated through tissue culture techniques. The parental rice Lines of the breeding project; Da'ma, Irat 109 and Marilla were obtained from the MATE ÖVKI served as control checks too. An additional rice variety, Nembo (Italian variety) was also used in the germination experiments.

3.2.1 Measuring on-field drought stress characteristics and yield parameters of 20 DH rice Lines

The twenty DH Lines were regenerated (sown on 18th May 2022) on the field using (Randomized Complete Block Design (RCBD) experimental design with four (4) repetitions for each DH rice Line (See Appendix 1). The field was divided into two with two major treatments; well-watered (Normal irrigation field) and Less watered (water irrigation shortage field). The difference between these two treatments was in the irrigation at flowering stage. Well-watered received irrigation from the drip lines for two weeks (60 mm) whereas less watered received no irrigation in that 2-week interval. Also each treatment has 2 repetitions of rice lines (Fig. 8). All other necessary agronomic practices were followed for growth and development of rice. Meteorological data linked with the lysimeter system at the site provided as with Climate data (Agromet Solar, Boreas Ltd., Érd, Hungary) and Evapotranspiration with which we used to time our irrigation accordingly in the aerobic rice production system.

On **14th August**, the Spectrophotometer (CI-710s SpectraVue Leaf Spectrometer, CID Biosciences, USA) was used to measure stress indices. Six stress indices were measured; IAD, CCI, CRI, CNDVI, PRI, and DCNI. For each rep of DH rice line, 10 measurements were taken for the Spectrophotometer.

On **18th October**, Using a meter rule, Plant Height data was measured. For each rep of DH rice line, five measurements were taken. Each treatment plot (either well-watered or drought) had 2 reps values for height. Average height calculated for each rice line. .

On **25th November**, rice was milled (using Wintersteiger LD 350 laboratory threshing machine, Wintersteiger Ltd., Austria) and rough rice weight (milled rice with husk) calculated. Each treatment plot (well-watered or drought treatment) had 2 reps for milled weight and the average milled weight calculated for each DH Line.

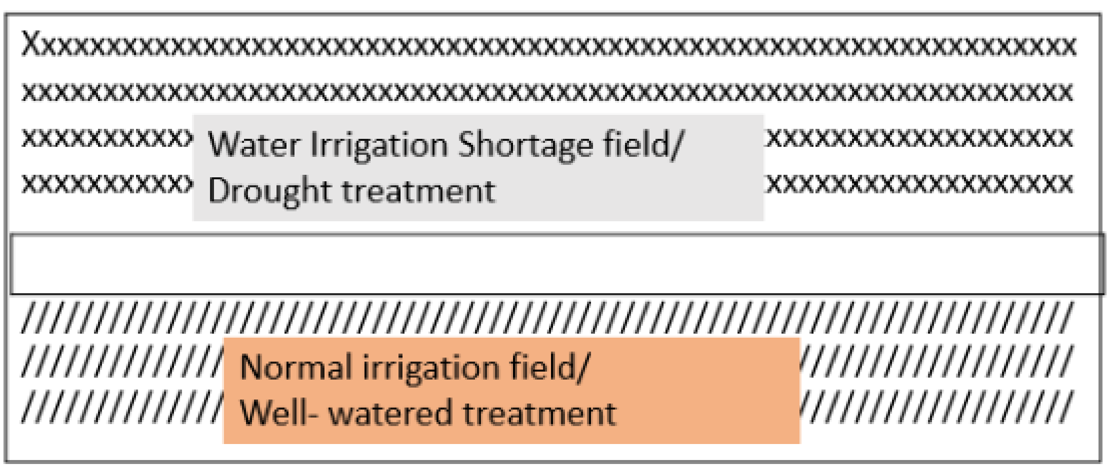

Fig 8: Rice Experimental Plot showing the induction of drought treatments.

3.3 Finding the limiting Polyethylene Glycol Concentration to use for screening the twenty rice lines in vitro.

Concentration of 0% mM (0g PEG/70 ml water), 5 % mM (3.5g PEG/66.5 ml water), 10% mM (7 g PEG/63 ml), 15% mM (10.5g PEG/ 59.5 ml water), 20% mM (14g PEG/56 ml water) and 25% mM (17.59PEG/52.5 ml water) PEG solutions were prepared and tested with rice cultivars to find the ideal PEG concentration to use for screening DH Lines. Two rice varieties were used; Nembo and Da'ma. Dama variety was our drought tolerant standard check in the experiment. Water treatment was set up as our 0% mM control. Forty seeds/ petri dish was used. Complete randomized Design (CRD) was the experimental design used with 3 repetitions for each rice line. This experiment took place in a growth climate chamber (KWBF 720, Binder Ltd., Germany) (See Fig. 9 and 10) at the MATE ÖVKI Galambos Rice Research Station (see Fig 6).

3.3.1 Screening the 20 lines under the limiting PEG concentration in vitro.

Twenty DH Lines were tested for their germination tolerance in 20% mM PEG (14g PEG/56 ml water) solution in the same growth Climate chamber as referred in 3.3 (see Fig 9 and 10) at 80% relative humidity and alternating temperatures cycles of 25°C and 30°C. The parental Lines; Da'ma, Irat 109 and Marilla were used as Control checks. Two main treatments existed; water treatment (control treatment) and 20% mM PEG treatment for the DH Lines for 40 seeds/petri dish/3 replications in a Completely Randomized Design (CRD) for 14 days. Periodic data was taken on germination count of DH Lines every 12 hours. Standard Germination Parameters; Median Germination time (MGT), Final Germination Percentage (FGP), Average shoot Length, Average radicle Length were calculated at the end of 14 days. Number of seeds that had germinated each day was recorded and a cumulative germination count reading was taken until there was no increase in cumulative germination count. Below were the formulas used in calculations:

Median germination time (MGT): time (in days) for 50% of germination:

MGT=ti+(N/2−ni)−(tj−ti)/(nj−ni),MGT=ti+(N/2−ni)−(tj−ti)/(nj−ni)

Where, N is the final number of germinated or emerged seeds and nj and ni are the cumulative number of seeds germinated by adjacent counts at times tj (day) and ti, (day) respectively, when ni < N/2 < nj.

Final Germination Percentage (FGP)

FGP= ((total number of seeds germinated/total number of seeds plated)*100)

Fig. 9: Rice seeds in Petri dish in growth Climate Chamber under Completely Randomized Design.

Fig. 10: Setting up temperature and Humidity for Germination Climate Chamber.

Fig. 11: DH Rice Line ready for Root Length and Shoot Length measurement.

3.4 Analysis of Data

The IBM SPSS V 23 software package was used to statistically evaluate our results. A one way T- test at 0.05 level of Significance was used to compare the means of rep average for height and milled weight data from the well-watered and less watered treatments on the field.

For some Parameters, graphs and simple arithmetic calculation values provided a means for some evaluation analysis of results.

Values for stress indices parameters (IAD, CCI, CRI, CNDVI, PRI and DCNI) were extracted from the Spectrophotometer. Values from well-watered treatment and drought treatment were subjected to ANOVA module from the IBM SPSS software at 0.05 level of significance. Means that were significantly different were separated with Tukey HSD ($p<0.05$).

4. RESULTS & DISCUSSION

This part assembles the main results, analyses and discussion from the various subsets of the conducted experiments:

4.1 From measuring on-field drought stress characteristics and yield parameters of 20 DH rice Lines.

4.1.1 Yield Parameters

One sample T-Test revealed significant differences in milled weight between well-watered treatment and Drought treatment ($p<0.05$)(see Appendix 2). As such these two treatments provided significant differences in the 20DH Lines and their response to yield (milled weight).

Table 2: Milled weight from the 20 DH Lines.

Rice Lines	Well-watered treatment (Average)(g)	Drought treatment (Average)(g)	Average for all treatments (g)
1 20	36.79	17.7	38.12
1 28	35.65	16.5	24.3
1 31	35.84	33.75	28.2
2 22	36.62	23.8	22.05
2 40	37.64	22.65	27.2
2 35	38.03	20.85	36.6
3 8	37.01	4.95	16.5
3 30	37.70	13.25	13.45
3 57	39.70	3.9	27.05
4 3	38.75	14.7	38.35
4 43	36.43	12.55	15.025
4 60	38.53	16.85	41.325
6 26	35.12	0.9	6.65
6 33	38.3	4.5	14.625
6 46	40.64	13.45	23.375
6 49	42.11	0.8	23.325
7 70	41.17	4	16.325
8 55	45.35	5.55	30.175
8 33	40.62	5.15	28.075
8 40	30.25	6.35	18.3

In well-watered treatment, the 5 top performing lines were 8 55(45.35g), 6 49(42.11g), 7 70(41.17g), 6 46(4.64g) and 8 33(40.62g). In drought treatment though, the order for 5 top performing lines took a different turn; **1 31(33.75g) was followed by 2 40 (22.65g), 2 35 (20.85g), and 1 20 (17.7g).** Averagely, when all treatments were compared for each DH line, the best performing DH lines for yield were 4 60, 4 3, 1 20, 2 35 and 8 55 and the poor performing lines were 6 26, 3 30, 6 33, 4 43 and 7 70 (see table 2).

Our results showing huge disparity in milled weight for well-watered and drought tolerant treatment is consistent with findings from literature and supports the work of Surendran et al. (2021). In their research, they mentioned that water stress at certain critical stages of growth (for instance at the active tillering) in rice could hamper development. The active tillering stage was a critical stage in rice growth where deprivation of water had serious consequences on final growth and development characteristics. In our experiment the two week deprivation of water (in the drought treatment) was during the active tillering stage of rice and although final yield could not be seen then, this brief period of drought had consequently affected number of tillers borne per plant and subsequently in the total milled weight for that rice line. Also, although all 20 DH lines in drought conditions were affected by the water stress period, **DH Line 1 31** recorded **weight (33.75g) in drought treatment that were almost comparable to well-watered treatment (35.84g)**. **The lines 2 22, 2 40 and 2 35** also recorded greater than 20 g (>20g) milled weight when some of the DH lines scored as low as 3.9g. With these marked differences **1 31, 2 22, 2 40 and 2 35** should be earmarked as lines that could be further studied for their drought tolerance ability.

One sample T-Test revealed significant differences in Height between well-watered treatment and Drought treatment ($p<0.05$) (see Appendix 3). These two treatments provided significant differences in the 20DH Lines and their response to height. In well-watered treatment, the 5 top performing lines were 1 20(76.5g), 6 49(73.5g), 8 40(66g), 6 26(64.6g) and 6 33(63.3g). In drought treatment though, the order for 5 top performing lines took a different turn; 1 20(70.5g) was followed by 6 33(58.4g), 8 40(57.7g), 2 22(57.5g), and 4 3(56.1g). Averagely, when all treatments were compared for each DH line, the **best performing DH lines for yield** were **1 20, 8 40, 6 33, 6 49 and 3 57** and the poor performing lines were 1 31, 8 33, 4 43, 3 8 and 3 30 (see table 3).

Table 3: Height data for the 20 DH Lines.

Rice Lines	Well-watered treatment (Average)	Drought treatment (Average)	Total Average
1 20	76.5	70.5	73.5
1 28	50	46	48
1 31	38.1	40.1	39.1
2 22	57.8	57.5	57.65
2 40	56.7	51.2	53.95
2 35	55.9	50.4	53.15
3 8	43.1	45.8	44.45
3 30	49.3	44	46.65
3 57	62.7	55.3	59
4 3	61	56.1	58.55
4 43	45.1	40.2	42.65
4 60	60.7	54.8	57.75
6 26	64.6	52.2	58.4
6 33	63.3	58.4	60.85
6 46	51.7	50.5	51.1
6 49	73.5	46.7	60.1
7 70	54.1	42.9	48.5
8 55	53.6	45.9	49.75
8 33	44.9	39	41.95
8 40	66	57.7	61.85

4.1.2 Stress Indices Parameters

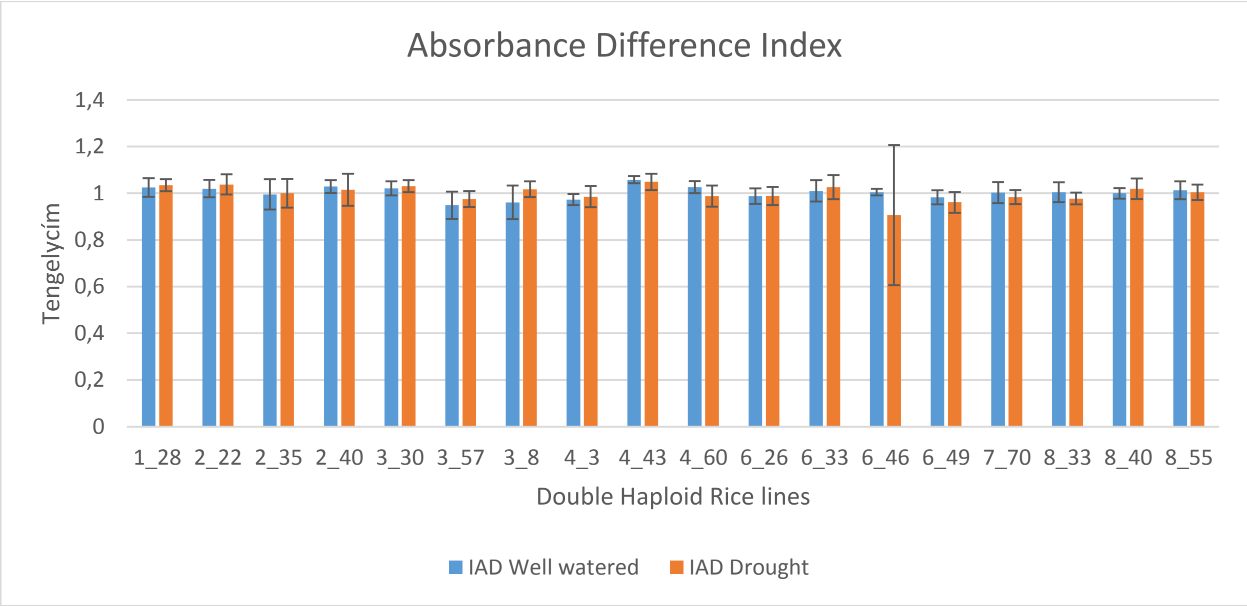

Fig. 12: Bar chart showing the Absorbance difference index between well-watered treatment and drought treatment

ANOVA revealed significant differences in the genotype means (at alpha level of 5%) and Tukey HSD was used to separate the means (See Appendix 4). Means in the same subset were not significantly different from each other. The DH Lines 4 43, 6 33, 1 28, 2 22 and 8 40 showed high IAD values in drought condition than in water. When treatment (well-watered and Drought) effect was compared for the 20 DH Lines at alpha level of 5%, the treatments did not have an effect on the stress parameter IAD. The combined effect of both Genotype and treatment also revealed that both Genotype and treatment interaction did not show significant effect on the stress index IAD. In this parameter, the genotype only had an effect on how it related to the parameter IAD.

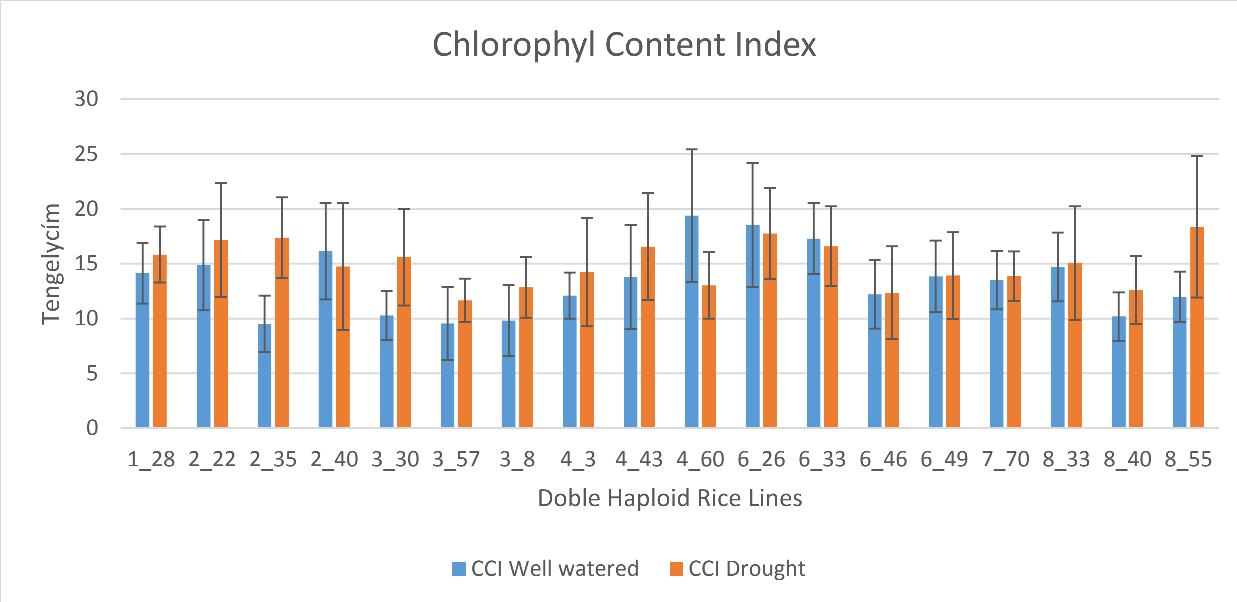

Fig. 13: Bar chart showing the Chlorophyll Content index between well-watered treatment and drought treatment

ANOVA revealed no significant differences in the genotype means (at alpha level of 5%) meaning the genotype did not have an effect on the stress parameter CCI. However, significant differences in means was seen in treatment for 20DH Lines for the stress parameter CCI. Tukey HSD was used to separate the means (See Appendix 5). Means in the same subset were not significantly different from each other. The combined effect of both Genotype and treatment also revealed that both Genotype and treatment interaction showed significant effect on the stress index CCI. The genotypes 6 26, 6 33, 8 33, 2 40 and 1 28 recorded appreciable increases in both water treatment and drought treatment and on CCI.

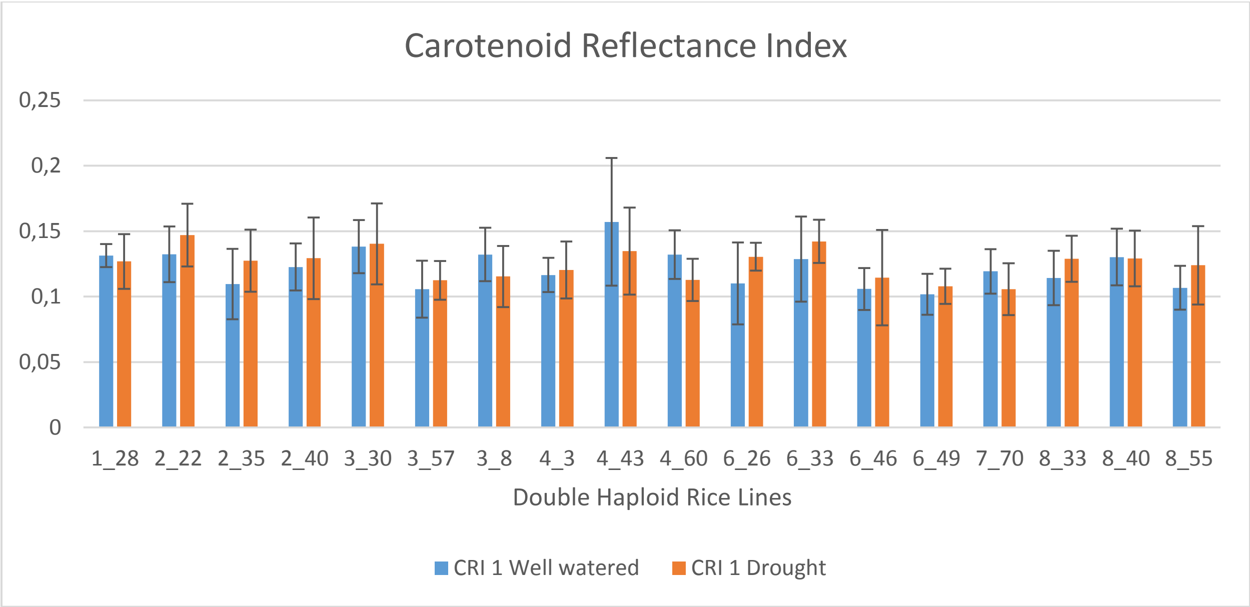

Fig. 14: Bar chart showing the Carotenoid Reflectance index between well-watered treatment and drought treatment

ANOVA revealed significant differences in the genotype means (at alpha level of 5%) on their response to the stress index CRI. Tukey HSD was used to separate the means (See Appendix 6). Means in the same subset were not significantly different from each other. The genotypes 2 22, 6 33, 3 30, 8 33 and 6 26 performed well on CRI. When treatment (well-watered and Drought) effect was compared for the 20 DH Lines at alpha level of 5%, the treatments did not have an effect on the stress parameter CRI. The combined effect of both Genotype and treatment also revealed that both Genotype and treatment interaction did not show significant effect on the stress index CRI.

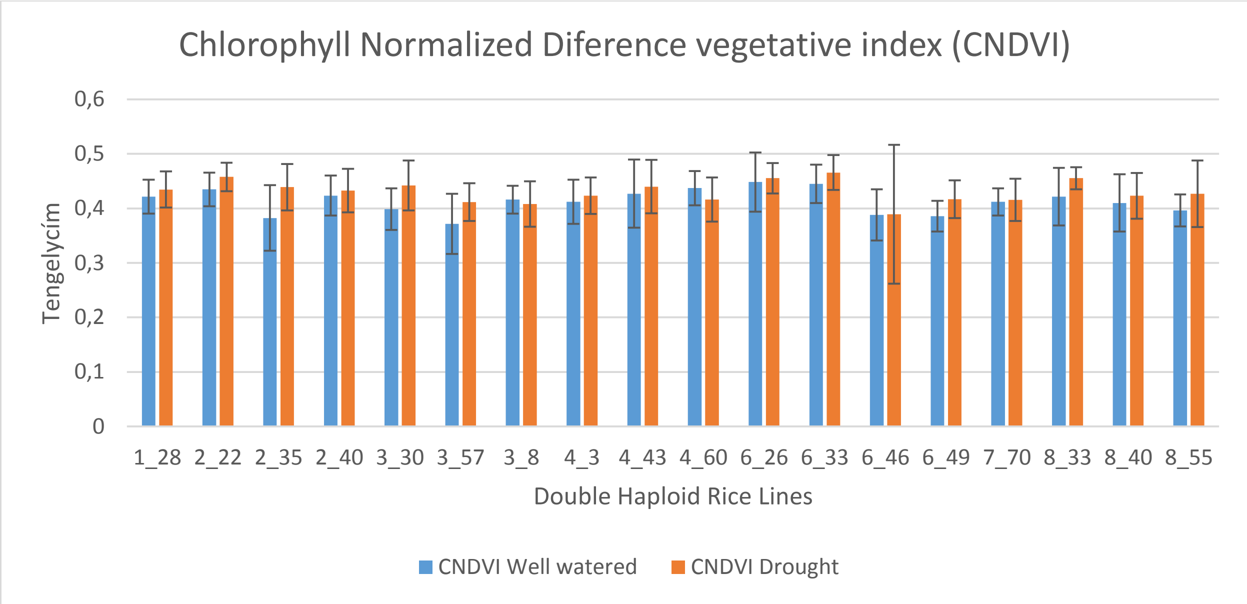

Fig. 15: Bar chart showing the Chlorophyll Normalized Difference Vegetative Index between well-watered treatment and drought treatment

ANOVA revealed significant differences in the genotype means (at alpha level of 5%) on the stress index CNDVI. Tukey HSD was used to separate the means (See Appendix 7). Means in the same subset were not significantly different from each other. When treatment (well-watered and Drought) effect was compared for the 20 DH Lines at alpha level of 5%, the treatments did have an effect on the stress parameter CNDVI. The DH lines 8 33, 6 33, 6 26, 3 30 and 2 20 showed elevated levels of drought stress and this showed on the CNDVI recorded for these lines. The combined effect of both Genotype and treatment however did not show significant effect on the stress index CNDVI.

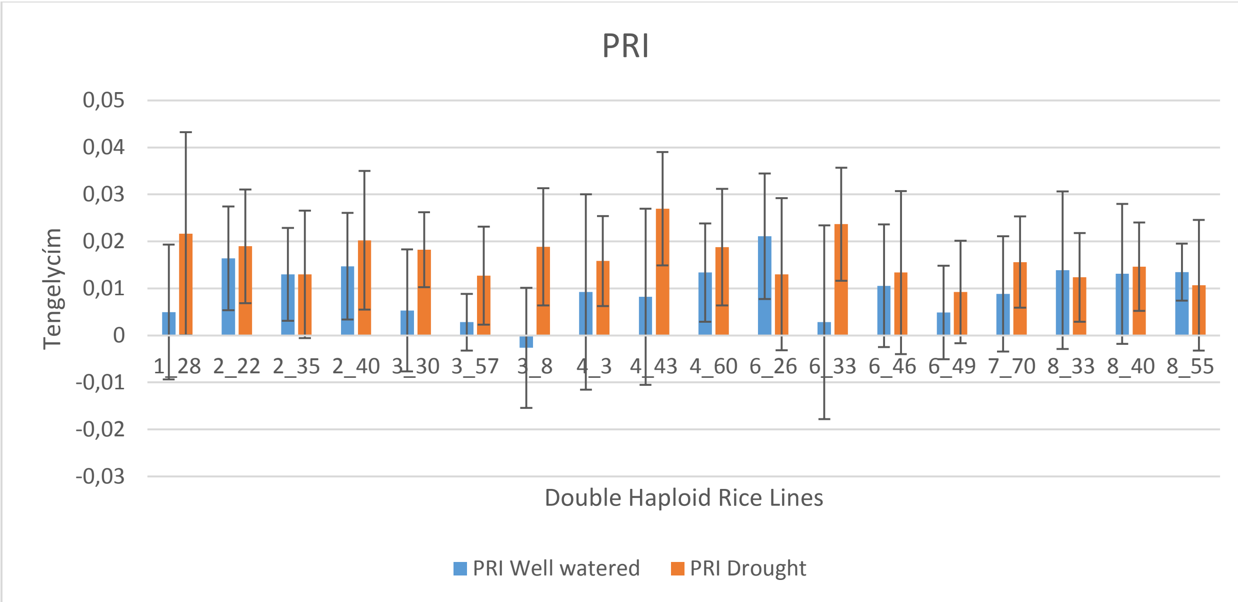

Fig. 16: Bar chart showing the Photochemical Reflectance Index between well-watered treatment and drought treatment

ANOVA revealed no significant differences in the genotype means (at alpha level of 5%) on the stress index PRI. Tukey HSD was used to separate the means (See Appendix 8). Means in the same subset were not significantly different from each other. When treatment (well-watered and Drought) effect was compared for the 20 DH Lines at alpha level of 5%, the treatments did have an effect on the stress parameter PRI. The DH lines 4 43, 6 33, 1 28, 2 40 and 3 30 showed elevated levels of drought stress when compared with well-watered counterparts on the PRI recorded for these lines (see Fig 16). The combined effect of both Genotype and treatment revealed significant effect on the stress index PRI (See Appendix 8).

ANOVA revealed significant differences in the genotype means (at alpha level of 5%) on the stress index DCNI. Tukey HSD was used to separate the means (See Appendix 9). Means in the same subset were not significantly different from each other. When treatment (well-watered and Drought) effect was compared for the 20 DH Lines at alpha level of 5%, the treatments did have an effect on the stress parameter DCNI.

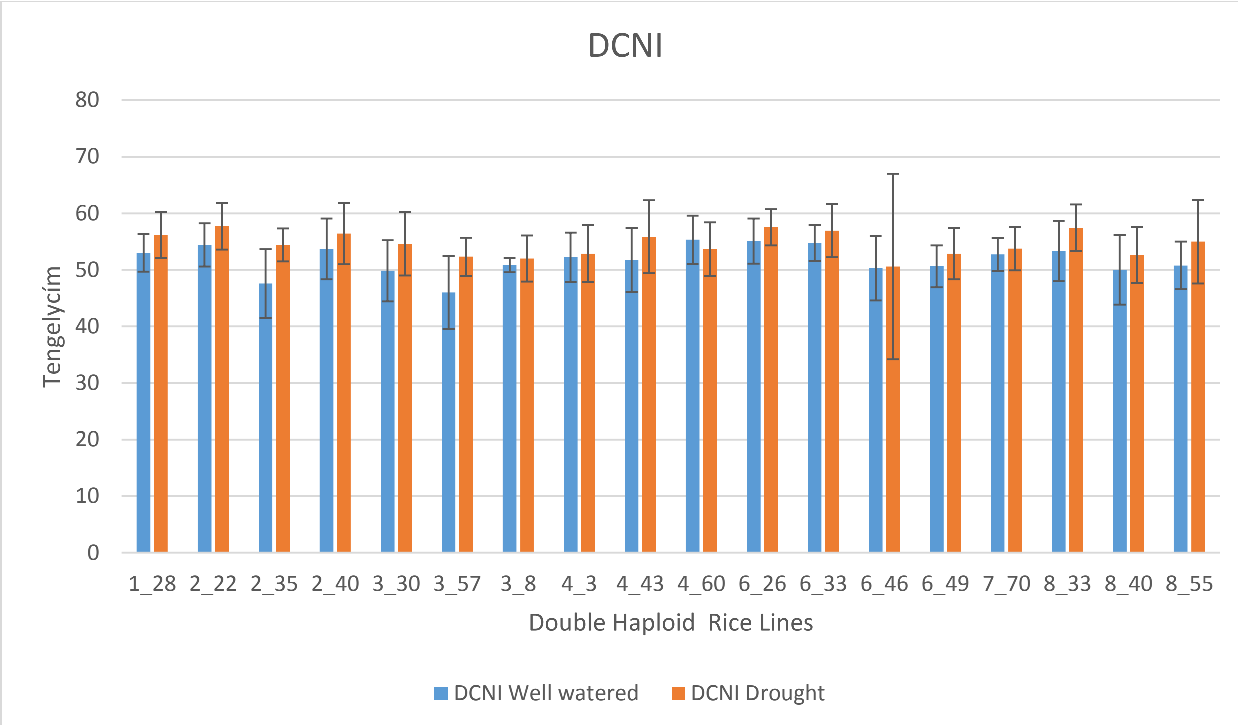

Fig. 17: Bar chart showing the Double Peak Canopy Nitrogen Index between well-watered treatment and drought treatment

The DH lines 6 26, 2 22, 8 33, 6 33 and 1 28 showed elevated levels of drought stress when compared with well-watered counterparts on the DCNI recorded for these lines (see Fig 17). However when the combined effect of both Genotype and treatment on DCNI, no significant effect on the stress index DCNI (See Appendix 9)

4.2.1 Finding the limiting Polyethylene Glycol Concentration to use for screening the twenty rice lines in vitro

At the end of the first lab experiment 20% mM concentration of PEG was selected as the ideal concentration for screening the DH Lines.

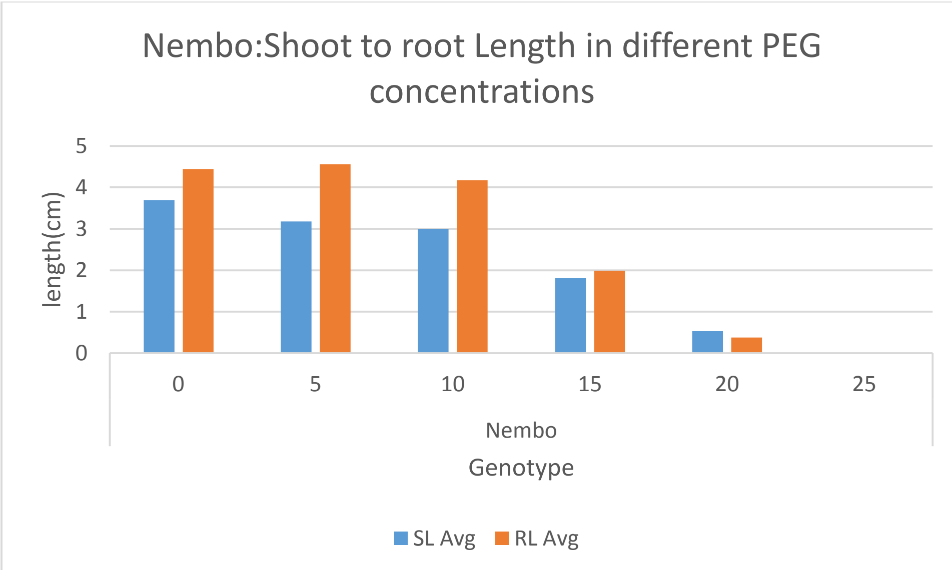

Fig 18: Bar chart showing shoot to root length ratio in Nembo variety

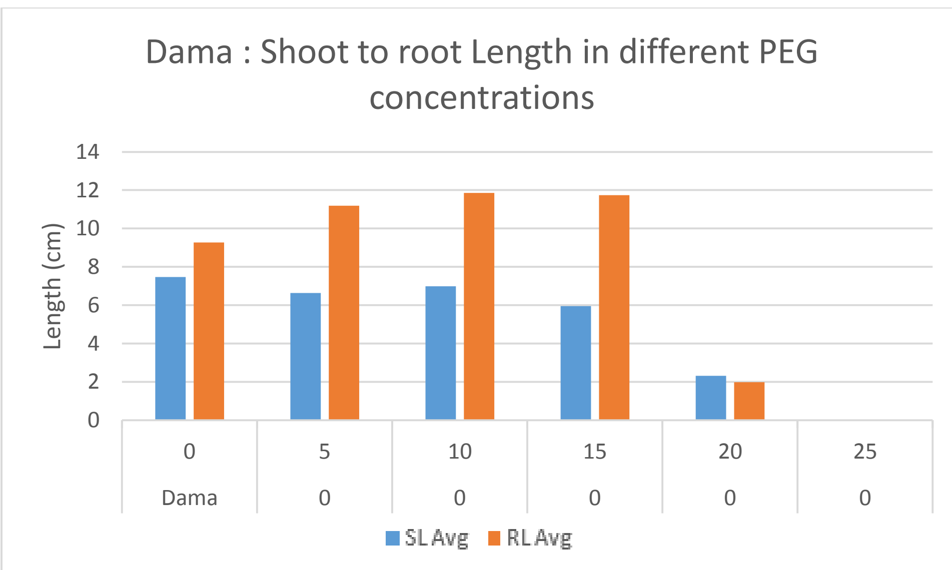

Fig 19: Bar chart showing shoot to root length ratio in Da’ma

Fig. 20: Performance of rice lines under different concentrations of PEG.

Comparison of shoot and root length in parentals Da'ma and Nembo revealed that as PEG concentration increased, germination number, shoot length, and root length decreased (See Fig 18, 19, 20). This result was consistent with findings in literature (Vibhuti *et al.*, 2015) on the effect of drought stress in reducing seed germination and seedling growth. Our results also supported the work of Sagar et al. (2020) where they found that increasing PEG concentrations showed a steady decrease in growth and germination parameters.

15% mM PEG was not ideally selected because a lot of shoot and root growth had been recorded and the length of the shoot and the root was quite long (See Fig 18, 19, 20). 25% mM was not selected because the seeds hardly germinated hence that concentration seemed too harsh to further screen the DH lines on. 20% mM PEG was selected as ideal screening concentration to conduct our DH screening because at the concentration, some growth was seen (though few) for both shoot and root length. The concentration was not too high that it prevented total emergence of shoot and radicle (as was seen in 25% mM) and it was not too low that it easily allowed increased length in shoot and root of seeds (as was seen in 15%). We wanted to see the inner characteristics of the 20 DH lines in how they would respond to drought stress and that inducing factor (in this case the limiting PEG concentration) was to be such as to enable the DH rice lines sense the high drought stress however allow great genotypes among the DH Lines to show their robust resilience characteristics in such drought simulated condition.

4.2.2 Screening of DH Lines at 20% mM PEG at 14 days;

For shoot to length ratio of the germinated seedlings in water treatment from the 20 DH Lines, the three top performing rice lines for total *shoot average* were 4 60, 6 46 and 6 49 whilst the worst performing lines were 4 3, 6 26 and 2 22 in descending order. For total *root average*, the three top performing rice lines were 2 40, 6 4, 3 30 whilst the worst performing lines were 8 33, 2 22 and 4 43 in descending order (See Fig. 21)

For shoot to length ratio of the germinated seedlings in PEG treatment, the three top performing rice lines for total shoot average were 8 40, 3 30 and 1 28 whilst the worst performing lines were 2 22, 8 33 and 1 31 in descending order. For total root average, the three top performing rice lines were 1 28, 8 40, 3 30 whilst the worst performing lines were 2 22, 2 35 and 1 31 in descending order (See Fig. 22). When total root length was compared to the shoot length in PEG, results from our experiment confirmed findings of Wu & Cosgrove (2000) and Raees et al.(2022) where they mentioned that when plants are stressed, on their adaptation measures for longer term survival strategy was to increase their root length. Rice lines in the drought induced PEG conditioned just adapted their survival strategy as they would have done in the soil looking for water by increasing root length whilst decreasing shoot length. Our results was also consistent with Zhu et al.(2020) findings on plant stress adaptation where they mentioned plant reduced photosynthetic activity (in our case, the shoot length which has more photosynthetic pigment so it could develop deeper and stronger root.

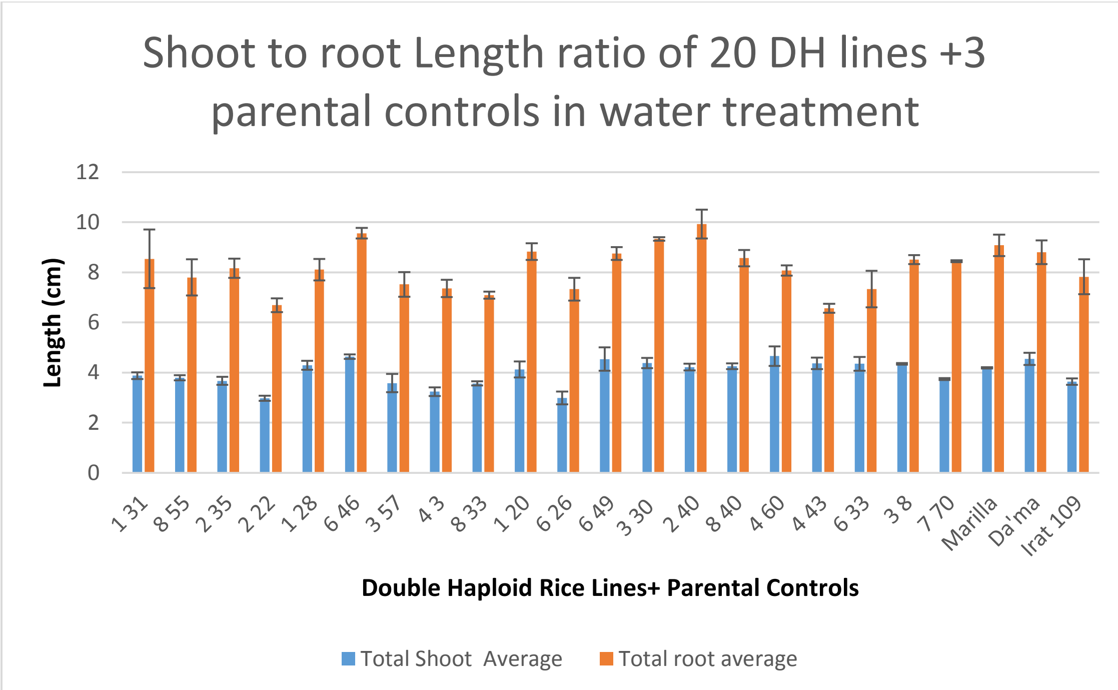

Fig 21: A bar chart showing Shoot to root Length ratio of 20 DH lines +3 parental controls in water treatment

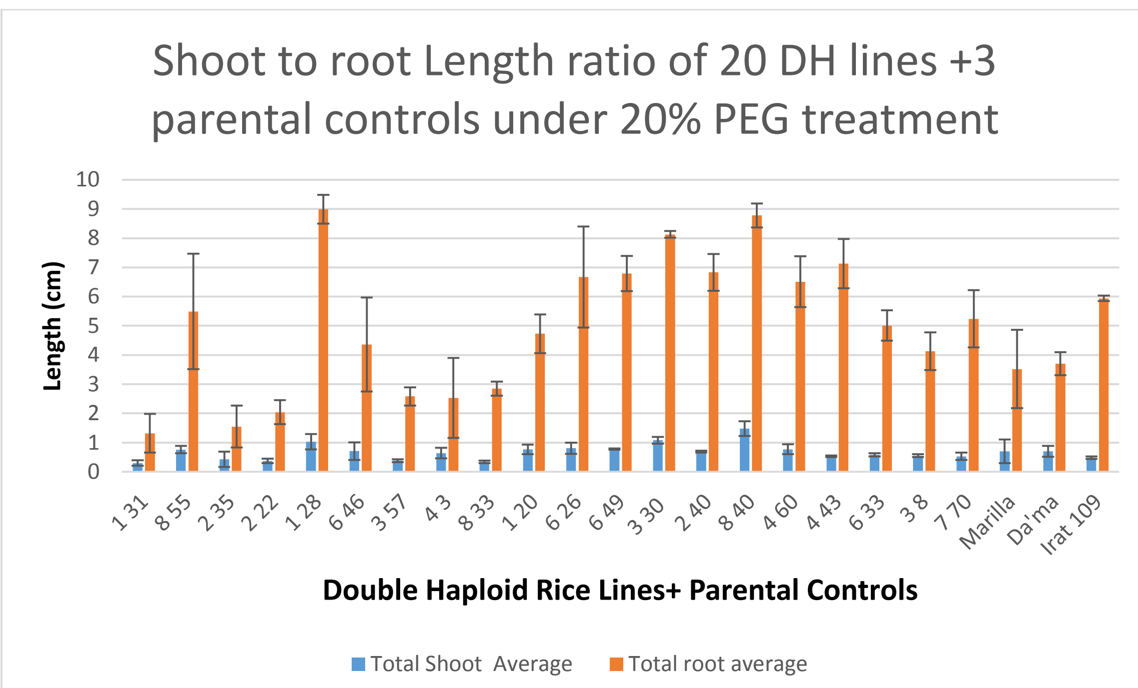

Fig 22: A bar chart showing Shoot to root Length ratio of 20 DH lines +3 parental controls under 20% PEG treatment

Treatments in Water and PEG when compared for the Shoot average Length revealed that **germination in water**, in all cases, showed approximately **a four-fold increase than in PEG**. The *parental lines* Marilla, Dama and Irat *outperformed the rice lines 1 31, 2 35, 2 22, 3 57 and 8 33* in PEG treatment. However, the **shoot length of rice** lines **1 28, 8 40 and 3 30** *outperformed the parental lines in PEG treatment* (see Fig. 23).

For most cases, **total radicle length** in **water treatment surpassed** that recorded *in* **PEG**. The **exception** of this was seen in rice lines **1 28, 4 43 and 2 40.** Here, the converse was true as radicle length showed a slight increase in PEG than in water. These 3 lines also exhibited higher radicle averages in PEG than the parental lines Marilla, Da'ma and Irat (see Fig. 24). In totality, five rice **lines (1 28, 8 40, 3 30, 4 43 and 2 40)** performed *significantly better than the parental Lines* in PEG however **1 28, 8 40 and 4 43** were the lines whose **growth in radicle length in PEG** showed *higher increase both over water treatment and over parental lines.*

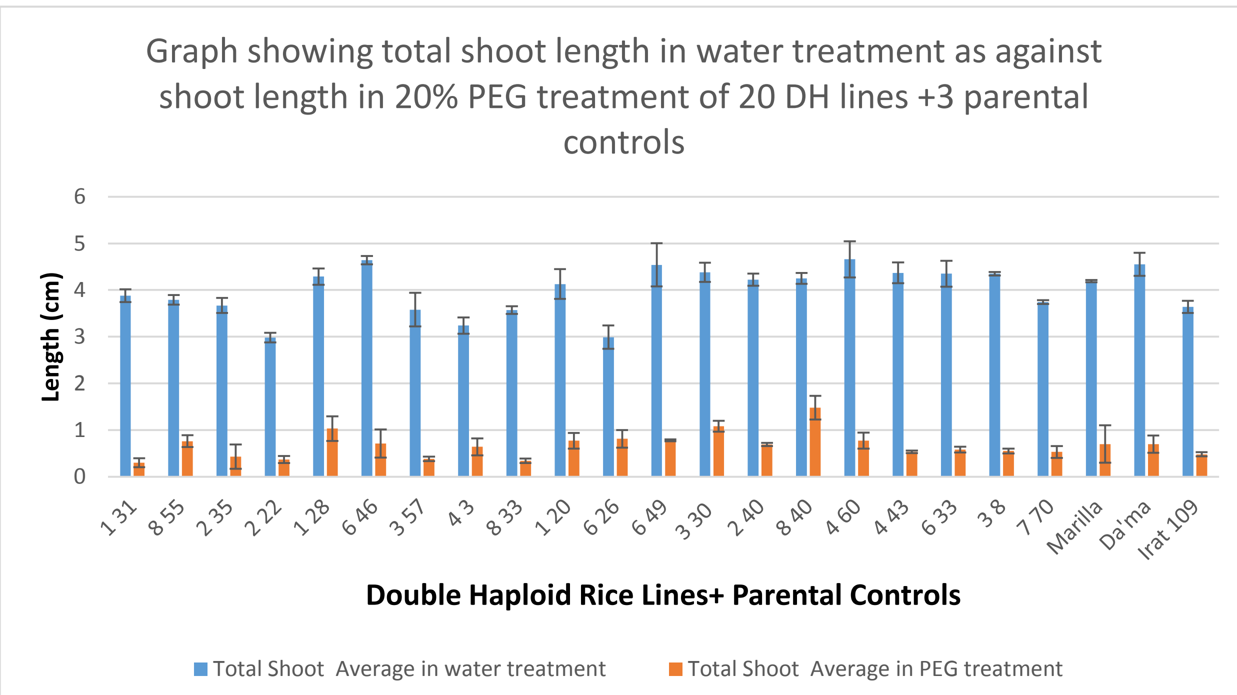

Fig 23: A bar chart showing total shoot length in water treatment as against shoot length in 20% PEG treatment of 20 DH lines +3 parental controls

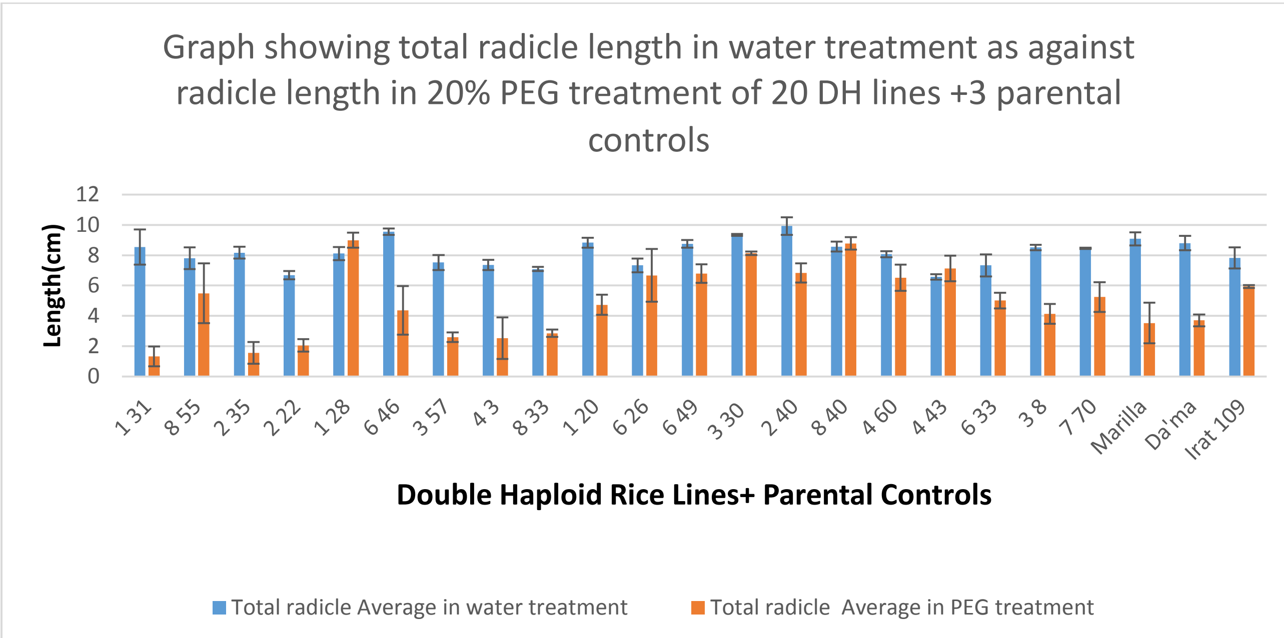

Fig. 24: A bar chart showing total radicle length in water treatment as against radicle length in 20% PEG treatment of 20 DH lines +3 parental controls

4.2.3 More on Germination Parameters: Final Germination Percentage

Final Germination Percentage for almost all 20 DH lines (95%) in water treatment performed very well (>90%) however with the exception of DH line 3 30, Parental line Irat 109 and Marilla, this trend was not the case in PEG treatment as greater than 90 % of DH Lines recorded FGP below 90%. (See Table 4). Nonetheless, **1 20(86.7%), 3 30 (94.2%), 8 40(89.2%)** *performed creditably well* **in PEG treatment.** These lines also outperformed the Parental line Da'ma in PEG. The DH lines 3 57(29.2%), 3 8(29.2%) and 6 46(24.2%) performed abysmally in PEG treatment although in water treatment their FGP were very good (87.5%. 90.8% and 90% respectively) (see Table 4)

When FGP ratio was compared for PEG treatment as against Water treatment for the 20 DH lines to bring them on the same relative scale, it was seen that **1 20(92.03%), 3 30 (97.41%), 8 40(91.45%), 6 33(82.60%) and 4 60(80.50%)** outperformed the Parental Line Da'ma. However, Parental lines Irat 109(94.91%) and Marilla (93.1%) outcompeted 1 20, 8 40, 6 33 and 4 60. The worst performing lines were 6 46(26.85%), 3 8 (32.11%), 3 57(33.33%) and 1 31(37.71%) (See table 5).

Table 4: Final Germination Percentage in PEG and in water for 20DH Lines

DH Genotype	PEG FGP	Water FGP
1 20	86.7	94.2
1 28	65.0	95.8
1 31	35.8	95.0
2 22	55.0	95.0
2 35	39.2	91.7
2 40	71.7	93.3
3 30	94.2	96.7
3 57	29.2	87.5
3 8	29.2	90.8
4 3	51.7	93.3
4 43	55.0	92.5
4 60	79.2	98.3
6 26	60.0	94.2
6 33	79.2	95.8
6 46	24.2	90.0
6 49	71.7	94.2
7 70	44.2	95.0
8 33	70.0	99.2
8 40	89.2	97.5
8 55	75.0	97.5
Da'ma	70.8	95.0
Irat 109	93.3	98.3
Marilla	90.0	96.7

Table 5: FGP ratio between PEG and water treatment for 20DH Lines

DH genotype	PEG/Water FGP ratio
1 20	92.03
1 28	67.82
1 31	37.71
2 22	57.89
2 35	42.72
2 40	76.78
3 30	97.41
3 57	33.33
3 8	32.11
4 3	55.35
4 43	59.45
4 60	80.50
6 26	63.71
6 33	82.60
6 46	26.85
6 49	76.10
7 70	46.49
8 33	70.58
8 40	91.45
8 55	76.92
Da'ma	74.56
Irat 109	94.91
Marilla	93.10

When relative Median Germination Time was compared in PEG as against Water treatment, the top 5 performing lines that took relative shorter for 50% of their viable seeds to germinate were **7 70 (2.9 days), 1 20(3 days), 6 46(3 days), 4 60 (3.2 days) and 4 43(3.3 days)**.These lines outcompeted the parental Lines Irat 109 and Marilla (4.5 days and 4.9 days respectively). The worst performing lines were 2 22 (6.3 days), 2 35(6.1 days), 1 31(5.9 days) and 8 33(5.1 days). Generally, the parental line Da'ma took the longest time (6.7 days) amongst all the studied lines for 50% of its viable seeds to germinate (see Table 6)

Table 6: Median Germination Time in PEG and in water treatment for 20DH Lines

Genotype(DH Line)	PEG	Water	Difference (PEG-Water)
	t50%	t50%	t50%
	Average (days)	Average (days)	Average (days)
1 20	5.8	2.3	3.4
1 28	5.3	2.3	3.0
1 31	8.2	2.3	5.9
2 22	9.0	2.7	6.3
2 35	9.1	3.0	6.1
2 40	6.1	2.3	3.8
3 30	5.8	2.3	3.4
3 57	7.5	3.0	4.5
3 8	6.8	2.3	4.4
4 3	7.4	3.0	4.3
4 43	5.0	1.8	3.3
4 60	5.4	2.2	3.2
6 26	6.2	2.6	3.6
6 33	6.6	2.1	4.5
6 46	5.2	2.2	3.0
6 49	5.6	2.3	3.4
7 70	5.2	2.3	2.9
8 33	7.9	2.7	5.1
8 40	5.9	2.4	3.5
8 55	6.8	2.4	4.4
Da'ma	8.5	1.9	6.7
Irat 109	6.8	2.3	4.5
Marilla	6.4	1.5	4.9

5. CONCLUSION AND RECOMMENDATIONS

The present master thesis which was based on the study of abiotic stress tolerance of rice for the production of aerobic rice production systems focused on screening the performance of twenty Double Haploid Rice lines under drought stress for some selected on-field and in vitro germination parameters. Our findings from our research affirmed many findings from literature that indeed, rice was sensitive to drought stress and even to the least sensing of stress environment (Jancsó *et al*., 2017) especially in active tillering stage.

A two weeks water deprivation interval between two on-field treatments caused significant differences in height and milled weight of DH Lines. Furthermore, although all 20 DH lines in drought conditions were affected by the water stress period, DH Line 1 31 recorded weight (33.75g) in drought treatment that were almost comparable to well-watered treatment (35.84g). The lines 2 22, 2 40 and 2 35 also recorded greater than 20 g (>20g) milled weight when some of the DH lines scored as low as 3.9g.

Also, Polyethylene Glycol (PEG) was able to induce drought stress conditions in germinating seeds. Comparison of shoot and root length in parentals Da'ma and Nembo revealed that as PEG concentration increased (from 0% mM PEG to 25% mM PEG), germination number, shoot length, and root length decreased. This result was consistent with findings in literature (Vibhuti *et al.*, 2015) on the effect of drought stress in reducing seed germination and seedling growth and on work by Sagar et al. (2020) where they found that increasing PEG concentrations caused a steady decrease in growth and germination parameters. Further, 20% mM PEG was an ideal screening concentration for DH rice genotype screening because that concentration was not too high that it prevented total emergence of shoot and radicle (as was seen in 25% mM or too low that it easily allowed increased length in shoot and root of seeds (as was seen in 15%).

More so, water treatment enhanced the growth and germination (>90%) of DH rice lines. However, inducing a drought stress (PEG treatment) brought out the drought resilience characteristics of DH Genotypes. Treatments in water when compared for the shoot average length revealed a four-fold increase than in PEG. Further, total radicle length in water treatment in most cases, surpassed that recorded *in* PEG. The shoot and root lengths of the genotype 1 28, 8 40, 3 3 outperformed the parental lines *Marilla, Da'ma and Irat 109)*.

FGP ratio of PEG to water treatment for the 20 DH lines showed genotypes 1 20 (92.03%), 3 30 (97.41%), 8 40(91.45%), 6 33(82.60%) and 4 60(80.50%) outperform parental Line Da'ma. However, Parental lines Irat 109(94.91%) and Marilla (93.1%) outcompeted 1 20, 8 40, 6 33 and 4 60. The worst performing lines were 6 46(26.85%), 3 8 (32.11%), 3 57(33.33%) and 1 31(37.71%).

Relative Median Germination Time compared in PEG as against water treatment revealed the top 5 performing lines as 7 70 (2.9 days), 1 20(3 days), 6 46(3 days), 4 60 (3.2 days) and 4 43(3.3 days). These lines outcompeted the parental Lines Irat 109 and Marilla (4.5 days and 4.9 days respectively). The worst performing lines were 2 22 (6.3 days), 2 35(6.1 days), 1 31(5.9 days) and 8 33(5.1 days). Generally, the parental line Da'ma took the longest time (6.7 days) amongst all the studied lines for 50% of its viable seeds to germinate.

Based on the accumulated findings from our study, we recommend DH genotypes 1 20, 1 28, 1 31, 8 40, 3 30, 7 70 and 6 33 as suitable candidates for further screening and evaluation for their drought tolerance characteristics and resilience in aerobic rice production systems; as they may possess innate characteristics that enhance their drought tolerance.

Also, a correlation test should be conducted in the subsequent study to assess the relationship between on-field parameters such as height and milled weight and in vitro germination parameter; shoot length, radicle length, median germination time and final germination percentage. When this is done, it will give a fine-tuning component to selecting drought tolerant DH lines.

6. SUMMARY

Thesis title: ABIOTIC STRESS TOLERANCE OF DH RICE LINES FOR THE DEVELOPMENT OF AEROBIC RICE PRODUCTION SYSTEMS

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Course: MSc Crop Production Engineering

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Rice (*Oryza sativa L.*), is a major staple for more than fifty percent of the world's population (Fuagawa & Ziska*,* 2019) and its consumption rate continues to increase (Statista, 2023). About one third of our freshwater resources is used in the production of flooded paddy rice (Surendran *et al*., 2021). With news of depleting freshwater resources (Tuong *et al*, 2005), unpredictable hydrology variations (Turral *et al*., 2011) and increasing surface temperature (Pauchari & Meyer, 2014) due to climate change, drought stress- constrained rice production will cause enormous economic losses and huge food security issues. Many have called for more sustainable water saving rice production technologies (Mandal *et al*., 2019) and robust drought tolerant cultivars.

The aerobic rice production system uses upland rice varieties and is able to save 60–90% more water than traditional flooded paddy rice (Çolak, 2021) however, there exist inadequate rice cultivars that can be grown under this production system. Over the years, rice breeders have faced numerous challenges breeding for rice that meets the pace of fast-changing drought stress conditions (Hernández-Soto *et al*., 2021). One of the acclaimed transformation approaches to accelerate rice breeding process in aerobic rice production systems, aid in the faster selection of homozygous lines, and finally screen for useful traits which cannot be easily attained with conventional methods (Pauk *et al*., 2009) is the Double Haploid technique.

This present study screened twenty double haploid (DH) rice lines for their on-field and in-vitro drought tolerance characteristics and suitability for use in aerobic rice production systems. The

research took place from 18th May 2022 to 20th February, 2023 at the MATE IES ÖVKI (Szarvas) and the studied parameters were on-field parameters (height, milled weight and the stress indices parameters IAD, CCI, CRI, CNDVI, PRI, and DCNI) as well as in vitro germination parameters (shoot length, root length, median germination time and final germination percentage). On-field experiment was set in Randomized Complete Block Design with four repetitions whereas in-vitro experiment was set in Completely Randomized Design with three repetitions. Results were analyzed with ANOVA IBM SPSS software at 0.05 level of significance and means that were significantly different were separated with Tukey HSD.

Our findings revealed that, two-week water deprivation between well-watered treatment and drought treatment caused significant differences in height and milled weight of DH Lines. Furthermore, 20% mM Polyethylene Glycol (PEG) was the ideal concentration for scouting for drought tolerance in DH genotypes. Whilst water enhanced the growth and germination (>90%) of DH rice lines in radicle length, shoot length, median germination time and final germination percentage PEG treatment brought out the drought resilience differences in genotypes.

Based on the accumulated findings from our study, we recommend DH genotypes 1 20, 1 28, 1 31, 8 40, 3 30, 7 70 and 6 33 as suitable candidates for further evaluation for their drought resilience and use in aerobic production systems. These genotypes outperformed the water control treatment as well as their parental lines (Irat and Da'ma) from which they were developed.

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APPENDICES

Appendix 1: RCBD on-field Spot Plan of 20 DH Lines

DH Line	Field Positioning			
	R1t1	**R2t1**	**R1t2**	**R2t2**
1 20	1//8	9//2	13//8	21//2
1 28	1//13	9//46	13//13	21//46
1 31	1//11	9//3	13//11	21//3
2 22	1//52	9//42	13//52	21//42
2 40	1//37	9//52	13//37	21//52
2 35	2//7	10//46	14//7	22//46
3 8	2//19	10//49	14//19	22//49
3 30	6//49	10//54	18//49	22//54
3 57	6//45	10//52	18//45	22//52
4 3	2//47	10//56	14//47	22//56
4 43	3//15	11//48	19//37	23//48
4 60	3//19	11//49	15//19	23//49
6 26	3//37	7//48	15//37	19//48
6 33	3//33	7//46	15//33	19//46
6 46	3//50	7//25	15//50	23//42
6 49	3//43	11//55	15//43	19//51
7 70	4//34	12//38	16//34	20//17
8 55	5//56	9//28	17//56	21//28
8 33	4//48	8//24	16//48	24//12
8 40	4//51	12//58	16//51	24//58

Appendix 2: One sample T-Test testing significance of means between Milled Weight of well-watered portions and milled weight of drought portions for 20 DH Lines

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
T1 AvgW	20	38.11943	3.111721	.695802
T2 AvgW	20	12.10750	8.873368	1.984145

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	T	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
T1 AvgW	54.785	19	.000	38.119430	36.66310	39.57576
T2 AvgW	6.102	19	.000	12.107500	7.95464	16.26036

Appendix 3: One sample T-Test testing significance of means between Height of well-watered portions and Height of drought portions for 20 DH Lines

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
T1 AvgH	20	56.43000	9.956120	2.226256
T2 AvgH	20	50.26000	7.823137	1.749307

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	T	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
T1 AvgH	25.347	19	.000	56.430000	51.77039	61.08961
T2 AvgH	28.731	19	.000	50.260000	46.59866	53.92134

Appendix 4: ANOVA amongst the 20 DH line for IAD stress Index

Tests of Between-Subjects Effects

Dependent Variable: IAD

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	279.495	1	279.495	########	.000
	Error	1.429E-05	1	1,429E-5[a]		
Genotype	Hypothesis	.146	17	.009	7.213	.000
	Error	.020	17	,001[b]		
Treatment	Hypothesis	1.429E-05	1	1.429E-05	.012	.914
	Error	.024	19.574	,001[c]		
Genotype * Treatment	Hypothesis	.020	17	.001	.889	.588
	Error	.361	269	,001[d]		

a. MS(Treatment)

b. MS(Genotype * Treatment)

c. ,939 MS(Genotype * Treatment) + ,061 MS(Error)

d. MS(Error)

IAD

Tukey HSD[a,b,c]

Genotype	N	Subset			
		1	2	3	4
3_57	19	.9706			
6_49	20	.9720			
4_3	20	.9794	.9794		
6_26	20	.9879	.9879	.9879	
8_33	10	.9907	.9907	.9907	
7_70	20	.9932	.9932	.9932	
2_35	10	.9977	.9977	.9977	
4_60	15	1.0005	1.0005	1.0005	
6_46	14	1.0024	1.0024	1.0024	
8_55	15	1.0070	1.0070	1.0070	1.0070
3_8	14	1.0093	1.0093	1.0093	1.0093
8_40	20	1.0097	1.0097	1.0097	1.0097
6_33	20		1.0184	1.0184	1.0184
3_30	20			1.0254	1.0254
2_22	20			1.0288	1.0288
1_28	14			1.0316	1.0316
2_40	19			1.0322	1.0322

4_43	15				1.0519
Sig.		.196	.202	.067	.058

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = ,001.
a. Uses Harmonic Mean Sample Size = 16,078.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = ,05.

Appendix 5: ANOVA amongst the 20 DH line for CCI stress Index

Tests of Between-Subjects Effects

Dependent Variable: CCI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	56520.703	1	56520.703	339.113	.035
	Error	166.672	1	166,672[a]		
Genotype	Hypothesis	1189.965	17	69.998	2.125	.065
	Error	559.976	17	32,940[b]		
Treatment	Hypothesis	166.672	1	166.672	5.239	.034
	Error	572.378	17.991	31,815[c]		
Genotype * Treatment	Hypothesis	559.976	17	32.940	2.265	.003
	Error	3912.781	269	14,546[d]		

a. MS(Treatment)

b. MS(Genotype * Treatment)

c. ,939 MS(Genotype * Treatment) + ,061 MS(Error)

d. MS(Error)

CCI

Tukey HSD[a,b,c]

Genotype	N	Subset 1	2	3	4
3_57	19	10.9408			
8_40	20	11.3996	11.3996		
3_8	14	12.3297	12.3297	12.3297	
6_46	14	12.9181	12.9181	12.9181	
3_30	20	12.9210	12.9210	12.9210	
4_3	20	13.1512	13.1512	13.1512	
2_35	10	13.4426	13.4426	13.4426	13.4426
7_70	20	13.6759	13.6759	13.6759	13.6759
6_49	20	13.8811	13.8811	13.8811	13.8811
8_33	10	14.8771	14.8771	14.8771	14.8771
4_60	15	15.1451	15.1451	15.1451	15.1451
1_28	14	15.2167	15.2167	15.2167	15.2167
4_43	15	15.6236	15.6236	15.6236	15.6236
2_40	19		16.0043	16.0043	16.0043
2_22	20		16.0091	16.0091	16.0091
8_55	15			16.2376	16.2376
6_33	20			16.9448	16.9448
6_26	20				18.1409
Sig.		.057	.067	.066	.055

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 14,546.
a. Uses Harmonic Mean Sample Size = 16,078.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = ,05.

Appendix 6: ANOVA amongst the 20 DH line for CRI stress Index

Tests of Between-Subjects Effects

Dependent Variable: CRI1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4.244	1	4.244	5388.228	.009
	Error	.001	1	,001[a]		
Genotype	Hypothesis	.037	17	.002	2.937	.016
	Error	.013	17	,001[b]		
Treatment	Hypothesis	.001	1	.001	1.091	.310
	Error	.013	18.576	,001[c]		
Genotype * Treatment	Hypothesis	.013	17	.001	1.434	.120
	Error	.138	269	,001[d]		

a. MS(Treatment)

b. MS(Genotype * Treatment)

c. ,939 MS(Genotype * Treatment) + ,061 MS(Error)

d. MS(Error)

CRI1

Tukey HSD[a,b,c]

Genotype	N	Subset 1	2	3	4
6_49	20	.1049			
3_57	19	.1103	.1103		
7_70	20	.1125	.1125	.1125	
8_55	15	.1182	.1182	.1182	.1182
6_46	14	.1184	.1184	.1184	.1184
4_3	20	.1184	.1184	.1184	.1184
2_35	10	.1185	.1185	.1185	.1185
4_60	15	.1192	.1192	.1192	.1192
6_26	20	.1203	.1203	.1203	.1203
3_8	14	.1215	.1215	.1215	.1215
8_33	10	.1216	.1216	.1216	.1216
2_40	19	.1264	.1264	.1264	.1264
1_28	14	.1286	.1286	.1286	.1286
8_40	20	.1297	.1297	.1297	.1297
6_33	20		.1355	.1355	.1355

3_30	20			.1393	.1393
2_22	20			.1397	.1397
4_43	15				.1423
Sig.		.158	.142	.070	.201

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = ,001.
a. Uses Harmonic Mean Sample Size = 16,078.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = ,05.

Appendix 7: ANOVA amongst the 20 DH line for CNDVI stress Index

Tests of Between-Subjects Effects

Dependent Variable: CNDVI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	49.456	1	49.456	1844.496	.015
	Error	.027	1	,027[a]		
Genotype	Hypothesis	.088	17	.005	4.147	.003
	Error	.021	17	,001[b]		
Treatment	Hypothesis	.027	1	.027	21.202	.000
	Error	.025	19.938	,001[c]		
Genotype * Treatment	Hypothesis	.021	17	.001	.782	.713
	Error	.428	269	,002[d]		

a. MS(Treatment)

b. MS(Genotype * Treatment)

c. ,939 MS(Genotype * Treatment) + ,061 MS(Error)

d. MS(Error)

CNDVI

Tukey HSD[a,b,c]

Genotype	N	Subset 1	2	3
3_57	19	.3968		
6_49	20	.4014	.4014	
3_8	14	.4100	.4100	.4100
2_35	10	.4107	.4107	.4107
7_70	20	.4139	.4139	.4139
6_46	14	.4144	.4144	.4144
8_40	20	.4165	.4165	.4165
8_55	15	.4168	.4168	.4168
4_3	20	.4176	.4176	.4176
3_30	20	.4205	.4205	.4205
4_60	15	.4233	.4233	.4233
2_40	19	.4257	.4257	.4257
1_28	14	.4346	.4346	.4346
4_43	15	.4357	.4357	.4357
8_33	10	.4386	.4386	.4386
2_22	20		.4464	.4464
6_26	20			.4519
6_33	20			.4555
Sig.		.222	.127	.114

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = ,002.
a. Uses Harmonic Mean Sample Size = 16,078.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = ,05.

Appendix 8: ANOVA amongst the 20 DH line for PRI stress Index

Tests of Between-Subjects Effects

Dependent Variable: PRI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	.049	1	.049	13.763	.168
	Error	.004	1	,004[a]		
Genotype	Hypothesis	.003	17	.000	.634	.822
	Error	.005	17	,000[b]		
Treatment	Hypothesis	.004	1	.004	12.298	.002
	Error	.005	18.264	,000[c]		
Genotype * Treatment	Hypothesis	.005	17	.000	1.781	.030
	Error	.045	269	,000[d]		

a. MS(Treatment)

b. MS(Genotype * Treatment)

c. ,939 MS(Genotype * Treatment) + ,061 MS(Error)

d. MS(Error)

PRI

Tukey HSD[a,b,c]

Genotype	N	Subset 1
6_49	20	.0071
3_57	19	.0084
8_55	15	.0116
3_30	20	.0118
7_70	20	.0122
6_46	14	.0125
4_3	20	.0125
3_8	14	.0130
2_35	10	.0130
8_33	10	.0131
6_33	20	.0132
8_40	20	.0139

4_60	15	.0170
6_26	20	.0171
2_22	20	.0177
2_40	19	.0178
1_28	14	.0199
4_43	15	.0207
Sig.		.216

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = ,000.
a. Uses Harmonic Mean Sample Size = 16,078.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = ,05.

Appendix 9: ANOVA amongst the 20 DH line for DCNI stress Index

Tests of Between-Subjects Effects

Dependent Variable: DCNI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	786315.731	1	786315.731	1254.457	.018
	Error	626.818	1	626,818[a]		
Genotype	Hypothesis	1094.927	17	64.407	4.341	.002
	Error	252.253	17	14,838[b]		
Treatment	Hypothesis	626.818	1	626.818	41.046	.000
	Error	311.762	20.415	15,271[c]		
Genotype * Treatment	Hypothesis	252.253	17	14.838	.677	.825
	Error	5895.971	269	21,918[d]		

a. MS(Treatment)

b. MS(Genotype * Treatment)

c. ,939 MS(Genotype * Treatment) + ,061 MS(Error)

d. MS(Error)

DCNI

Tukey HSD[a,b,c]

Genotype	N	Subset 1	Subset 2
3_57	19	49.8601	
2_35	10	50.9780	50.9780
8_40	20	51.3337	51.3337
3_8	14	51.6997	51.6997
6_49	20	51.7460	51.7460
3_30	20	52.2206	52.2206
4_3	20	52.5447	52.5447
7_70	20	53.2330	53.2330
8_55	15	53.5738	53.5738
6_46	14	53.7469	53.7469
4_60	15	54.1903	54.1903
4_43	15	54.4878	54.4878
2_40	19	54.6271	54.6271
8_33	10	55.3912	55.3912
1_28	14	55.5245	55.5245
6_33	20		55.8557
2_22	20		56.0396
6_26	20		56.3147
Sig.		.066	.116

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 21,918.
a. Uses Harmonic Mean Sample Size = 16,078.
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = ,05.

STUDENT DECLARATION

I, Obirih-Opareh Jennifer, MSc Crop Production Engineering student of the Szent István Campus of the Hungarian University of Agriculture and Life Science, hereby declare that this Thesis is my own work and I have cited and quoted literature in agreement with the appropriate legal and ethical rules. I recognize that my one page-summary of my thesis will be loaded onto the website of the Campus/Institute/Course and my Thesis will be accessible at the Host Department/Institute and in the repository of the University in accordance with the relevant legal and ethical rules.

Confidential data are presented in the thesis: yes no*

Date: 2023____4_ month _20 day

Student

SUPERVISOR'S DECLARATION

As primary supervisor of the author of this thesis, I hereby declare that review of the thesis was done thoroughly; student was informed and guided on the method of citing literature sources in the dissertation, attention was drawn on the importance of using literature data in accordance with the relevant legal and ethical rules.

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Date: 24th April 2023

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