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

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops and has contributed greatly to the world's food security. Asia has a leading role in global rice production, being responsible for 90%, but the yield is stagnant (Bandumula, 2018). Although it is predicted that there will be 9.1 billion people on the planet by the year 2050, agricultural productivity is not growing at the same pace. To feed 2.3 billion more people then, 70% more food would need to be produced (Wani & Kumar Sah, 2014). The growing rate of the world's population makes the demand an emerging challenge, especially for developing countries. Besides that, the cultivation has inherent difficulties, such as pests and drought. For this reason, the development of high yielding rice variety with a high degree of resistance to both biotic and abiotic stresses is required (Oladosu et al., 2019).

The main abiotic stresses worldwide are high salinity, submergence, cold and drought. Among these, drought affects rice growth and productivity in several ways, including morphological changes in the germination stage, leaves and roots, and physiological changes in the photosynthesic system, membranes and osmotic control (Panda et al., 2021). Water is a resource directly impacted by climate change, which means that shifts in the regularity and level of hydrological fluctuations will lead to more issues in agriculture. Therefore, water management is needed to ensure sustainable development (Surendran et al., 2021).

Rice grows in a wide variety of locations, and most are under persistent flood conditions. By nature, it is a semiaquatic plant. In such a way that, to produce one kilogram of rice, 3,000 to 5,000 liters of water are needed, much more than for wheat and corn (Dey et al., 2018). Alternative irrigation systems need to be established to meet the demand for higher yields while using less water. A study carried out in an extremely dry area of Brazil has shown that through aerobic rice cultivation it is possible to obtain equivalent or even better results in crop performance, nitrogen recovery and water productivity than continuously flooded or any other alternative rice system. This could be replicated in areas of South America, West Africa, or another region with similar climatic conditions (Froes de Borja Reis et al., 2018).

When the ground is flat or terraced, the soil can regularly be irrigated by rainfall, additional irrigation, or when the land is sloping but frequent rainfall can keep the soils moist throughout the growing season, aerobic rice is more likely to thrive. Wherever there is enough water for aerobic rice but not enough for lowland rice, the latter can be used in place of the

former. Both aerobic and upland rice are adapted to aerobic soil conditions, although aerobic rice types have better yields and are more input-responsive than conventional upland varieties (Parthasarathi et al., 2012). Higher water productivity can be attained under alternate irrigation circumstances, but without the right rice types that can withstand droughts, production can potentially be drastically decreased (Jancsó et al., 2022).

Selection for drought tolerance is quite challenging since plants usually experience several stresses (drought, salt, low temperature, mechanical damage, etc.). Traditional pedigree breeding and innovative biotechnology-based techniques can be coupled to successfully select novel high yielding and abiotic stress tolerant genotypes for aerobic rice farming (Jancsó et al., 2022). The breeding process can be sped up by using more effective methods, the doubled haploid (DH) plant production is an effective tool (Lantos et al., 2022).

Advances in rice breeding will enable us to enhance the varieties that farmers are now employing. Using high yielding and drought tolerant rice genotypes, better water use efficiency can be achieved. In this piece, points about rice drought tolerance will be addressed to explore its production. The objectives are:

I. Measure the evapotranspiration of rice in aerobic cultivation.
II. Evaluate double haploid (DH) rice lines regarding the variety of genotypes that are most suited to drought conditions.
III. Molecular fingerprinting of the genotypes with regards to drought tolerance.

2. LITERATURE REVIEW

2.1 Importance and production of rice worldwide

Rice is grown in all the six continents of the world (Asia, Africa, Australia, Europe, North America, South America). For some nations, it is now a source of foreign exchange that helps support their economy. For example, India, Thailand, Pakistan, the United States, Socialist Republic of Vietnam, Italy, Uruguay, Brazil, China, and Australia are the countries where rice has an important economic value (Prasad et al., 2017). The market is clearly influenced by regional and cultural preferences, so there needs to be a balance between production, consumer choice, farmer livelihood and use of the environment in each place (Fukagawa & Ziska, 2019).

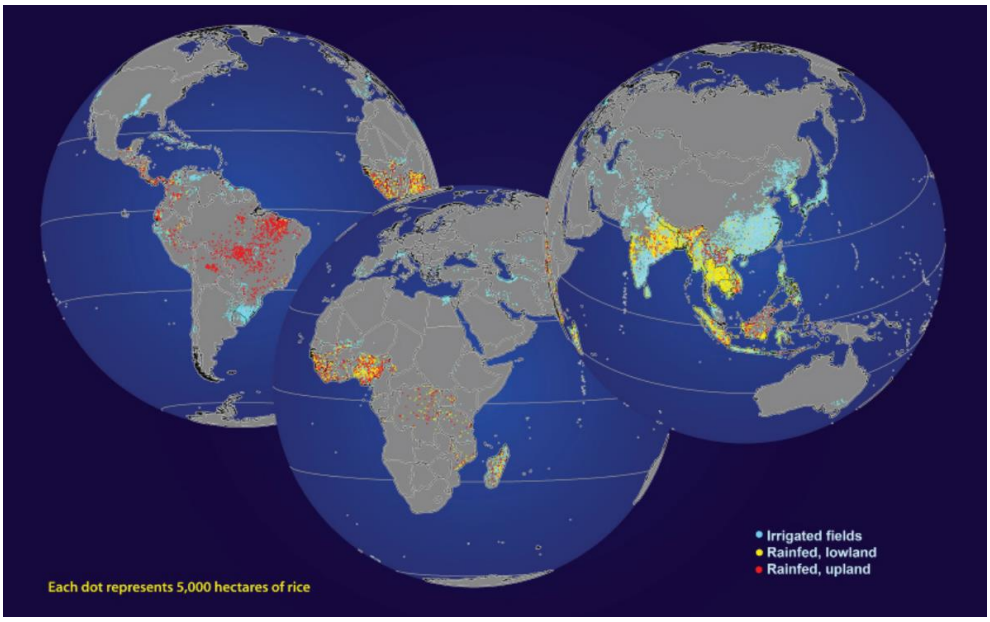

Figure 1: Major global rice-growing areas and ecosystems (GRiSP, 2013).

In addition to being significant for the economy, rice has influenced the diets and cultures of countless millions of people (Gnanamanickam, 2009). Several celebrations are held in honor of the rice harvest to express gratitude for a plentiful harvest. These celebrations also feature traditional rice dishes, e.g., Harvest Moon in China and Chu Suk in Korea (Prasad et al., 2017).

Even though rice characteristics depend on the variety, the nutritional aspects are more related to the way this food is prepared. Rice is often seen as the solution to meet the nutritional

demands of countries in Africa, South Asia, and Latin America. For that, biofortification is an effective method to delivery more nutrients and several rice cultivars have been studied (Majumder et al., 2019).

In the face of environmental forces, malnutrition will remain an issue. The quantity and quality of food we produce as well as our capacity to distribute it fairly will be impacted by human-caused climate change. The crop is affected by variations in temperature, precipitation, and atmospheric carbon dioxide concentration, which also have an impact on how pests and pollinators interact with the plant (Myers et al., 2017). Experiments have demonstrated that food crops cultivated at high CO_2 levels had lower protein content in their edible parts. The protein content of C3 grains and tubers, such as rice, wheat, barley, and potatoes, shows declines of 7 to 15% (Myers et al., 2014).

The adoption of innovations encouraged by what became known as the "green revolution" in the 1960s led to a huge increase in rice production. As a result, the yield per area substantially increased (Bandumula, 2018). To repeat the event, efforts are currently being made to enhance growth conditions while considering the impact on the environment and biological processes.

2.2 Water role in rice production

Rice is a plant that, while it can grow in a variety of environments, has evolved to grow faster and more vigorously in humid, warm conditions (Gnanamanickam, 2009). The ideal irrigation water requirement to grow 1 kilogram of rice was calculated to be roughly 3000 liters, therefore cultivation in certain regions is limited. Furthermore, in a wider perspective, plants are using a large portion of freshwater worldwide, which becomes a concern due to the imminent shortage of supply (Oladosu et al., 2019).

Considering the availability of water, 97.3% of the water on Earth is saline, and only 2.7% is available as fresh water (Patle et al., 2020). Of this portion, approximately 70% of freshwater consumption is used for agriculture. Although loss prevention methods have improved, most of the agricultural water wastage is still caused by plant-specific processes like evapotranspiration (Alberti et al., 2022).

Additionally, the discharge of untreated water coming from agriculture into natural water bodies is regarded as an environmental issue. That is the case of Brazil, where the quality

of the tap water is impacted especially in large metropolitan areas along the coast. Besides, the surplus of nutrients may cause eutrophication in water bodies, making them difficult to use as extra irrigation sources (Alberti et al., 2022).

Changes to the hydrological cycle will influence water resources. Watershed depletion is anticipated to have an impact on the majority of agricultural land (Patle et al., 2020), thus declining rice production. Crop growth factors are negatively impacted by drought, which eventually reduces production. This harm depends on the severity, duration, and stage of the plant's growth. There are two distinct categories of drought conditions: terminal and intermittent. While the "intermittent" state occurs when rainfall or irrigation are insufficient, the "terminal" state refers to the circumstance where a plant will die due to extreme water stress brought on by water shortage or reduction in the amount of water available. Drought tolerance or resistance mechanisms are those that enable plants to endure and carry on functioning in the face of terminal or intermittent drought conditions (Oladosu et al., 2019).

Pandey & Shukla (2015) and Panda et al. (2021) discussed the aspects involved in physiological, biochemical, and molecular adaptation of rice to drought tolerance. In the germination stage, drought stress affects metabolic processes at the cellular level, and reduces ATP synthesis and respiration, which results in a decrease of seedling strength. Poor cell development, reduced leaf area, and roots growth are also responses to disrupted water flow. The occurrence of natural antioxidants may be an alternative mechanism to prevent or reduce potential damage.

Fundamentally, the interaction of a plant's genotype and environment determines its growth and development. Given that the plant's metabolism can respond through several methods, involving molecular and biochemical pathways as well as morphological and physiological changes (Figure 2), the process of acquiring drought tolerance in rice is complex.

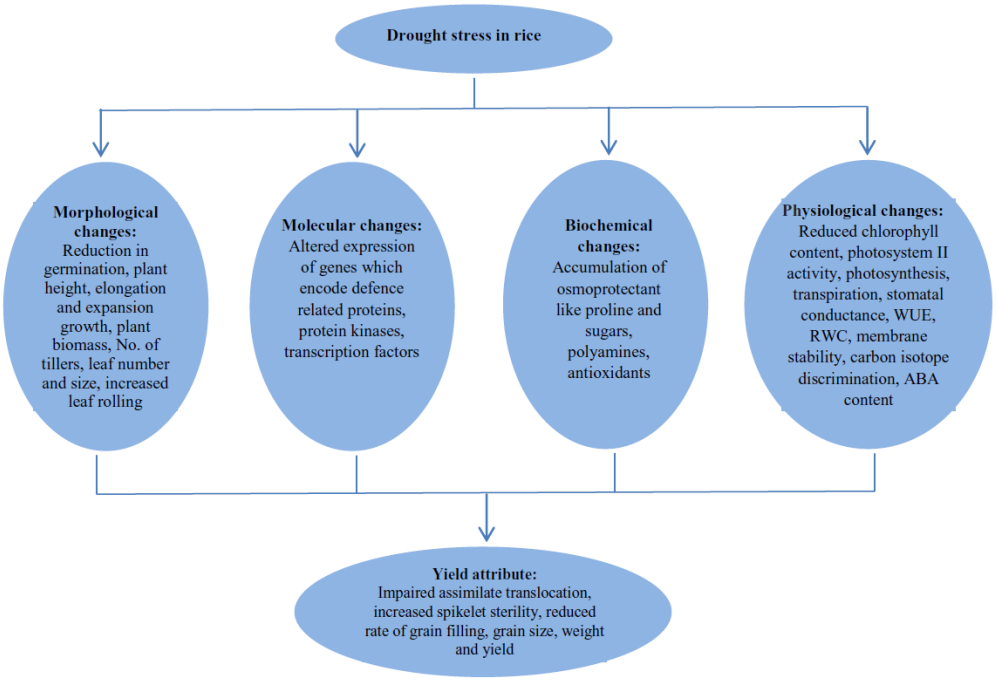

Figure 2: Drought induced responses in rice which ultimately affect yield (Pandey & Shukla, 2015). WUE, Water use efficiency; RWC, Relative water content; ABA, Abscisic acid.

According to Standard Evaluation System for Rice (International Rice Research Institute, 2002), drought sensitivity (DRS) can be estimated by repeated measurements as the drought progresses. During this period, leaf rolling precedes leaf drying. The leaf rolling and the leaf drying at vegetative stage are presented on a scale of 1 to 9. For the first case, 1 represents that the leaves are healthy and 9 they are tightly rolled in V-shape. While for the next stage, 1 represents that the plant was without symptoms and 9 that it is apparently dead and the length of most of the leaves is completely dry.

Another way to measure the effects of the environment is the use of spectroradiometers to optimize plant health and yield. Through modeling, spectral data from leaves in response to stressors can generate a wide variety of insights. Besides, it is a non-destructive and fast assay (Kebede et al., 2022). Some indices that can be calculated are:

- The variance in absorbance levels at two specific wavelengths (670 and 720 nm) is referred to as the Absorbance Difference Index (IAD). This index shows a significant correlation with the concentration of chlorophyll-a (Székely et al., 2023).
- Relative to chlorophyll concentration, the Chlorophyll Content Index (CCI) represents the ratio of radiation transmission from a light-emitting diode (LED)

centered at 931 nm to that from an LED centered at 653 nm. Various studies have utilized CCI measurements to formulate prediction equations for chlorophyll content (Parry et al., 2014).

- Carotenoids, plant pigments crucial for functions like shielding against oxidative damage and providing photoprotection, play a vital role in plant physiology. The Carotenoid Reflectance Index (CRI), which reflects the difference in reciprocal reflectance at 510 nm, serves as a sensitive indicator not only of carotenoid levels but also potentially of chlorophyll content within plants. Its measurement not only provides insights into plant health and physiological status but also sheds light on their response to environmental stressors (Gitelson et al., 2007).
- Chlorophyll Normalized Difference Vegetation Index (CNDVI) uses the normalized difference between near-infrared reflectance (NIR) and red reflectance (RED) to determine chlorophyll density in plant leaves (J. J. Chen et al., 2021).
- The Photochemical Reflectance Index (PRI) serves as a metric for assessing photosynthesis efficiency in plants, utilizing spectral reflectance data. It's computed by normalizing the difference between reflectance values in two adjacent spectral bands, typically green (531 nm) and red (570 nm), and is indicative of the photochemical activity of photosynthetic pigments, primarily chlorophyll. PRI demonstrates sensitivity to alterations in chlorophyll quantity and light utilization efficiency, making it a valuable measurement of plant health and physiological condition. Particularly, it proves beneficial in monitoring plant reactions to environmental pressures like drought, cold, or high solar radiation (Gamon et al., 1992).
- Plant water status, indicated by the Water Band Index (WBI), can be assessed by analyzing reflectance within the 950–970 nm range, a parameter closely associated with relative water content (RWC). When plants experienced considerable water stress, resulting in RWC dropping below 80–85%, notable changes occurred. These changes were most pronounced alongside a reduction in the flexibility of the cell wall (Penuelas et al., 1993).
- Dry Canopy Nitrogen Index (DCNI) is an index used to estimate the amount of nitrogen present in plant leaves. It is calculated from the difference in reflectance in two nearby spectral bands, generally in the red and near infrared. Since nitrogen is

an essential component for plant growth and protein synthesis, DCNI can provide valuable information about plant health and nutrition (P. Chen et al., 2010).

2.3 Aerobic rice system

Alternative irrigation techniques must be studied to determine if they can meet the demand for improved production and decreased water use. Some strategies are a) saturated soil systems, where soil pores are kept saturated but without ponding water; b) alternate wetting and drying (AWD), where the crop is subjected to sporadic periods of flooding and drying; c) aerobic rice, where fields are not flooded and soil is kept unsaturated for most of the growing season, typically through rainfed or sprinkler irrigation. However, while every one of these strategies reduces water use, in some circumstances it also results in decreased yields (Froes de Borja Reis et al., 2018). The most promising method for conserving water among these is thought to be aerobic rice. Due to less water being used for land preparation, seepage, percolation, and evaporation, aerobic rice uses on average 51% less water than flooded rice (Dey et al., 2018).

Growing rice as an upland crop on non-flooded aerobic soils is an alternative strategy that eliminates continuous seepage and percolation, besides reducing evaporation significantly. Traditional upland rice was developed for the adverse uplands to produce a consistent crop with minimal outside assistance (Parthasarathi et al., 2012). Most farming types are unsuitable for aerobic conditions due to their significant yield penalty. Previous studies on the water-saving potential of cultivating high-yielding lowland rice under aerobic conditions revealed a significant yield penalty. To produce large yields under high-input aerobic conditions, a new variety of rice is required, combining the drought tolerance of upland cultivars with the high yield of lowland cultivars (Dey et al., 2018).

The two main agronomical systems used to grow rice are transplanted puddled rice (TPR) and direct seeded rice (DSR). TPR, the traditional approach, mostly relies on flooding with water. The DSR consists of direct sowing of seeds in dry soil without puddles (Shekhawat et al., 2020). The benefit of DSR is that planting, weeding, and other tasks may be mechanized, requiring less labor overall, apart from reducing water use (Surendran et al., 2021). Nearly 22% of the total rice land in Asia is now under DSR due to its advantages (Shekhawat et al., 2020).

Nevertheless, weed insurgence in DSR is exacerbated by the lack of standing water during the initial crop establishment phase. Competition for nutrients, sunlight, and water

occurs as a result onwards, which means that either manual weeding, usage of pesticides, or a combination of both is required to control weeds (Shekhawat et al., 2020). Thus, by using the correct aerobic rice cultivar and management technique, it is possible to ensure yields that are equal to the to the conventional model while consuming up to 60% less water (Surendran et al., 2021).

2.4 Biotechnology approach

2.4.1 Genetic background

Rice, *Oryza sativa*, is a member of the grass family (*Poaceae*) of the plant world. It is known to grow easily in the tropics, and it was likely grown initially without being submerged, but it is thought that genetic changes caused it to develop into a semi-aquatic plant (Gnanamanickam, 2009).

In summary, the domestication of rice happened in Asia 10.000 years ago and led to two major subspecies: *indica* and *japonica*. These varieties can be recognized by their phenotypes, such as grain shape and texture. Besides, the plant height, germinating rate, and disease resistance, among other traits, are significantly different. The reproductive isolation can be confirmed by around 70% sterility in the F_1 crossing the two cultivars. Furthermore, genome sequence was used to analyze the phylogenetics, in this process molecular markers helped to determine that these two groups are predominant in the databases and the differences between them. For example, the United States Department of Agriculture (USDA) collection consists of 35% *indica*, 27% temperate *japonica*, 24% tropical *japonica*, 10% *aus*, and 4% *aromatic* (Wei & Huang, 2018).

The *indica* mainly grows in tropical Asia, while *japonica* in temperate East Asia. The geographical difference is because they are autogamous species, so the gene flow is restricted (Wei & Huang, 2018). Apart from this trait, modern inbred techniques and artificial selection managed to develop hybrid rice. A study concluded that, despite the possibility of hybrid varieties having a greater number of superior alleles, in terms of grain yield, grain quality, and disease-resistance traits, benefits may not add up due to epistatic interactions (Huang et al., 2015).

The domestication of African rice (*O. glaberrima*) is particularly significant due to its unique properties. It is currently nearly entirely restricted to West Africa, and from genetic data it is possible to infer that domestication happened 6000 years after the *O. sativa*. As a result of

the shorter time frame, it has a smaller gene pool and less genetic variety. In terms of yield and nutritional content, *O. glaberrima* does not show superiority, however it is more adapted to stress conditions, including drought, salinity, iron, and diseases (Wei & Huang, 2018).

The improvement of *O. sativa* could be forthcoming by inserting superior genes from *O. glaberrima* genome, despite the crossover difficulty. Understanding the mechanisms of domestication was the first step towards elucidating the behavior of genes in this population, especially those linked to agronomic traits. Based on this characterization, other methods can be used.

2.4.2 Classical and modern plant breeding methods

The natural genotypic variation in rice can be investigated to discover novel genotypes with desired drought tolerant traits. Different genotypes can be used in conventional breeding programs to produce rice varieties that are tolerant to drought through marker-assisted selection (Panda et al., 2021). Plant breeders use MAS for three main reasons: (a) to accumulate advantageous alleles by tracking the desirable alleles as dominant or recessive through generations; (b) to identify advantageous individuals from segregated breeding lines based on the entire genome or part of the allelic composition; and (c) to introgress advantageous alleles by disabling the unfavorable linkage loci (Oladosu et al., 2019).

The traditional breeding methods are important for maintaining genetic diversity, fostering hybridization between genetically distinct parents, and introducing novel genetic traits. The three major techniques employed are induced mutation, backcrossing, and pedigree selection (Oladosu et al., 2019). The issue is, as stated earlier, since drought conditions influence so many biochemical and physiological aspects, it is difficult to determine an effective selection criterion. The most often used, though, is rice grain yield (Rasheed et al., 2020).

Pedigree selection is one of the oldest and most popular techniques. It has the potential to combine multiple genes controlling biotic and abiotic processes, which is one of its main benefits (Oladosu et al., 2019). The main drawback is that it takes a lot of time and needs periodic evaluation numerous lines across planting seasons, while maintaining a record of the selection criteria (Rasheed et al., 2020). Figure 3 illustrates that many rounds of crossing are required for the desired traits to be successfully selected, according to the interaction of genotype and environment.

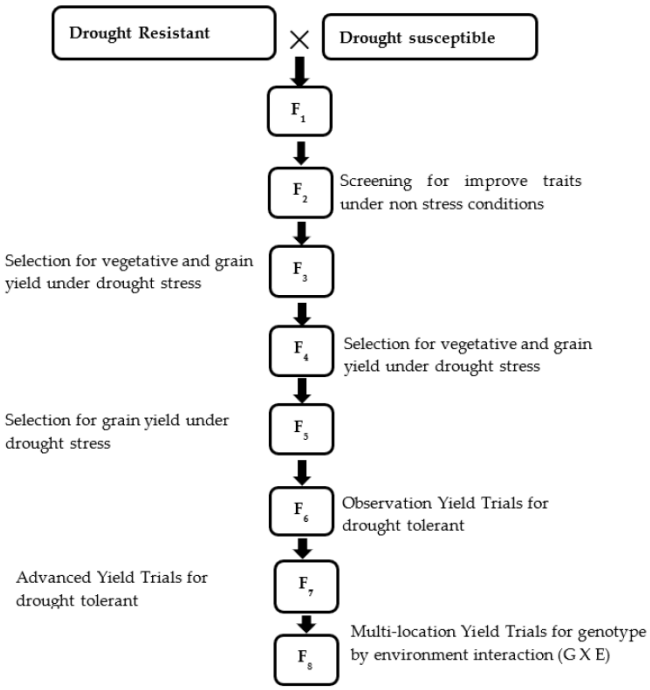

Figure 3: Modified method for conventional yield trail in rice (Oladosu et al., 2019).

Modern molecular technology can speed up crop improvement after the relationship between phenotypic and molecular data has been clarified. For the creation of novel rice types, some biotechnological methods can be applied (Miah et al., 2013). The development of effective tissue culture and transformation procedures, genome editing technology, and genome sequences have recently been shown as alternatives to help the rice breeding process (Figure 4) (Hernández-Soto et al., 2021). Moreover, considering that DNA markers open the possibility of finding superior genotypes in the first generations, this has a significant impact on breeding programs, reducing the number of progenies examined and, consequently, accelerating reproductive cycles (Oladosu et al., 2019).

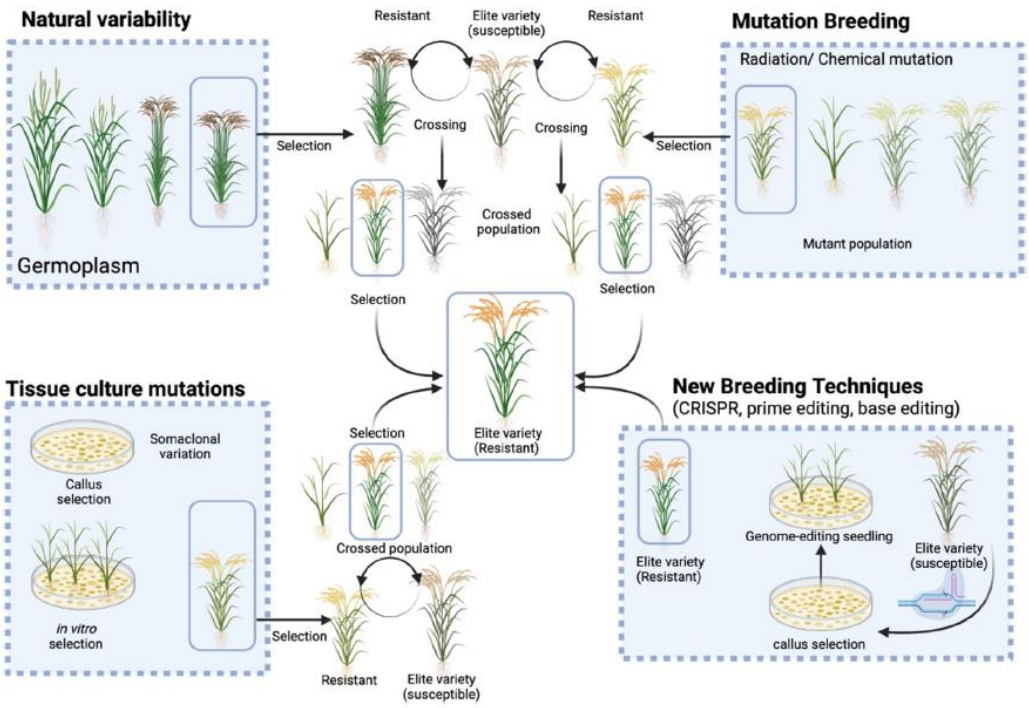

Figure 4: Schematic representation of different systems used for breeding rice: natural variability, mutation breeding, tissue culture mutation, and new breeding techniques (Hernández-Soto et al., 2021).

Another method for accelerating the procedure is the doubled haploid (DH) plant. Within a single generation, homozygous DH lines can be created using anther culture (AC) procedures. As a result, recessive alleles could be gathered and selected early since DH lines are homozygous. The effectiveness of in vitro AC is influenced by several variables, including genotype, donor plant growth conditions, microspore developmental stage, pre-treatments, and media compositions for induction and plant regeneration. It is a promising approach for breeding projects, particularly when combined with plant biotechnology methods like molecular markers (Lantos et al., 2023).

2.4.3 Molecular markers used in rice

In crop genetics, the random amplified polymorphic DNA (RAPD) approach has been widely employed since the early 1990s for many purposes, including mapping quantitative trait loci (QTLs) and evaluating genetic diversity. Two disadvantages of this type of marker are their low repeatability and the fact that they are not usually located in gene regions. Hence, a strategy to mitigate this drawback involves focusing on conserved regions. Typically, these conserved regions correspond to functional domains that align with conserved DNA sequences located in

genes. The rationale behind this approach is that these brief conserved genetic sequences should exist at numerous locations within plant genomes, consequently offering multiple primer binding sites. This approach is called the conserved DNA derived polymorphism (CDDP) method (Collard & Mackill, 2009). The studies carried out in recent years regarding rice genomics have different approaches. In this work, some cases using CDDP will be addressed.

Alterations in transcriptional regulation represent a significant mechanism through which plants react to environmental challenges. Transcription factors (TFs) and elements of stress-triggered signaling pathways are pivotal in stimulating gene expression when confronted with stress. By controlling the activity of numerous stress-responsive genes, specific transcription factors like ethylene response factor (ERF), MYB, and WRKY are important to enhance plants' ability to endure drought. Particularly, ERFs are crucial for both biotic and abiotic stress responses, a study has shown that transgenic expression of it may be a viable means to develop new rice varieties with greater drought tolerance (Joo et al., 2013).

ERF has already been implicated as a regulator of responses to dehydration stress during the reproductive and vegetative phases. Plants that were overexpressing TFs from this family demonstrated greater survival and seed setting rates compared to the wild type when facing water stress during the reproductive phase. Physiological analyses confirmed the enhancement of their pollen fertility. Conversely, knockout mutant and RNAi lines exhibited diminished pollen fertility and decreased drought tolerance during the reproductive stage (Jin et al., 2018).

MYB transcription factors also have demonstrated to participate in abiotic stress responses in rice. It has already been reported to have a critical function under conditions of drought and salinity stress (Yin et al., 2017). Based on the information that plants subjected to salt and drought stress often accumulate proline, wild-type and transgenic plants under normal growth conditions and drought stress were investigated. The findings showed that there was no difference in proline levels between transgenic and wild-type plants grown under typical growth circumstances. In contrast to the wild-type plants, the transgenic plants showed significantly higher proline level following exposure to drought stress, thus the greater expression of MYB increase the resistance of transgenic plants to drought stress (Tang et al., 2019).

The WRKY gene family stands out as one of the largest groups of transcription factors found in higher plants. These genes play a crucial role in enhancing plant stress tolerance, particularly through a complex network of signaling pathways activated by abiotic stressors

such as drought. Research has identified a significant association between at least 17 rice WRKY genes and their response to drought stress (Jiang et al., 2017). Moreover, studies have demonstrated that under challenging conditions, the overexpression of *OsWRKY11* leads to improved drought tolerance (Lee et al., 2018).

3. MATERIALS AND METHODS

3.1 Description of experimental site

The Research experimental site took place at the Research Center for Irrigation and Water Management (ÖVKI), located in the city of Szarvas. The exact location is shown with their coordinates by the digital map (Figure 5). There was on-field research at the MATE ÖVKI Lysimeter Research Station in Szarvas (south-east Hungary, latitude 46°86'N, longitude 20°52'E) followed by a lab experimental setup at the Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences (MATE).

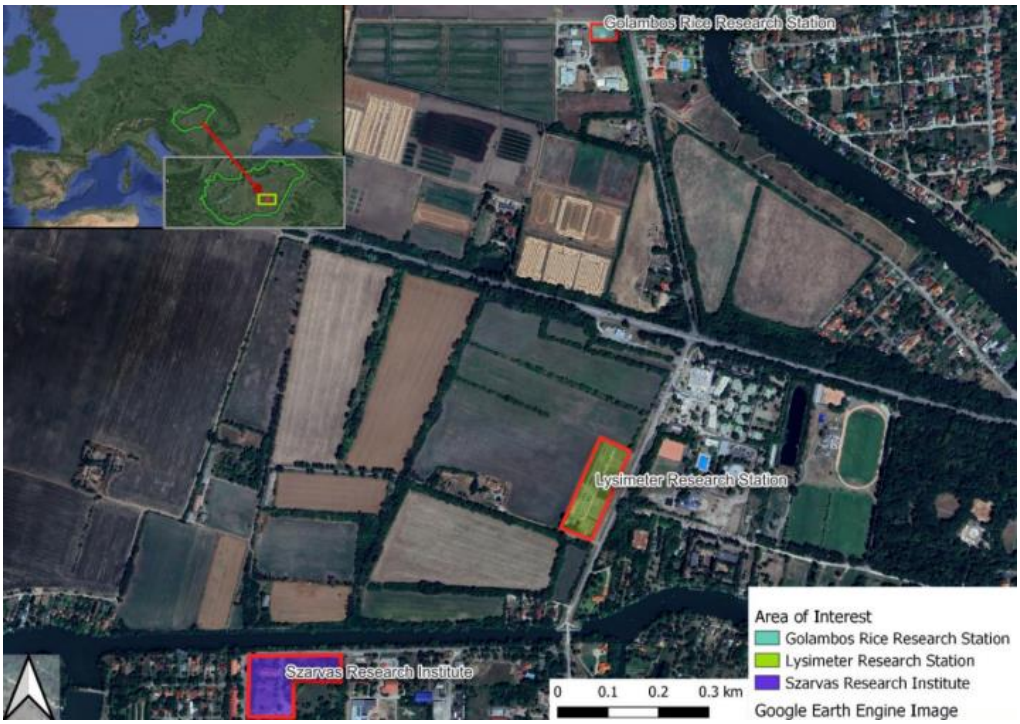

Figure 5: Experimental site for rice research in Szarvas.

During the on-field research, the regeneration and multiplication of Doubled Haploid (DH) lines occurred, accompanied by the measurement of stress indices and other agronomic data. Subsequently, in the laboratory experimental setup, the screening of samples derived from the on-field regenerated DH lines took place.

3.2. Plant material

Double haploid (DH) rice seeds, obtained from the Cereal Research Non-profit Company in Szeged, were utilized for the experiment. 32 genotypes were used, which were the product of crosses between the Irat 109, from Ivory Coast, and the Hungarian rice varieties Dáma and Marilla. The breeding project's parental rice lines, Dáma, Irat 109, and Marilla, were also acquired and functioned as control measures, which totals 35 genotypes analyzed.

Table 1: Code and pedigree of the genotypes used

1	Marilla	DH (Marilla x IRAT 109)
2	Irat 109	DH (Marilla x IRAT 109)
3	Dáma	DH (Marilla x IRAT 109)
4	1080/10/6	DH (Marilla x IRAT 109)
5	1080/10/27	DH (Marilla x IRAT 109)
6	1080/10/29	DH (Marilla x IRAT 109)
7	1080/10/30	DH (Marilla x IRAT 109)
8	1080/10/42	DH (Marilla x IRAT 109)
9	1080/10/49	DH (Marilla x IRAT 109)
10	1080/10/52	DH (Marilla x IRAT 109)
11	1080/11/14	DH (Marilla x IRAT 109)
12	1080/11/26	DH (Marilla x IRAT 109)
13	1080/11/50T	DH (Marilla x IRAT 109)
14	1080/11/58	DH (Marilla x IRAT 109)
15	1080/10/15	DH (Marilla x IRAT 109)
16	1080/10/25	DH (Marilla x IRAT 109)
17	1080/10/41	DH (Marilla x IRAT 109)
18	1080/10/47	DH (Marilla x IRAT 109)
19	1080/10/51	DH (Marilla x IRAT 109)
20	1080/11/1	DH (Marilla x IRAT 109)
21	1080/11/4	DH (Marilla x IRAT 109)
22	1080/11/16	DH (Marilla x IRAT 109)
23	1080/11/23	DH (Marilla x IRAT 109)
24	1080/11/43	DH (Marilla x IRAT 109)
25	1080/11/62	DH (Marilla x IRAT 109)

26	1087/1_20	DH (Dama x IRAT 109)
27	1087/3_30	DH (Dama x IRAT 109)
28	1087/3_37	DH (Dama x IRAT 109)
29	1087/4/60	DH (Dama x IRAT 109)
30	1087/6_33	DH (Dama x IRAT 109)
31	1087/6_46	DH (Dama x IRAT 109)
32	1087/8_40	DH (Dama x IRAT 109)
33	1087/8_55	DH (Dama x IRAT 109)
34	1080/1/5/4/2/3	Marilla x IRAT 109
35	1085/1/4/1/2/2	Dama x IRAT 109

3.3 Stress indices

The CI-710s SpectraVue Leaf Spectrometer (Cid-Bioscience, Camas, WA, U.S.A.) was used to collect data. This device is designed to measure a plant's light absorption, transmission, and reflection across a broad spectrum of wavelengths, all at once. It has two broad spectrum light sources integrated into the leaf spectrometer. One, located inside the leaf clamp, evaluates light transmission, while the other, housed inside the casing, quantifies light reflection. The spectral values from 400 nm to 1100 nm have been used to determine indices according to the formulas below:

I_{AD}: Chlorophyll Absorbance Index

$$IAD = (A670 - A720)$$

CCI: Canopy Chlorophyll Content Index

$$CCI = \left(\frac{R750 + R705}{R750 - R705}\right) \times R750$$

CRI1: Carotenoid Index

$$CRI1 = \frac{1 - R510}{1 - R550}$$

CNDVI: Corrected Normalized Difference Vegetation Index

$$CNDVI = \frac{R750 - R705}{R750 + R705}$$

PRI: Photochemical Reflectance Index

$$PRI = \frac{R531 - R570}{R531 + R570}$$

WBI: Water Band Index

$$WBI = \frac{R900}{R970}$$

DCNI: Dry Canopy Nitrogen Index

$$DCNI = (\frac{R720 - R700}{R700 - R670})/(\frac{R700 - R670}{R720 - R670 + 0{,}03})$$

3.4 DNA isolation

Grains and leaves were used for DNA extraction, following the protocol from NucleoSpin Plant II by Macherey-Nagel.

3.5 DNA assessment

Following extraction, the sample was assessed qualitatively with an 1% agarose gel in Tris–borate–ethylenediaminetetraacetic acid (TBE) buffer produced with SeaKem ® LE Agarose and stained with ethidium bromide, and quantitatively using the NanoDrop -1000 apparatus to measure the concentration. This stage was crucial to confirm the success of the extraction since it demonstrated the presence of DNA and an uncontaminated profile.

3.6 PCR Condition

PCR was conducted using a T100™ Thermal Cycler BioRad. The reaction mixture contained 4.25 µL of water, 1 µL Buffer x10, 1 µL of dNTPs 2 mM, 0.2 µL of $MgCl_2$ 25 mM, 1.5 µL of a primer 100 pM, 1 U of DreamTaq™ DNA polymerase (Thermo Fisher Scientific™), added 2 µL of each sample template, performed within a total volume of 10 µL. The reaction parameters consisted of an initiation cycle at 94 ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, primer annealing at 50 ºC for 30 s and extension at 72 ºC for 1 min. The reaction was completed with a post-polymerization extension cycle at 72 ºC for 5 min.

All PCR amplification products were separated on 1% agarose gels in TBE buffer stained with ethidium bromide and visualized under UV light. The GeneRuler 1 kb (Thermo Fisher Scientific™) DNA ladder was used to scale the size of the fragments.

3.7 Molecular markers

The molecular markers used in this work were chosen based on the conserved DNA-derived polymorphism (CDDP) method (Collard & Mackill, 2009). This methodology uses a single primer as a forward and reverse primer, allows small amounts of DNA template and simple agarose gel electrophoresis to be used, having the advantage of concentrating on gene regions. In this instance, these markers are based on DNA sequences found in several well-characterized plant genes that are mostly involved in the response to biotic and abiotic stressors or plant development. Therefore, conserved gene sequences should be found in the genomes at various locations, providing multiple primer binding sites. The list of primers, their sequence and function of the corresponding gene is shown in Table 2.

Table 2: Conserved DNA sequence targets and primer sequences (Collard & Mackill, 2009).

Gene	Gene function	Amino acid motif	Primer name	Primer sequence (5′ to 3′)	GC (%)
WRKY	Transcription factor for developmental and physiological roles	WRKYGQ	WRKY-F1	TGGCGSAAGTACGGCCAG	67
		GEHTC	WRKY-R3	GCASGTGTGCTCGCC	73
MYB	Unknown (implicated in secondary metabolism, abiotic and biotic stresses, cellular morphogenesis)	GKSCR	MYB1	GGCAAGGGCTGCCGC	80
		GKSCR	MYB2	GGCAAGGGCTGCCGG	80
ERF	Transcription factor involved in plant disease resistance pathway	HYRGVR	ERF1	CACTACCGCGGSCTSCG	77
		AEIRDP	ERF2	GCSGAGATCCGSGACCC	77
KNOX	Homeobox genes that function as transcription	KGKLPK	KNOX-1	AAGGGSAAGCTSCCSAAG	61

	factors with a unique homeodomain	HWWELH	KNOX-2	CACTGGTGGGAGCTSCAC	67
MADS	Involved in controlling floral organ initiation and development	MGRGKV	MADS-1	ATGGGCCGSGGCAAGGTGC	74
		LCDAEV	MADS-4	CTSTGCGACCGSGAGGTG	72
ABP1	Auxin-binding protein	TPIHR	ABP1-1	ACSCCSATCCACCGC	73

3.8 Statistical Analysis

The fragments were scored as either present or absent, and subsequent association analysis was conducted to identify markers that were correlated with the drought tolerance.

The iMEC: Online Marker Efficiency Calculator program, an online analytical tool based on R software, was utilized to assess the results (Amiryousefi et al., 2018). Table 4 displays the values for the following metrics: The expected Polymorphic Information Content (PIC), Effective multiplex ratio (EMR), Marker index (MI), Discriminating power (DP) and Resolving power (RP). Greater levels of polymorphism between genotypes were indicated by higher values of the indices.

The Past 4.03 (Paleontological Statistics) software was used to construct the Jaccard index (Table 5). Based on the existence or lack of alleles, the Jaccard index calculates genetic similarity between genotypes on a scale of 0–1, where 1 denotes total similarity and 0 denotes complete divergence. On this same platform, a Principal Components Analysis (PCA) was generated using variance-covariance matrix, which allowed the genotypes to be plotted on a graph (Figure 9). It is, therefore, ways of evaluating the degree of difference between parental lines among themselves and among other genotypes.

Furthermore, using IBM SPSS Statistics 25.0 software, a dendrogram was created to examine the genetic similarity between the genotypes. The "Between groups linkage" method to classify the genotypes into hierarchical clusters was used based on the binary data.

4. RESULTS

According to Standard Evaluation System for Rice (International Rice Research Institute, 2002), the biomass, yield, and harvest index were assessed. Regarding Drought Sensitivity (DRS), we can see that the Hungarian varieties were badly affected while Irat 109 showed higher level ofresistance. In the first stage we could find Marilla and Dáma having leaves margins touching (0-shape) and they dried completely in the next stage. While Irat 109 only had the leaves folded (deep V-shape) followed by the tips drying up to 1/4. As for DH, the results were varied, but Marilla x Irat 109 presented better indices than those resulting from the cross Dáma x Irat 109.

Table 3: Assessment according to Standard Evaluation System for Rice.

Genotype		Biomass (g)	Yield (g)	Harvest index (%)	DRS (leaf rolling at vegetative stage)	DRS (leaf drying at vegetative stage)
1	Marilla	970	400	41,2%	7	9
2	Irat 109	760	150	19,7%	3	4
3	Dáma	380	60	15,8%	8	8
4	1080/10/6	930	450	48,4%	2	2
5	1080/10/27	680	270	39,7%	2	3
6	1080/10/29	610	180	29,5%	2	2
7	1080/10/30	480	100	20,8%	2	3
8	1080/10/42	1110	440	39,6%	2	2
9	1080/10/49	400	30	7,5%	1	1
10	1080/10/52	680	180	26,5%	1	2
11	1080/11/14	540	160	29,6%	2	2
12	1080/11/26	770	270	35,1%	2	2
13	1080/11/50T	300	50	16,7%	1	1
14	1080/11/58	680	200	29,4%	2	3
15	1080/10/15	390	30	7,7%	5	5
16	1080/10/25	840	350	41,7%	4	5
17	1080/10/41	580	190	32,8%	5	6
18	1080/10/47	510	90	17,6%	5	6
19	1080/10/51	970	450	46,4%	8	8
20	1080/11/1	730	260	35,6%	4	4
21	1080/11/4	810	240	29,6%	5	5
22	1080/11/16	520	110	21,2%	6	7
23	1080/11/23	820	310	37,8%	5	5
24	1080/11/43	580	220	37,9%	6	6
25	1080/11/62	840	310	36,9%	6	6
26	1087/1_20	860	310	36,0%	6	6
27	1087/3_30	440	120	27,3%	5	5
28	1087/3_37				2	2

29	1087/4/60	460	150	32,6%	3	3
30	1087/6_33	200	90	45,0%	3	3
31	1087/6_46				8	8
32	1087/8_40	480	190	39,6%	7	7
33	1087/8_55	340	110	32,4%	8	9

The data presented in Table 4 portray the outcomes of the crossbreeding of Dáma x Irat 109 under drought and well-irrigated conditions. As it can be seen from the results, significant differences – highlighted in bold – were hardly scored. The Water Band Index only differed in case of 1087/6_33. Regarding the Chlorophyll Absorbance Index (IAD), no significant differences were observed. However, in terms of the Canopy Chlorophyll Content Index (CCI), samples 1087/3_30 and 1087/4/60 exhibited variations, indicating substantial differences in chlorophyll concentration under these conditions. This is also ratified by the Chlorophyll Normalized Difference Vegetation Index (CNDVI) that showed differences in chlorophyll density in samples 1087/3_30 and 1087/6_46. The Photochemical Reflectance Index (PRI) is another way of approaching chlorophyll quantity, significant differences were noted in the case of 1087/3_30 and 1087/3_37, but these genotypes managed to resist the drying process. The Carotenoid Reflectance Index (CRI1) highlighted a notable distinction in sample 1087/6_46, which dried almost completely, thus corroborating its critical health status.

Table 5 presents a comparison of genotypes under identical treatment conditions. In case of the drought treatment plants showed similar patterns in terms of all the indicis. However, in the case of the well-watered plants it becomes evident that indices related to chlorophyll exhibit greater variability under well-watered conditions. As anticipated, the Water Band Index (WBI) effectively discerned the water disparity between treatments. Furthermore, the Dry Canopy Nitrogen Index (DCNI) illustrated that genotypes retain varying levels of nitrogen in the presence of water, which is therefore crucial to sustaining their growth.

Table 4: Stress indices comparing each genotype under drought and well-irrigated conditions.

Genotype	Irrigation	IAD	CCI	CRI1	CNDVI	PRI	WBI	DCNI
1087/1_20	Drought	1,034±0,008a	15,832±0,805a	0,127±0,007a	0,435±0,011a	0,022±0,007a	0,966±0,003a	56,180±1,299a
	Well-watered	1,025±0,018a	14,121±1,234a	0,131±0,004a	0,422±0,014a	0,005±0,006a	0,962±0,006a	52,995±1,478a
1087/3_30	Drought	1,030±0,008a	**15,576±1,385a**	0,140±0,010a	**0,442±0,014a**	**0,018±0,003a**	0,969±0,003a	54,607±1,775a
	Well-watered	1,021±0,009a	**10,266±0,706b**	0,138±0,006a	**0,399±0,012b**	**0,005±0,004b**	0,964±0,004a	49,834±1,705a
1087/3_37	Drought	0,975±0,011a	11,652±0,630a	0,113±0,005a	0,411±0,011a	**0,013±0,003a**	0,966±0,002a	**52,311±1,068a**
	Well-watered	0,949±0,019a	9,539±1,059a	0,106±0,007a	0,372±0,017a	**0,003±0,002b**	0,965±0,002a	**46,002±2,037b**
1087/4/60	Drought	0,988±0,014a	**13,023±0,964a**	0,113±0,005a	0,416±0,013a	0,019±0,004a	0,965±0,004a	53,626±1,503a
	Well-watered	1,026±0,011a	**19,389±2,698b**	0,132±0,008a	0,437±0,014a	0,013±0,005a	0,965±0,004a	55,319±1,904a
1087/6_33	Drought	1,026±0,017a	16,595±1,152a	0,142±0,005a	0,466±0,010a	0,024±0,004a	**0,958±0,002a**	56,943±1,499a
	Well-watered	1,010±0,015a	17,295±1,019a	0,129±0,010a	0,445±0,011a	0,003±0,007a	**0,976±0,005b**	54,768±1,011a
1087/6_46	Drought	1,001±0,009a	13,309±1,035a	**0,125±0,004a**	**0,429±0,007a**	0,014±0,006a	0,960±0,005a	**55,662±1,126a**
	Well-watered	1,005±0,007a	12,214±1,406a	**0,106±0,007b**	**0,388±0,021b**	0,011±0,006a	0,966±0,003a	**50,300±2,548b**
1087/8_40	Drought	1,020±0,014a	12,618±0,976a	0,129±0,007a	0,423±0,013a	0,015±0,003a	0,964±0,003a	52,648±1,575a
	Well-watered	1,000±0,007a	10,181±0,695a	0,130±0,007a	0,410±0,017a	0,013±0,005a	0,966±0,002a	50,020±1,947a
1087/8_55	Drought	1,004±0,010a	18,367±2,039a	0,124±0,009a	0,427±0,019a	0,011±0,004a	0,973±0,002a	54,972±2,336a
	Well-watered	1,012±0,017a	11,980±1,030a	0,107±0,007a	0,397±0,013a	0,013±0,003a	0,968±0,005a	50,777±1,891a

Note: Values in the same coloumn and subtable not sharing the same subscript are significantly different at p< 0,05 in the two-sided test of equality for column means. Samples are colour coded based on their leaf drying values.

Table 5: Stress indices comparing the genotypes to the same treatment, under drought and well-irrigated conditions.

Irrigation	Genotype	IAD	CCI	CRI1	CNDVI	PRI	WBI	DCNI
Drought	1087/1_20	1,034±0,008a	15,832±0,805a,b	0,127±0,007a	0,435±0,011a	0,022±0,007a	0,966±0,003a,b	56,180±1,299a
	1087/3_30	1,030±0,008a	15,576±1,385a,b	0,140±0,010a	0,442±0,014a	0,018±0,003a	0,969±0,003a,b	54,607±1,775a
	1087/3_37	0,975±0,011b	11,652±0,630a	0,113±0,005a	0,411±0,011a	0,013±0,003a	0,966±0,002a,b	52,311±1,068a
	1087/4/60	0,988±0,014a,b	13,023±0,964a,b	0,113±0,005a	0,416±0,013a	0,019±0,004a	0,965±0,004a,b	53,626±1,503a
	1087/6_33	1,026±0,017a,b	16,595±1,152a,b	0,142±0,005a	0,466±0,010a	0,024±0,004a	0,958±0,002a	56,943±1,499a
	1087/6_46	1,001±0,009a,b	13,309±1,035a,b	0,125±0,004a	0,429±0,007a	0,014±0,006a	0,960±0,005a,b	55,662±1,126a
	1087/8_40	1,020±0,014a,b	12,618±0,976a	0,129±0,007a	0,423±0,013a	0,015±0,003a	0,964±0,003a,b	52,648±1,575a
	1087/8_55	1,004±0,010a,b	18,367±2,039b	0,124±0,009a	0,427±0,019a	0,011±0,004a	0,973±0,002b	54,972±2,336a
Well-watered	1087/1_20	1,025±0,018a	14,121±1,234a,c,d,e,f	0,131±0,004a	0,422±0,014a,b	0,005±0,006a	0,962±0,006a	52,995±1,478a,c,d
	1087/3_30	1,021±0,009a	10,266±0,706a,b	0,138±0,006a	0,399±0,012a,b	0,005±0,004a	0,964±0,004a	49,834±1,705a,c,d
	1087/3_37	0,949±0,019b	9,539±1,059b,d	0,106±0,007a	0,372±0,017b	0,003±0,002a	0,965±0,002a	46,002±2,037c
	1087/4/60	1,026±0,011a	19,389±2,698f	0,132±0,008a	0,437±0,014a,b	0,013±0,005a	0,965±0,004a	55,319±1,904b,d
	1087/6_33	1,010±0,015a	17,295±1,019c,f	0,129±0,010a	0,445±0,011a	0,003±0,007a	0,976±0,005a	54,768±1,011a,b
	1087/6_46	1,005±0,007a,b	12,214±1,406a,c,d,e	0,106±0,007a	0,388±0,021a,b	0,011±0,006a	0,966±0,003a	50,300±2,548a,c,d
	1087/8_40	1,000±0,007a,b	10,181±0,695b,e	0,130±0,007a	0,410±0,017a,b	0,013±0,005a	0,966±0,002a	50,020±1,947a,c,d
	1087/8_55	1,012±0,017a,b	11,980±1,030a,c,d,e	0,107±0,007a	0,397±0,013a,b	0,013±0,003a	0,968±0,005a	50,777±1,891a,c,d

Note: Values in the same coloumn and subtable not sharing the same subscript are significantly different at p< 0,05 in the two-sided test of equality for column means.
Samples are colour coded based on their leaf drying values.

In this study, thirty-five rice genotypes were fingerprinted using eleven CDDP markers. On the gel photos (Figures 6, 7, 8), the red arrows indicate polymorphic fragments generated with ERF1, ERF2 and ABP1-1, respectively. Based on the presence and absence of the fragments, a binary table was generated, which was used for the following analyzes.

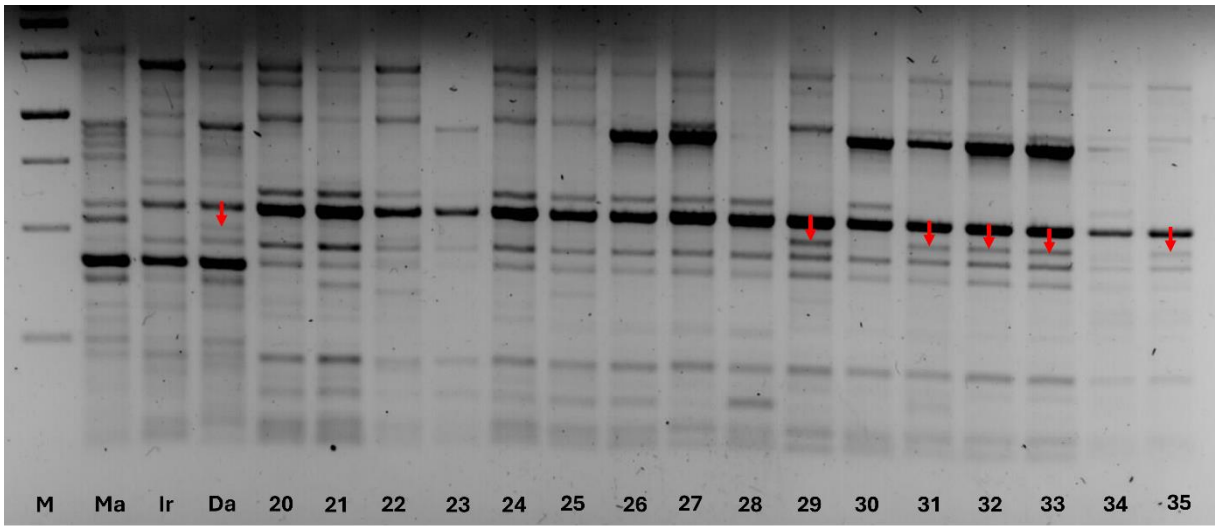

Figure 6: 1% TBE agarose gel, pattern generated with ERF1 marker, order of the samples: Marilla, Irat 109, Dáma, 20-35. M: Thermo Scientific GeneRuler 1 kb DNA Ladder.

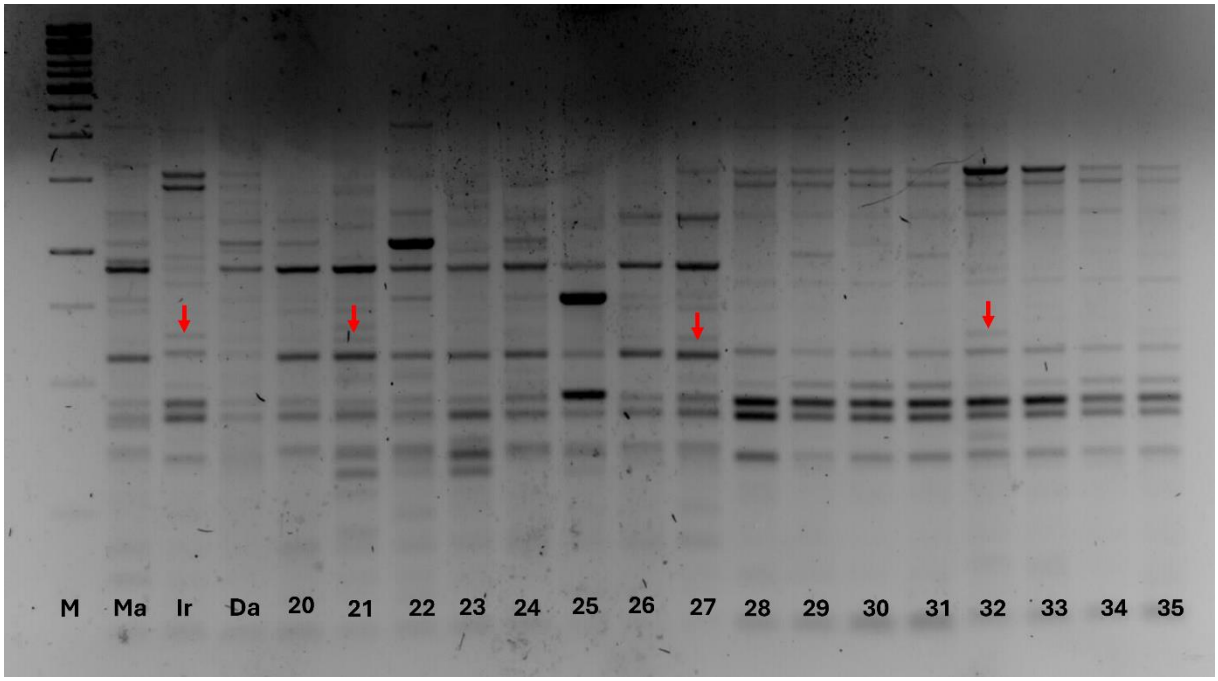

Figure 7: 1% TBE agarose gel, pattern generated with ERF2 marker, order of the samples: Marilla, Irat 109, Dáma, 20-35. M: Thermo Scientific GeneRuler 1 kb DNA Ladder.

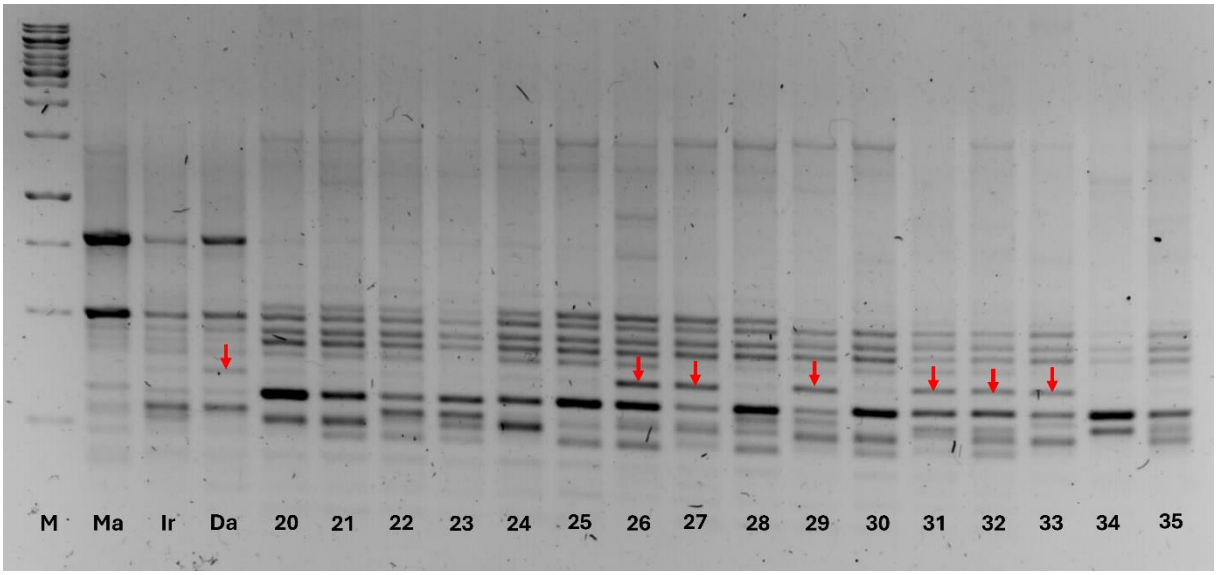

Figure 8: 1% TBE agarose gel, pattern generated with ABP1-1 marker, order of the samples: Marilla, Irat 109, Dáma, 20-35. M: Thermo Scientific GeneRuler 1 kb DNA Ladder.

Table 6: Summary of the results of all samples regarding total number of alleles and the range of allele sizes.

Locus	Number of Alleles	Allele Size Range (bp)
ABP1-1	9	75-1000
ERF1	11	75-5000
ERF2	11	75-4000
KNOX-1	8	75-3000
KNOX-2	5	500-1500
MADS-1	7	75-700
MADS-4	5	75-700
MYB1	2	75-2000
MYB2	8	300-3000
WRKY-F1	8	75-4000
WRKY-R3	11	75-3000

When it comes to the ERF1 pattern (Figure 6), we can observe that a fragment about 1500 bp is unique to Dáma among the parent lines. Samples 29, 31, 32, 33, and 35 all contain the same fragment, indicating a closer relation with Dáma. This particular marker is within those that detected the highest quantity of alleles (Table 6). Similarly, ERF2 (Figure 7) was able to

identify an allele only present in Irat 109 in between 700 and 1000 bp. Samples 21, 27 and 32 have that fragment. In another instance, ABP1-1 (Figure 8) found a Dáma-specific fragment roughly 300 bp and samples 26, 27, 29, 30, 31 and 32 have it. Consequently, these markers have shown to be useful for tracking distinctive alleles of the parental lines, providing a way to detect variations amongst samples.

In order to provide solid information regarding each CDDP marker's ability to discriminate across samples using statistical analysis, all eleven of them were computed together to provide five polymorphism indices through iMEC: Online Marker Efficiency Calculator.

The polymorphic information content (PIC) values of KNOX2 (PIC = 0.389), WRKY-R3 (PIC = 0.375), ABP1 (PIC = 0.373), and MYB2 (PIC = 0.369) were the highest among the eleven markers that were evaluated. The efficacy of the primer-marker system is shown by the effective multiplex ratio (EMR), which has a range of 1,314 to 8,886. Higher values denote more efficacy. Similar to EMR, the Marker Index (MI) is viewed as better performance being indicated by higher numbers. The discriminating power indicates that KNOX2 (DP=0.286) has the lowest ability to differentiate individuals in a population, while ERF2 (DP=0.709), ERF1 (DP=0.689), and KNOX1 (DP=0.624) have the best ability to separate individuals in a population, minimizing the risk of confusion between individuals. The capacity to discriminate between the analyzed samples is correlated with the resolving power, which is based on the distribution of alleles within the genotypes examined. In this case, ERF1 (RP=5,600) has the greatest ability to distinguish the genotypes (Table 7).

As a member of the AP2/ERF superfamily, the ERF family is a large gene family of transcription factors. The AP2/ERF proteins have been demonstrated to have significant roles in the transcriptional regulation of numerous biological processes associated with development and growth, as well as diverse responses to environmental stimuli, including hormone signal transduction, biotic and abiotic stress response, metabolic control, and developmental processes in different plant species (Nakano et al., 2006). Thus, it is important to observe how the drought state affects the functioning of transcription factors in different genotypes.

Table 7: Index generated with the iMEC online tool (Amiryousefi et al., 2018).

Loci	PIC	EMR	MI	DP	RP
ABP1-1	0,373	7,229	0,007	0,355	2,343
ERF1	0,301	6,143	0,008	0,689	5,600
ERF2	0,299	5,943	0,008	0,709	3,829
KNOX1	0,311	4,914	0,008	0,624	1,829
KNOX2	0,389	4,229	0,006	0,286	0,457
MADS1	0,339	5,000	0,008	0,491	2,000
MADS4	0,314	3,143	0,008	0,606	1,886
MYB1	0,321	1,314	0,008	0,571	0,629
MYB2	0,369	6,343	0,007	0,372	1,486
WRKY-F1	0,355	6,057	0,008	0,427	3,371
WRKY-R3	0,375	8,886	0,007	0,348	3,371

The principal component analysis was a tool that allowed positioning each genotype on an Eigenvalue scale. The figure 9 displays the relationships between all 35 genotypes at the same time. The distance between points implies greater sample variation. Figure 9's PC1 (component 1) and PC2 (component 2) each account for 17,48% and 12.62% of the variation, respectively. We can notice that Marilla and Dáma, the Hungarian varieties, are close together while the African variety, Irat 109, is the furthest away, the hybrids are located inbetween the parental genotypes.

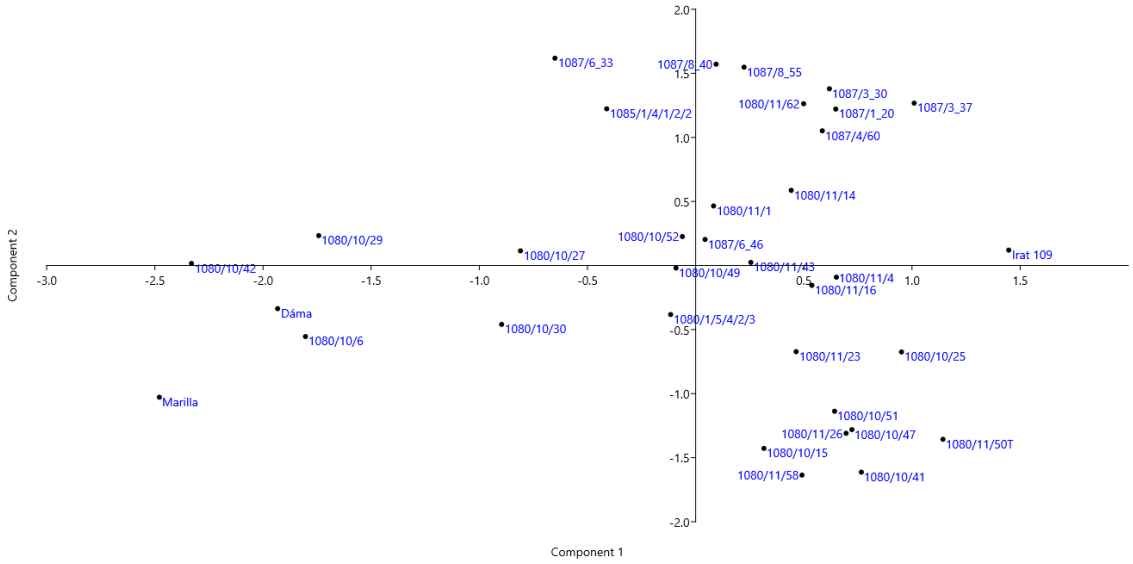

Figure 9: PCA score plot of the rice genotypes.

The most related genotypes are shown by the Jaccard index. In table 8, the darker the color, the more closely the genotypes are associated with each other. The greatest similarity is found between 1087/8_40 and 1087/8_55 with an index of 0.94. Followed by 1087/1_20 and 1087/3_30 with an index of 0.91. Among the parental lineages, Irat 109 and Marilla are the most distant with only 0.49 similarity.

Among the samples resulting from the crosses between Dáma and Irat 109, 1087/3_30 and 1087/3_37 exhibit the highest resemblance to Irat 109, with values of 0.83 and 0.82 respectively. They demonstrate milder effects regarding drought sensitivity, as indicated in Table 3, which may be associated with the presence of the resistance capacity of Irat 109. The samples 1087/6_46, 1087/8_40, 1087/8_55, which showed less similarity, with values below 0.80, suffered severe drought injury.

In the case of samples from the crossing between Marilla and Irat 109, 1080/11/26 exhibited the highest similarity to Irat 109, with a value of 0.82, showcasing remarkable drought resistance. Intriguingly, despite displaying the lowest similarity, at a value of 0.60, 1080/10/42 also demonstrated robust resilience, even ranking among the top for biomass, yield, and harvest index among all evaluated genotypes.

Using the CDDP data, a dendrogram was created to determine genetic relatedness. In the dendrogram, there are two main groups. Irat 109 and most of the crosses are found in the

first and largest group. Marilla, Dáma, and certain hybrids between Marilla and Irat 109 make up the second major group. Overall, 1087/8_40 and 1087/8_55 are the closest ones, being the first to be grouped, which was also seen previously as they had the higher Jaccard index. Again, 1087/1_20 and 1087/3_30 are shown as the second closest. The Marilla genotype is the most distinct and genetically remote from the other samples in its cluster, as indicated in Figure 10.

Table 8: Genetic relationship of rice genotypes based on the Jaccard index.

	Marilla	Irat 109	Dáma	1080/10/6	1080/10/27	1080/10/29	1080/10/30	1080/10/42	1080/10/49	1080/10/52	1080/11/14	1080/11/26	1080/11/50T	1080/11/58	1080/10/15	1080/10/25	1080/10/41	1080/10/47	1080/10/51	1080/11/1	1080/11/4	1080/11/16	1080/11/23	1080/11/43	1080/11/62	1087/1_20	1087/3_30	1087/3_37	1087/4/60	1087/6_33	1087/6_46	1087/8_40	1087/8_55	1080/1/5/4/2/3	1085/1/4/1/2/2
Marilla	1,00																																	
Irat 109	0,49	1,00																																
Dáma	0,72	0,58	1,00																															
1080/10/6	0,74	0,64	0,79	1,00																														
1080/10/27	0,63	0,76	0,71	0,84	1,00																													
1080/10/29	0,74	0,66	0,75	0,83	0,80	1,00																												
1080/10/30	0,65	0,70	0,74	0,76	0,82	0,78	1,00																											
1080/10/42	0,77	0,60	0,78	0,83	0,78	0,85	0,82	1,00																										
1080/10/49	0,59	0,78	0,67	0,72	0,75	0,79	0,75	0,72	1,00																									
1080/10/52	0,58	0,75	0,69	0,68	0,82	0,75	0,77	0,69	0,81	1,00																								
1080/11/14	0,58	0,79	0,68	0,73	0,81	0,70	0,74	0,68	0,80	0,82	1,00																							
1080/11/26	0,60	0,82	0,63	0,73	0,78	0,70	0,77	0,66	0,72	0,69	0,78	1,00																						
1080/11/50T	0,59	0,78	0,62	0,67	0,67	0,64	0,68	0,61	0,71	0,70	0,75	0,86	1,00																					
1080/11/58	0,61	0,77	0,64	0,68	0,71	0,68	0,80	0,71	0,75	0,69	0,74	0,82	0,78	1,00																				
1080/10/15	0,66	0,75	0,65	0,71	0,74	0,71	0,75	0,72	0,71	0,68	0,72	0,85	0,84	0,85	1,00																			
1080/10/25	0,61	0,77	0,62	0,66	0,71	0,73	0,70	0,62	0,73	0,75	0,76	0,82	0,83	0,82	0,85	1,00																		
1080/10/41	0,58	0,77	0,66	0,68	0,68	0,65	0,74	0,63	0,70	0,71	0,76	0,85	0,84	0,82	0,80	0,80	1,00																	
1080/10/47	0,58	0,80	0,64	0,63	0,69	0,70	0,77	0,64	0,81	0,80	0,74	0,77	0,81	0,83	0,78	0,80	0,80	1,00																
1080/10/51	0,58	0,77	0,66	0,68	0,71	0,63	0,72	0,64	0,68	0,69	0,77	0,82	0,81	0,77	0,78	0,77	0,83	0,77	1,00															
1080/11/1	0,63	0,76	0,63	0,72	0,75	0,69	0,74	0,70	0,69	0,71	0,78	0,75	0,72	0,71	0,76	0,76	0,71	0,71	0,71	1,00														
1080/11/4	0,59	0,75	0,65	0,69	0,77	0,66	0,70	0,65	0,76	0,81	0,86	0,77	0,76	0,78	0,76	0,78	0,75	0,75	0,73	0,77	1,00													
1080/11/16	0,59	0,75	0,67	0,74	0,70	0,69	0,70	0,67	0,76	0,73	0,80	0,75	0,82	0,75	0,76	0,78	0,78	0,75	0,78	0,77	0,76	1,00												
1080/11/23	0,61	0,76	0,70	0,69	0,75	0,71	0,76	0,65	0,79	0,82	0,84	0,78	0,77	0,81	0,76	0,84	0,78	0,85	0,79	0,75	0,82	0,79	1,00											
1080/11/43	0,63	0,76	0,66	0,73	0,76	0,70	0,77	0,70	0,77	0,79	0,81	0,78	0,77	0,76	0,79	0,76	0,76	0,74	0,77	0,81	0,80	0,77	0,78	1,00										
1080/11/62	0,59	0,80	0,62	0,69	0,76	0,73	0,75	0,71	0,81	0,80	0,85	0,74	0,73	0,74	0,70	0,75	0,69	0,75	0,72	0,74	0,81	0,81	0,78	0,79	1,00									
1087/1_20	0,60	0,80	0,65	0,67	0,72	0,71	0,73	0,70	0,79	0,75	0,82	0,75	0,76	0,77	0,78	0,80	0,70	0,75	0,70	0,74	0,76	0,84	0,79	0,82	0,88	1,00								
1087/3_30	0,58	0,83	0,65	0,69	0,77	0,73	0,75	0,69	0,73	0,75	0,79	0,74	0,76	0,75	0,78	0,80	0,70	0,75	0,70	0,79	0,76	0,84	0,76	0,77	0,88	0,91	1,00							
1087/3_37	0,55	0,82	0,61	0,66	0,74	0,70	0,72	0,64	0,78	0,77	0,84	0,76	0,78	0,71	0,72	0,82	0,74	0,74	0,72	0,81	0,83	0,78	0,78	0,82	0,85	0,83	0,85	1,00						
1087/4/60	0,59	0,77	0,64	0,66	0,71	0,70	0,72	0,66	0,72	0,74	0,76	0,76	0,75	0,67	0,72	0,74	0,74	0,72	0,74	0,76	0,78	0,75	0,73	0,76	0,82	0,80	0,80	0,85	1,00					
1087/6_33	0,64	0,73	0,70	0,75	0,78	0,76	0,73	0,75	0,74	0,73	0,80	0,68	0,64	0,68	0,71	0,71	0,65	0,66	0,68	0,82	0,71	0,74	0,72	0,80	0,76	0,81	0,81	0,81	0,73	1,00				
1087/6_46	0,63	0,71	0,73	0,73	0,73	0,75	0,74	0,68	0,75	0,74	0,76	0,73	0,77	0,74	0,74	0,79	0,76	0,77	0,74	0,70	0,75	0,77	0,78	0,71	0,79	0,77	0,82	0,79	0,82	0,72	1,00			
1087/8_40	0,61	0,76	0,71	0,70	0,75	0,74	0,76	0,73	0,69	0,71	0,81	0,75	0,72	0,71	0,74	0,79	0,73	0,69	0,76	0,77	0,74	0,74	0,75	0,78	0,81	0,84	0,87	0,84	0,81	0,82	0,81	1,00		
1087/8_55	0,58	0,76	0,68	0,68	0,73	0,74	0,74	0,70	0,69	0,69	0,75	0,75	0,72	0,71	0,74	0,76	0,73	0,69	0,74	0,75	0,74	0,72	0,72	0,73	0,79	0,82	0,84	0,84	0,86	0,82	0,81	0,94	1,00	
1080/1/5/4/2/3	0,69	0,69	0,68	0,70	0,70	0,74	0,73	0,70	0,77	0,76	0,75	0,75	0,80	0,76	0,74	0,79	0,70	0,82	0,73	0,72	0,77	0,77	0,80	0,78	0,79	0,77	0,76	0,78	0,71	0,75	0,78	0,72	0,70	1,00
1085/1/4/1/2/2	0,67	0,67	0,73	0,65	0,68	0,74	0,71	0,73	0,72	0,76	0,78	0,68	0,72	0,66	0,69	0,76	0,66	0,71	0,74	0,73	0,72	0,72	0,75	0,75	0,79	0,82	0,76	0,78	0,76	0,80	0,75	0,85	0,80	0,83	1,00

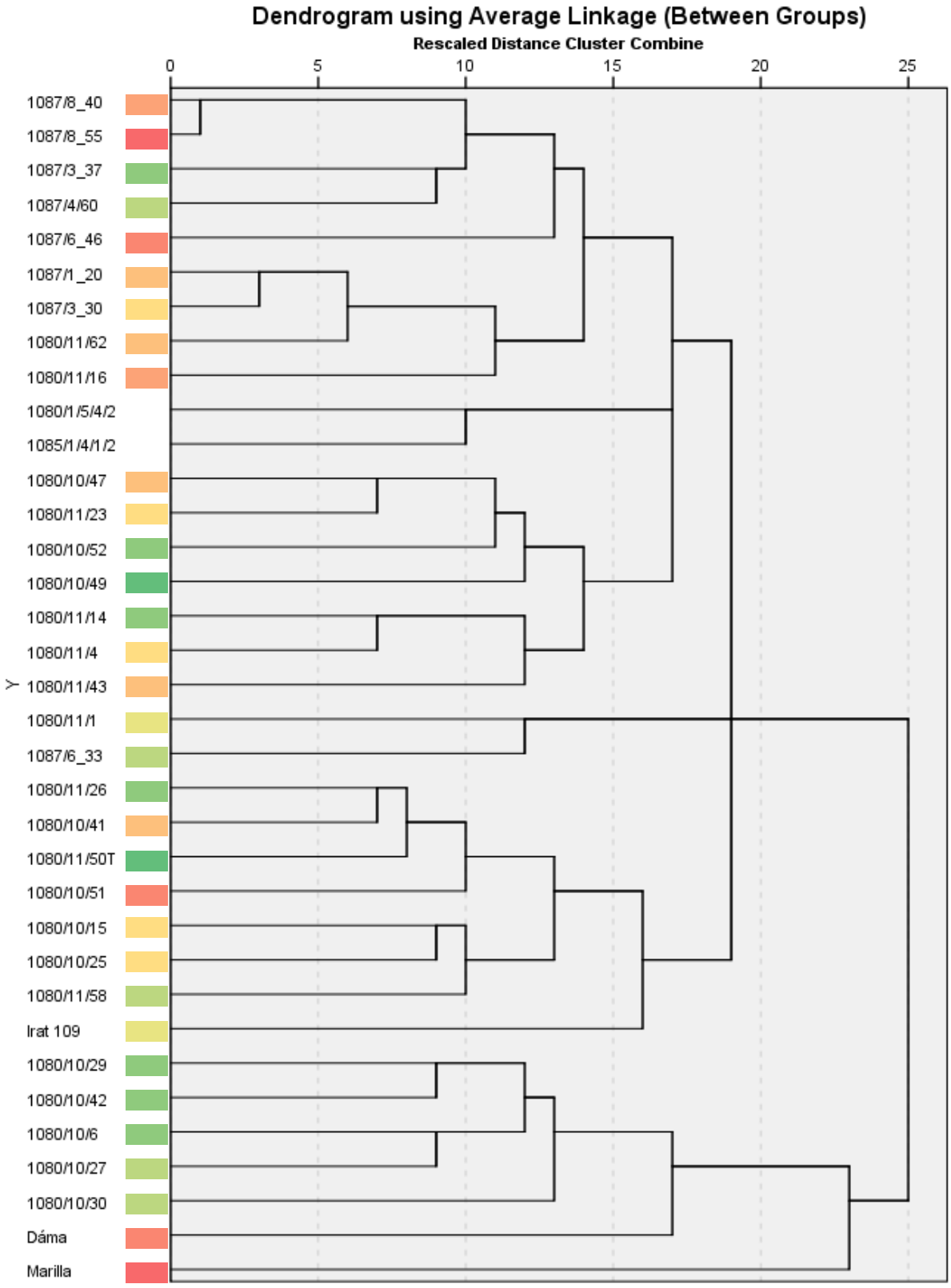

Figure 10: Dendrogram using between group linkage as the clustering method, samples are colour coded based on their leaf drying values

5. CONCLUSION AND RECOMMENDATIONS

The present master thesis examines double haploid rice lines that were exposed to drought stress on-field. Analyzing drought sensitivity, while Irat 109 exhibited resilience, the Hungarian parental lines, Marilla and Dama, were more affected as expected. Notably, certain crosses displayed improved phenotypes, with reduced leaf curling or drying. Subsequent analysis using the Jaccard index revealed that genotypes exhibiting greater similarity to Irat 109 also demonstrated enhanced drought resistance.

By screening conserved DNA-derived polymorphism (CDDP) markers, Marilla and Irat 109 are the parental lines that are most distant from one another, according to the marker data and principal component analysis. This is evident in both the dendogram, where Marilla and Irat 109 are forming two distinct groups, and the table of percentage comparison from the Jaccard index.

Utilizing leaf spectrometer data, various indices were computed to assess how different genotypes responded to varying conditions of well-irrigated and drought growth. The analysis revealed significant impacts on chlorophyll and nitrogen levels.

The selection of markers is largely influenced by the expected degree of polymorphism and the genome coverage of the molecular markers. The MADS showed the worst indices out of the eleven that were examined, and it is advised that they not be considered for further analysis. ABP1-1, MYB, WRKY, ERF, and KNOX met expectations. Markers designed for WRKY, ERF and KNOX stand out, which are related to transcription factors acting in stress response.

Since Irat 109 is described in the literature as a drought tolerant genotype (Anyaoha et al., 2018), it would be crucial for the traits of this variety to be widely distributed as a goal of a plant breeding effort. The set of genotypes in this work that showed a high Jaccard index and proximity to Irat 109 in the dendrogram, indicating a high degree of similarity, should be investigated further. It is recommended that in order to enhance the selection of drought tolerant DH lines, more field data should be cross-referenced with molecular markers in the future. And it is anticipated that in aerobic growth, those genotypes will exhibit improved tillers, maturity, and average leaf area, among other traits, compared to observations of genotypes susceptible to drought stress.

6. SUMMARY

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

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Course: MSc Agricultural Biotechnology

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Rice (*Oryza sativa* L.) is vital for global food security, with Asia producing 90% of it, but yields are stagnant (Bandumula, 2018). By 2050, with a projected global population of 9.1 billion, agricultural productivity needs to increase by 70% (Wani & Kumar Sah, 2014). Adding to this complexity is the pervasive threat of drought, a major abiotic stressor known to disrupt rice growth and productivity through diverse morphological and physiological alterations (Panda et al., 2021). Compounding these issues is climate change, exacerbating water scarcity and underscoring the urgent need for effective water management strategies to sustain agriculture (Surendran et al., 2021).

Rice, a semiaquatic plant, typically grows in flooded conditions, requiring 3,000 to 5,000 liters of water per kilogram, much more than wheat and corn (Dey et al., 2018). To mitigate the water-intensive nature of rice cultivation and bolster yields, the adoption of alternative irrigation systems emerges as imperative. Among these alternatives, aerobic rice cultivation, adaptable to flat or terraced landscapes with ample rainfall or irrigation, offers a viable solution, particularly in regions where water is scarce for flooded rice cultivation (Parthasarathi et al., 2012). While alternate irrigation can improve water productivity, without drought-resistant rice varieties, production may suffer (Jancsó et al., 2022).

Natural genotypic variation in rice offers a promising avenue for discovering novel drought-tolerant traits (Panda et al., 2021). A method to accelerate breeding procedures is the doubled haploid (DH) plant technique. By utilizing anther culture (AC) procedures, homozygous DH lines can be generated within a single generation, facilitating the early selection of recessive alleles due to the homozygosity of DH lines. This approach holds considerable promise for breeding initiatives, particularly when complemented by molecular marker-assisted techniques (Lantos et al., 2023).

The utilization of the Conserved DNA Derived Polymorphism (CDDP) method presents another avenue for enhancing drought resilience in rice. This strategy, proposed by Collard and Mackill (2009), leverages multiple primer binding sites across plant genomes to identify conserved regions within genes. The regulation of stress-responsive genes is important to fortify plants against drought, with specific transcription factors assuming pivotal roles (Joo et al., 2013).

In this study, phenotypic data were collected during plant growth, biomass, yield, and harvest index assessments, as well as drought sensitivity (DRS). During this period, spectrometry data were also collected and was processed to calculate the indices IAD, CCI, CRI1, CNDVI, PRI, WBI, DCNI.

Following this, thirty-five rice genotypes underwent fingerprinting using a panel of eleven CDDP markers known for their association with abiotic resistance. The resulting binary table, structured based on the presence or absence of fragments, laid the groundwork for subsequent analyses. Five polymorphism indices were calculated using iMEC: Online Marker Efficiency Calculator to properly evaluate how effective the CDDP markers were. Expectations were met by ABP1-1, MYB, WRKY, ERF, and KNOX. And through Principal Component Analysis, Jaccard index and a dendrogram, the genetic relationships between the samples were explored.

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Signed below, _____, student of the Szent István Campus of the Hungarian University of Agriculture and Life Science, at the BSc/MSc Course of _____ declare that the present Thesis is my own work and I have used the cited and quoted literature in accordance with the relevant legal and ethical rules. I understand that the one-page-summary of my thesis will be uploaded on the website of the Campus/Institute/Course and my Thesis will be available at the Host Department/Institute and in the repository of the University in accordance with the relevant legal and ethical rules.

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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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