THESIS OF PHD DISSERTATION

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THESIS OF PHD DISSERTATION

ADAPTATION OF DOUBLED HAPLOID RICE LINES FOR THE DEVELOPMENT OF ABIOTIC AND BIOTIC STRESS TOLERANCE OF HUNGARIAN RICE IN TRADITIONAL AND AEROBIC CONDITIONS

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The candidate has fulfilled all the requirements of the Doctoral Regulations of the Hungarian University of Agriculture and Life Sciences, and has taken into account the comments and suggestions made during the home defence when revising the thesis, therefore the thesis may be submitted for the public defence procedure.

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PREVIOUS WORKS AND OBJECTIVES

Rice (*Oryza sativa* L.) is a special element of Hungarian agriculture and plant breeding. This plant is one of the basic foods of humanity, and its cultivation range extends from 35 degrees southern latitude to 50 degrees northern latitude, adapting to a wide range of ecological conditions from coastal marshes to mountainous areas. Hungary represents the northern limit of rice cultivation in Europe, therefore the marginal climatic conditions determine the range of rice varieties that can be grown. In Hungary, among abiotic stress factors, periodically low temperatures, high soil salinity, and increasingly hot summers can reduce crop yields. Although rice cultivation benefits from flood irrigation, providing shallow water habitats during the hottest summer months, globally, it is an important goal to improve the efficiency of irrigation water use, reduce methane emissions, and enhance the drought tolerance of plants that can be grown under aerobic conditions.

The evapotranspiration values of rice crops vary between 500-800 mm worldwide, and only rarely exceed 1000 mm. However, irrigation water usage exceeds 1000 mm even in Hungary, and in some regions of the world, this value exceeds 3000 mm (Simonné Kiss and Ipsits, 1992). Consequently, rice fields use 35-45% of all irrigation water (Bouman et al., 2006). These values clearly show that reducing the amount of irrigation water would have a significant impact.

Europe accounts for only 0.4% of the world's rice-growing areas, with two countries sharing 64% of that (Italy with 229.5 thousand hectares and Russia with 180.2 thousand hectares). The average rice consumption in Europe is also relatively low, at just 4.6 kg per person per year. Currently, Hungary is considered a small rice-producing country, with domestic producers cultivating just over 3,000 hectares.

Following Hungary's accession to the European Union, local producers and breeders must compete in a larger market with a more intense competition. Foreign breeding companies, primarily from Italy, which have larger rice-growing areas and therefore greater financial resources (Kraehmer et al., 2017), have introduced new, modern varieties for the Hungarian producers.

These rice varieties periodically achieve outstanding yields in Hungarian growing areas. However, their long-term cultivability is strongly affected by cold periods throughout the growing season, particularly in early spring and early autumn. For varieties with longer duration than current Hungarian-bred varieties, quality deterioration and even partial or total crop loss due to the increased sterility are common. However, according to climate change forecasts affecting Hungary (IPCC, 2023), warming temperatures may improve rice cultivation conditions in the future.

Most traditional plant breeding methods rely on creating high variability within a population and then selecting from it. With classical methods, it can take many years, even decades, to develop a new plant variety. The significant advantage of effective doubled haploid (DH) methods is that homozygous lines/lines can be produced in a single generation at any stage of the breeding program. Incorporating these methods into classical breeding can shorten the breeding time of a variety or a hybrid parent.

(Jena, 2010) (Jennings et al., 1979).

This time factor is particularly crucial when breeders need to respond to new challenges as quickly as possible. Introducing new gene sources (Jena, 2010) through international connections is effectively achievable in Hungary as well. However, the direct use of most genotypes may be problematic due to the duration or, in most cases, their photoperiodic sensitivity. To increase the efficiency of crossbreeding, we introduced the use of vacuum emasculators based on the recommendations of the International Rice Research Institute (IRRI) (Jennings et al., 1979). In recent years, we have created a large number of segregating populations with diverse genetic backgrounds. To test their competitivity, we have initiated selection trials in greenhouse in addition to the nursery conditions.

Our primary goal in the research and breeding activity is to create modern, high-quality rice varieties for the Hungarian rice production and consumption. To achieve this, we use both traditional breeding tools and biotechnological methods along with modern selection techniques. Our experiments aim to comprehensively study genotypes that can be effectively applied under the temperate climatic conditions and to develop selection methods for both traditional and water-saving (aerobic) production systems as follows:

1. **Preselection for the abiotic and biotic stress tolerance of Hungarian and international genotypes** and the development of a broader segregating population through their crossing, enabling efficient selection of genotypes resistant to environmental stresses. Improvement and automation of the Hungarian rice crossing method by the adaptation of vacuum emasculator devices.

2. Integrating the **androgenesis induced (anther culture) homogeneous (DH) rice lines** from genotypes with good responsivity and their offsprings into the rice breeding process, thereby increasing the efficiency of the breeding program.
3. Initiate multi-year field experiments using **DH lines in both traditional and aerobic rice cultivation**, and analyze the results in comprehensive performance tests.
4. Aerobic cultivation technology is an important opportunity for water-saving rice production. **Determine the differences in water use efficiency** among various rice varieties and candidates using large-scale precision field lysimeters.
5. Utilize our scientific results on general stress tolerance to **breed new, state-recognized rice varieties** that can be directly utilized in production practice.

MATERIALS AND METHODS

Structure of the Hungarian Traditional Rice Breeding Program and Integration of DH Plants into the Process

The fundamental goal during the planning of our experiments was to ensure that new scientific results could be directly utilized in our rice breeding program, thus making them available for practical use as soon as possible. In our traditional crossing program, we utilize the most promising materials from the rice variety collection of MATE IES ÖVKI, aiming to select lines that are effective and have good general adaptability under local conditions.

The traditional rice breeding process in Hungary primarily follows the classical Pedigree method, which is the most common breeding method for self-pollinating plants. This technique involves individual selection over multiple generations using a diverse initial population. Through successive generations, the homogeneity of the populations increases, ultimately resulting in uniform populations that comply with legal requirements and can be the basis for registering a new variety. This process can take several years (up to 9-10 years) for temperate zone rice cultivation, where only one generation can be grown per year using traditional methods. Rapidly changing environmental (climate change) and economic (consumer and processing industry demands) conditions necessitate foresight in breeding planning and shortening the process as much as possible.

Several possible methods are known for this, including:

1. **Shuttle Breeding:** Breeding in multiple ecological environments, potentially utilizing the different seasons of various continents.
2. **Molecular Markers:** Using these markers to select traits that are difficult to test traditionally (e.g., nutritional values, aroma).
3. **Rapid Generation Advance (RGA):** Techniques to accelerate generation cycles.
4. **DH Technique:** Application of double haploid techniques to produce homogeneous lines.

In our case, the breeding process starts traditionally by creating segregating populations through sexual crosses. The location for growing the parent pairs and performing the crosses is the MATE ÖVKI Galambos Rice Research Station in Szarvas. This station is uniquely designed in Hungary for rice breeding and seed production processes. Its main characteristic is that it allows for rice testing and cultivation on much smaller areas (averaging 2500-5000 m^2) than the typical plot sizes found in local farms.

Based on the knowledge acquired from the IRRI rice breeding training, we constructed our own vacuum emasculation equipment in 2015, which enables us to cross genotypes with desired traits much faster than before. The broad genetic base for this work is provided by the rice variety collection of MATE IES ÖVKI.

Throughout our work, we have created dihaploid plants at various stages of the breeding process (F1-F6) using in vitro androgenesis at the GK Biotechnology Laboratory in Szeged. The starting point for haploid production is anthers at the appropriate developmental stage. The process of in vitro androgenesis (anther and microspore culture) and the necessary conditions have been detailed previously. The natural flowering period of rice genotypes that can reliably mature in Hungary is from mid-July to mid-August. For the experiments, except for the F1 generation, donor plants were grown at the MATE ÖVKI Galambos Rice Experimental Station. Based on previous domestic experience, in vitro anther culture efficiency is higher for healthy plants grown in field conditions than for those grown in greenhouses or laboratories.

The produced green plants are grown in vitro in a culture chamber until the end of February, and after acclimatization in the greenhouse, cultivation continues until the end of May. Planting in the experimental field in Szarvas takes place afterward. Individual plants of the DH_0 generation undergo preliminary selection, where the goal is to pre-screen the population based on directly selectable traits (e.g., awn presence, grain type, plant habit, plant height, lodging, earliness, resistance, etc.). To facilitate more effective plant growth, we established a smaller (300 m^2) intermittently flooded cage at the MATE ÖVKI Lysimeter Station, where plants can be grown as long as possible to harvest the maximum amount of seeds. At this stage, field verification of the ploidy level of plants is also performed based on plant habit, panicle and grain morphology, and fertility.

The height of haploid (n) rice plants is lower compared to diploid plants of the same origin, the size of the panicles is smaller, and most importantly, the panicles do not contain fertile seeds. Additionally, the appearance of these plants is significantly characterized by the development of more tillers (up to 30-40 per plant) than diploid and tetraploid plants (Figure 1).

Tetraploid (4n) plants are receiving increasing attention worldwide for their breeding potential. However, they possess several traits that have so far hindered their utilization.

Tetraploid rice plants are similar in height or taller than diploids. In many cases, their stem strength is weaker, making lodging a common risk during the growing season. The number of tillers is usually similar to or fewer than that of diploids. The panicles generally contain fewer grains, and the degree of sterility is significant, although the size of the fertile grains exceeds that of diploid plants. The presence of awns is common, which is a disadvantage in domestic cultivation.

Currently, the practical significance for breeding lies with diploid (2n) plants, among which genotypes suitable for agricultural practice and consumer demands can be selected.

Figure 1. Tetraploid (A), diploid (B) and haploid (C) DH_0 plants in the nursery (Szarvas, 2019)

The DH1 Generation and Small Plot Experiments

For the DH1 generation, the quantity of seeds usually allows the initiation of micro plot experiments. In our program, this involves planting 1.5-meter rows under both flooded and aerobic conditions with 2-3 replications. Due to the large number of genotypes, the need to reduce mixing, and the leaf surface area required for certain analyses (e.g., surface temperature analysis), individual replications are typically placed directly next to each other. In this generation, in addition to general selection surveys, preliminary yield and quality assessments can be conducted.

Performance Tests in DH_{2-3} Generations

In the DH_{2-3} generations, we conduct multi-replication, small-plot performance comparison experiments primarily under traditional conditions using the previously best-rated lines. The agronomic characteristics and general stress tolerance of the new genotypes are compared to the most widely grown rice varieties and the parent genotypes. The basis for evaluation is our comprehensive examination method, emphasizing high yield, high harvest index, secure flowering time under domestic conditions, and suitability for intensive cultivation.

Research Equipments and Analysis

For these studies, we utilized available measuring instruments (CI-710s leaf spectrometer, Testo 885 thermal camera, SSR1 SunScan, soil moisture sensors, etc.). These tools enable us to describe the nutrient utilization efficiency, as well as drought and cold tolerance characteristics of the genotypes. For determining drought tolerance, we employ simple physiological tests that are easily applicable in breeding (e.g., IRRI SES evaluation, determination of relative water content).

Plant materials

Our experiments primarily relied on the rice cultivar collection of the Institute of Horticultural Sciences, MATE IES ÖVKI. This collection includes most rice varieties previously acclimated or bred in Hungary (e.g., Dunghan shali, Kákai 203, Oryzella, Sandora), several globally recognized control genotypes (e.g., Nipponbare, M202, Mangala), and additional lines from various countries around the world.

We tested numerous genotypes to increase the efficiency of DH (doubled haploid) production and successfully apply the refined methodology to the progeny of crossed plants. The substantive part of this dissertation consists of a detailed analysis of the field adaptability of rice varieties selected in previous preliminary experiments and the DH lines produced after crossing.

Key Genotypes

Five key genotypes were crucial for the implementation of the experiments, sexual crosses, and the creation of DH lines via androgenesis induction: Dáma, Marilla, IRAT 109, Sandora, and SZV Tünde.

Dáma (HSC-2)

Recognition: First rice variety in Hungary produced using biotechnological tools, recognized in 1992.

Characteristics: Long growing season, high yield potential, but sensitive to high nitrogen doses leading to lodging. Resistant to blast and has good general stress tolerance, though not competitive under non-flooded conditions.

Use in Breeding: Employed in several crosses (e.g., 1087 combination). SZV Tünde, our new rice variety, also originates from a cross involving Dáma and GB2005.

Marilla

Origin: From the collection of Dr. Ibolya Simonné Kiss.

Characteristics: Early maturing, long-grain variety aimed at producing material similar in quality to the Risabell variety but maturing significantly earlier. It is one of the most drought-sensitive genotypes under aerobic conditions.

Use in Breeding: Used as a drought-sensitive control and parent in crosses (e.g., 1080 combination).

IRAT 109

Origin: From Ivory Coast, included in our collection through Brigitte Courtois (CIRAD, Montpellier).

Characteristics: Late-maturing under Hungarian climatic conditions, efficiently uses available water resources, strong root development, and one of the most drought-tolerant materials in our collection.

Use in Breeding: Used as a drought-tolerant control and parent in crosses (e.g., 1080 and 1087 combinations).

Sandora (HSC55)

Recognition: Recognized in Hungary in 1993. Its recognition was later withdrawn due to decreasing significance in production and the introduction of the Janka variety with similar quality traits but better agronomic suitability.

Significance: Used in both the Sanoryza non-flooded rice cultivation system developed by Dr. Ibolya Simonné Kiss and her colleagues, and in international breeding and research programs due to its early maturity and excellent cold tolerance.

SZV Tünde

Recognition: The latest state-recognized rice variety by MATE KÖTI ÖVKI (2021).

Characteristics: Belongs to temperate japonica rice, medium-late maturing in Hungary, with a height of 75-80 cm, and resistant to local lines of blast. Noteworthy for its exceptional yield among Hungarian rice varieties, with small plot trials showing yields over 7 t/ha, and in some farms exceeding 10 t/ha.

DH Line Production

The DH lines from pollen sources originated from multiple rice varieties and cross combinations. The DH lines were produced at the Biotechnology Laboratory of Gabonakutató Nonprofit Kft. in Szeged, led by Dr. János Pauk and Dr. Csaba Lantos. The homogeneous DH plants and lines cultivated there were propagated under flooded conditions in Szarvas. Besides basic phenotypic selection where awned and lodging-prone materials were removed, no significant selection was performed.

In my experiments, the combinations used were: 1009: Köröstáj 333/Nembo; Tünde: Dáma/GB2005; 1080: Marilla/IRAT 109; 1087: Dáma/IRAT 109.

Microplot experiments with DH lines from combinations 1080 and 1087 were established under aerobic (non-flooded) conditions at the MATE ÖVKI Lysimeter Station in 2022 and 2023. For detailed studies, 18 DH lines from the 1087 combination were selected randomly. These lines were designated as 1_28, 2_22, 2_35, 2_40, 3_30, 3_57, 3_8, 4_3, 4_43, 4_60, 6_26, 6_33, 6_46, 6_49, 7_70, 8_33, 8_40, and 8_55.

The experimental setup aimed to assess the adaptability and performance of these DH lines under non-flooded conditions, focusing on their agronomic traits and stress tolerance.

Experiments Conducted Under Conventional Cultivation Conditions

Traditional rice cultivation typically takes place in built-up rice fields, which consist of embanked fields surrounded by dikes. The main components include irrigation channels for water delivery and drainage channels for water discharge. Water levels are regulated using structures like simple gates, which control the depth of flooding. The key characteristic of the rice fields is their relatively uniform terrain, which allows for consistent regulation of water depth across the entire area. This uniformity ensures that irrigation water can be uniformly supplied at a depth suitable for the developmental stage of the crop throughout the field.

Our experiments in a traditional cultivation environment were conducted at the MATE ÖVKI Galambosi Rice Experimental Station in Szarvas. The total area of the station is 20 hectares, with 15 hectares dedicated to the equipped rice fields. Due to the regulated irrigation options, the area is divided into two larger units: a 10-hectare section and a 5-hectare section. These units are alternated for use from year to year. The station uniquely facilitates research and seed production related to rice under small-scale, micro-plot conditions in Hungary.

The DH2-3 comparative experiments were conducted in experimental plots of the B-lines used for variety maintenance at MATE ÖVKI, with each lines planted in plots sized at 3.6 m^2, replicated four times.

Preliminary experiments for selecting lines suitable for aerobic cultivation

In the initial phase of the research program, several preliminary experiments were conducted. These primarily aimed to identify differences in drought tolerance among state-recognized rice varieties and to compare the research methods used.

Greenhouse comparative tests were conducted in the GK greenhouse in Szeged. A complex stress diagnostic system was set up, and small, enclosed breeding containers were used to compare the characteristics of two previously selected rice varieties, Sandora and Marilla. Based on the experience gained and the results of tests by Dr. Ibolya Simonné Kiss (unpublished data), small-plot field comparative experiments were initiated under aerobic cultivation conditions.

During these tests, we developed the key steps of cultivation technology and selected the most important methods for comparing drought tolerance (SES, RWC, WUE).

Experiments in aerobic cultivation technology

We conducted our experiments at the MATE ÖVKI Lysimeter Station (46°51'44.7"N 20°31'35.5"E, 81 m), using a non-flooded, aerobic rice cultivation system (formerly known as "dry rice"). This technology's advantage during selection lies in its heightened sensitivity to both water scarcity and the adverse effects of low temperatures. Critical aspects of this technology include uniform seeding, effective weed control, and ensuring irrigation tailored to the plants' requirements. Enhancing tolerance to abiotic stresses is a primary breeding objective, given Hungary's short growing season, variable weather (cold periods during early vegetative and reproductive stages), and weaker soil conditions (heavily compacted, saline soils), which can significantly reduce production efficiency due to these stress factors.

In our micro-plot comparative experiments, we ensured water supply through drip irrigation. The irrigation water source was from the Holt-Körös, which was deemed excellent for irrigation purposes based on analyses. Row spacing was 25 cm, and plant spacing was 2-3 cm. Soil moisture content was monitored using SM300 soil moisture and temperature sensors (Delta-T Devices, Cambridge, United Kingdom). Following manual harvesting, threshing, cleaning (LD 350, Wintersteiger AG, Ried im Innkreis, Austria), and drying, we analyzed the harvested grains under laboratory conditions (using THU laboratory dehusker and polisher, Satake, Japan) to assess the most critical milling properties of rice (percentage of brown rice, whole and broken polished white rice).).

Selection methods for drought tolerance under aerobic conditions

The most important trait for aerobic cultivation conditions in terms of cultivability is the tolerance to periodic water deficit, which also influences the applied selection methods. The simplest method for this is to assess leaf rolling and wilting during the early vegetative growth period. Based on the unified evaluation scale developed by IRRI, the most tolerant genotypes (0) show no symptoms indicating water deficiency, with the leaf blade intact. Conversely, the most sensitive plants exhibit complete leaf rolling and wilting (9).

Surface temperature assessment has successfully been used to detect water deficiency in rice under both aerobic conditions and Alternate Wetting and Drying (AWD). Given similar weather conditions, leaf surface temperature primarily depends on genotype, rice cultivation method, and the occurrence of water deficiency. Therefore, it can be suitable for determining the degree of drought tolerance in large breeding materials. In our case, differences in leaf surface temperature were determined during various summers when natural rainfall scarcity allowed for selection following at least 7 days of water deprivation, with daytime temperatures exceeding 30°C. Testo 885 thermal cameras (Testo SE & Co. KGaA, Titisee-Neustadt, Germany) were used for these assessments, with data extraction performed using software provided by the manufacturer.

To determine the degree of water deficit, we followed the protocol of relative water content (RWC), which is a straightforward method applicable to large plant populations, providing direct data on plant hydration levels. Following the protocol of González and González-Vilar (2001) we calculated RWC values.

Water use of rice in large weighing lysimeters

A The MATE ÖVKI Lizimeter Station began operating in 1971 and underwent significant renovations last in 2018, which also included updates to its instruments. Situated on

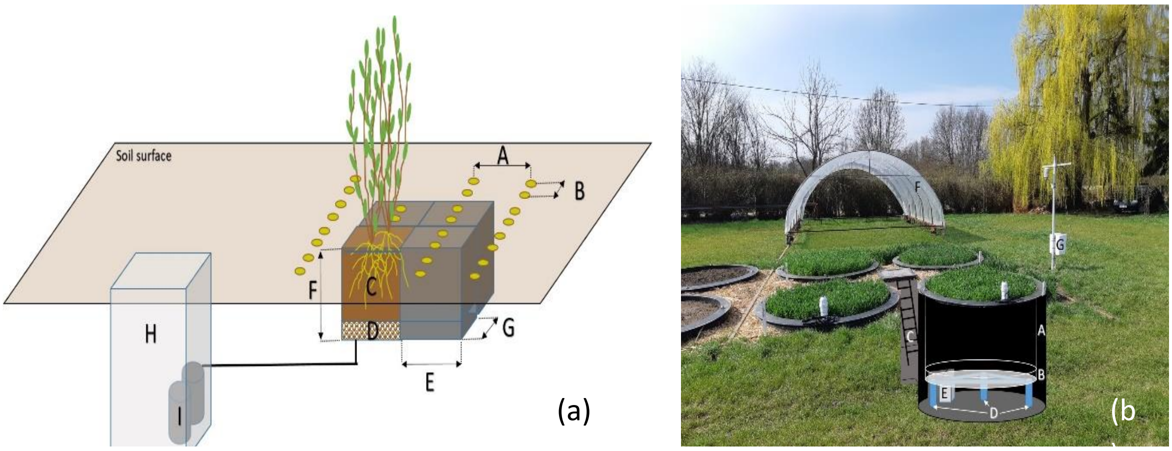

Figure 2. Gravitational lysimeter (a) and weighing lysimeter (b). a/A-B: row/plant spacing, C: soil column in the lysimeter, D: gravel, E-G: lysimeter width (1m), F: lysimeter height (1m), H: measuring pit, I: container;
b/A: soil column in the lysimeter, B: gravel, C: measuring pit, D: weighing cells, E: container, F: rainshelter, G: meteorological and soil measurement station

one hectare of land, the station houses 320 large gravity/compensated lysimeters, connected to 5 measurement pits, making it the largest research infrastructure of its kind in Europe (lysimeter.at). Each gravity/compensated lysimeter has a volume of 1 m3, with 80% of its volume occupied by the soil column being studied. These lysimeters rest on a 10 cm gravel layer, facilitating precise collection of water passing through the system.

Using these gravity/compensated lysimeters, which operate in a partially enclosed manner, allows for the precise monitoring of the movement of various substances in the soil and their uptake by plants installed in the lysimeters (e.g., nutrients, pesticides, heavy metals). Each lysimeter is connected to individual measuring vessels in the measurement pits, enabling the collection and determination of both the quantity and quality of percolating water. While the monitoring of soil moisture is somewhat limited, these systems are also suitable for evapotranspiration studies, with the accuracy of measurements enhanced by modern sensors and the compensatory systems integrated into the lysimeters.

At the MATE ÖVKI Lysimeter Station, alongside 320 gravitational/compensating lysimeters, there are 8 large precision weighing lysimeters (Type S 6048, Metrisystems Ltd.), each with a surface area of 2.7 m2 and a depth of 1.2 m, custom-built in 2018 (Figure 2). These weighing lysimeters enable tracking of mass changes with a resolution of 100 g (0.05% accuracy), facilitating precise measurement of evapotranspiration and thereby accurate determination of plant water requirements. Data collection occurs automatically at hourly intervals, managed by specialized software on a Windows PC-based EMX100 electronic weighing unit.

Table 1. Soil quality paramethers in the lysimeters at the beginning of the experiments (Szarvas, 2020)

	pH (H_2O)	K_a* (cm^3)	Total salinity (%)	Lime (%)	Humus (%)	N (KCl) mg/kg	P (AL) mg/kg	K (AL) mg/kg
Average	7.34	42	0.045	2.57	2.16	14.9	362.2	365.2
SD	0.1	2.4	0.007	0.68	0.25	11.95	85.91	73.05

* soil plasticity according to Arany

The soil columns within the weighing lysimeters were established through backfilling in 2018 using clay loam soil, characterized by key parameters outlined in Table 1. The soil depth is 100 cm, with a 10 cm gravel layer beneath for collecting any percolating water. Percolated water from the soil columns accumulates in individual tanks within the measurement pit. For reference evapotranspiration (ET) determination, one lysimeter maintains a grass cover while another keeps a bare soil surface.

ETc and Kc calculation and the environmental factors

In the thesis, two experiments determining ETc are presented. The experiments were conducted with the SZV Tünde rice variety in 2020, and with three control varieties (Dáma, Marilla, IRAT 109) in 2022 under natural weather conditions. Since detailed results of ET determination for the SZV Tünde are presented, I also provide a detailed overview of the weather conditions of that year for easier interpretation of the data.

Meteorological data were collected using the automatic weather station (Agromet Solar, Boreas Kft., Érd) located at the MATE ÖVKI Lysimeter Station. During the growing season, precipitation amounted to 460.9 mm. Reference evapotranspiration (ETo) values were calculated using daily average temperature (°C), daily average relative humidity (RH%), average wind speed (m/s), and daily irradiation (MJ/m2/day). Data were collected at 10-minute intervals, and hourly and daily averages and totals were calculated from these basic data.

ETo was calculated using the EToCalculator 64bit version 3.2 software (FAO), which computes ETo based on the Penman-Monteith equation and meteorological data (Raes, 2012). ETo and ETc values were reported as 5-day averages to smooth out variations between larger days and make the dataset more manageable. Based on these, ETc values associated with different developmental stages of aerobic rice were determined, and the crop coefficients were computed.

Crop coefficients for the initial growth stage (Kcini), mid-season stage (Kcmid), and end of the growing season (Kcend) were calculated following Allen et al. (1998).

$$Kc = \frac{ETc}{ETo}$$

, where Kc represents the crop coefficient, ETc denotes the actual evapotranspiration, and ETo stands for the reference evapotranspiration value for the studied stage of the growing season.

Leaf spectroscopy for the measurement of plant physiological status

From 2021 onwards, our investigations were supplemented with a CI-710s portable leaf spectrometer (CID-Bioscience, USA). We measured light absorption, transmittance, and reflectance in the 400-1100 nm range for each genotype with a minimum of 10 replications. In this study, we present the characteristics of 18 selected DH lines based on measurements taken after a prolonged water withholding period on August 3, 2022. Various widely recognized indices (CCI, CNDVI, CRI 1, DCNI, IAD, PRI, SPAD, and WBI) were computed from the available data, and following statistical analysis, we found significant differences among genotypes and treatments in PRI, CRI 1, and CCI indices, which are detailed herein.

The Photochemical Reflectance Index (PRI) was calculated using the formula: PRI = (R531-R570)/(R531+R570), where Rn represents the measured reflectance value at wavelength n nanometers (Gamon et al., 1992).

The Carotenoid Reflectance Index (CRI 1), indicating carotenoid content, was determined based on Gitelson et al. (2001): CRI 1 = (1/R510) - (1/R550), where Rn denotes the measured reflectance value at wavelength n nanometers.

The Chlorophyll Content Index (CCI), calculated from transmittance values, was derived using the formula: CCI = T931/T653 (Parry et al., 2014), where Tn denotes the measured transmittance value at wavelength n nanometers.

Statistical analysis and data presentation

In all experiments, regardless of the data collection method, collected data were summarized in tables using Microsoft Excel Professional Plus 2021. The diagrams and tables presented in the dissertation were also created using Microsoft PowerPoint Professional Plus 2021 software.

For verification of results, where feasible due to data quantity, statistical analyses were conducted using IBM SPSS Statistics 22. Normal distribution of data was verified (homogeneity of variance – Levene's test), and differences between genotypes and treatments were determined using analysis of variance (ANOVA). Post hoc tests (Tukey's test) were employed to determine differences between genotypes. Results of these tests are indicated in figures above columns with lowercase and uppercase letters, where different letters denote significantly different values. To explore linear relationships between various study parameters, Pearson correlation analysis was used..

RESULTS AND DISCUSSION

Efficiency of DH production and their integration into the breeding program

The efficiency and implementation costs of a breeding method significantly influence its general applicability. In our work, we initially started with various genotypes to improve the efficiency of in vitro DH (doubled haploid) production, aiming to generate a large quantity of DH plants from offspring generations of planned crossing combinations in subsequent experiments. During the initial phase of laboratory development of DH production, we primarily worked with traditionally bred rice varieties and to a lesser extent with offspring generations of previous crosses, until we developed the appropriate segregating populations through sexual crosses.

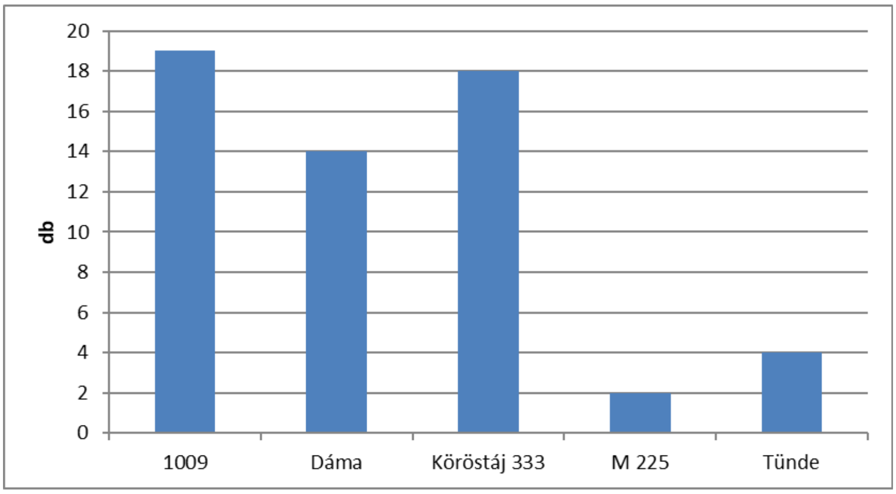

Figure 3. Spontaneous DH plants at the beginning of the experiments detected in the nursery (Szarvas, 2017)

In 2017, the greenhouse checks revealed a relatively low number of spontaneously occurring diploids in the DH0 generation (Figure 3). We identified a total of 57 fertile plants, among which 19 originated from the 1009 combination (Köröstáj 333/Nembo), while 4 were from the Tünde variety in the F8 generation, later recognized as a state-approved breed. Strong genotype dependence was evident, as we identified 14 and 18 fertile DH plants in the Dáma and Köröstáj 333 rice varieties, respectively, whereas only 2 were identified in the M 225 variety.

To increase the number of DH plants, colchicine treatment was applied to the previously identified haploid plants at the Szeged laboratory. This resulted in an increased number of fertile plants usable in subsequent tests. Despite treatment, no fertile plants were obtained for the Bioryza H, M 488, M 60, and M 225 rice varieties, while low efficiency was observed for 1009, Dáma, Karola, and Tünde varieties. Treatment of Köröstáj 333 haploids alone yielded results, with an additional 36 fertile plants produced alongside spontaneously occurring diploids.

The initial results underscored the need to enhance the method's efficiency to ensure DH lines were available in a quantity suitable for breeding practices. This task was successfully accomplished later during laboratory experiments by the Szeged research team (Lantos et al., 2022).

Year by year, the number of DH plants created through androgenesis induction has increased. Results from plants planted in 2019 indicate significant improvement in efficiency for the studied genotypes. The proportion of spontaneously occurring diploids exceeded 50% for two varieties: 57.9% for Dáma and 52.9% for Karola, despite the low number of plants produced. Among the genotypes originating from three different crosses, particularly the 1009 combination (444 plants) and Mirko/Karola (531 plants), a substantial number of green plants were produced, 38.1% and 44.1% of which were fertile.

As a result of these developments, by 2020, 1412 plants were housed in the DH0 cage at the Szarvas breeding facility (Table 2). This plant count provided a suitable foundation not only for more effective execution of breeding tasks but also for conducting drought tolerance tests under aerobic conditions in the future.

Table 2 Ploidity of in vitro plants detected in the nursery (Szarvas, 2020)

Ploidity	**Haploid**	**Diploid**	**Tetraploid**	**Mixoploid**	**Total**
Number	710	600	80	22	1412
Ratio (%)	50.3	42.5	5.7	1.6	100

The proportion of haploid plants in the entire DH0 population remains relatively high at 50.3%, but this ratio can be further improved with increased effectiveness of colchicine treatment. The percentage of diploids, which is crucial for breeding purposes, reached 42.5%, equivalent to 600 DH plants. This number provides a sufficient foundation for a medium-sized breeding program to successfully utilize lines with desirable traits in future selection procedures. A smaller portion of the produced plants (5.7%) exhibited characteristics of tetraploids during field checks (taller plants, larger panicle size, and partial fertility within the panicle). These plants, traditionally known for their low fertility, have recently gained increasing attention in rice breeding due to their developmental potential (large grain size, large panicle size, strong stem), which our research team has been intensively studying in recent years.

Evaluation of DH lines in conventional and aerobic conditions

The DH0 generation plants underwent simple selection, where we filtered out plants with undesirable traits for production and processing industries (height over 80 cm, late maturity) and undesirable grain types. This step is crucial to ensure that the DH1 generation lines are manageable and suitable for cost-effective experiments.

Continuous screening of DH1 generations is integral to our breeding program, and here I present the results of our 2018 microplot experiment. By this time, we had sufficient plants from fertile DH lines to sow continuous rows in multiple repetitions (typically 3 rows per DH lines). In agricultural practice, one of the most important traits is yield, which determines the economic viability of production. In the DH0 generation, using traditional breeding methods, it is challenging to estimate future yields accurately. This is evidenced by the fact that 37.9% of DH lines showed lower yield results compared to control varieties on average.

Based on the yield results of DH1 lines and field surveys, we initiated further small-plot comparative performance experiments with the best-performing lines in recent years (Table 3). Due to limited availability of field space, only the top-performing DH lines are included in the four-repetition tests, which typically now comprise 2-3 lines in combination.

The choice of control varieties for experiments depends on breeding goals, parental pairs, and the proportion of domestic cultivation areas. In the 2018 comparative experiment, these three control varieties were Janka, M 488, and M 225. For easier comparability with yield results available in agricultural practice, the data is presented per hectare.

Based on the overview of yield results, it can be stated that the conditions of the experiment were favorable under Hungarian production conditions. Based on yield averages, the weakest genotype was found to be the Janka variety, which was more widely present in Hungarian field cultivation at that time. Despite its excellent quality, the variety showed significant susceptibility to sheath blight, hence it plays a decreasing role in Hungarian rice cultivation.

Table 3 Yield performance of DH_2 lines in a small-scale experiment under conventional paddy environment (Szarvas, 2018)

Genotype	Yield [1] (kg/ha)	Min (kg/ha)	Max (kg/ha)	Ratio to controls %	Selection [2]
Janka control	5597.2 [a]	5027.8	6444.4	84.3	na.
Tünde 19.II/1	5663.0 [a]	4611.1	6200.0	85.3	-
Tünde 14.VIII/1	6290.7 [ab]	5877.8	6722.2	94.7	+
Tünde 14.I/1	6437.1 [ab]	5877.8	7266.7	96.9	-
Tünde 17. I/1	6659.3 [ab]	6016.7	7500.0	100.3	+ + +
Tünde 14.VII	6864.8 [ab]	6244.4	7205.6	103.4	+
M 488 control	7037.0 [ab]	6875.0	7291.7	106.0	na.
Tünde 19.III.	7053.7 [ab]	6483.3	7355.6	106.2	-
Tünde 17.II.	7233.3 [ab]	6400.0	8805.6	108.9	-
M 225 control	7287.0 [ab]	7027.8	7750.0	109.7	na.
1009/1/17	8470.4 [ab]	7383.3	10227.	127.6	+ + +
1009/1/12	8644.4 [b]	6988.9	10961.	130.2	+ +
Karola 11.I.	8688.9 [b]	8500.0	9222.2	130.8	+ +
Karola 11.I/3.	8835.2 [b]	8505.6	9094.4	133.1	+ + +
Karola 11. I/1	8872.2 [b]	7988.9	9238.9	133.6	+ + +
Control average	6640.4	5027.8	7750.0	100.0	

[1] DH_2 lines average yield results (n=3), different letters in the table indicate significant differences between lines at a confidence level of p=0.05 (ANOVA Tukey post hoc test).

[2] Value determined based on complex selection analysis of lines (early vegetative vigor, cold tolerance, resistance to sheath blight, earliness, phenotype, milling quality), where "+" indicates positive selection and "-" indicates line elimination.

Highlighting the yield results is important because the primary criterion for growers in variety selection is reliable and high yield. Among the examined DH lines, eight proved to have higher yield averages than the control varieties (6640.4 kg/ha). Furthermore, five DH lines even exceeded the yield average of the best control variety, M 225 (7287.0 kg/ha).

Although the results were obtained under small-plot conditions, potential yield averages exceeding 8 t/ha are considered outstanding in Hungary. Average operational yield results have ranged between 3 and 4 t/ha in recent years, with only the most successful producers achieving yields above 5 t/ha.

Based on agronomic properties, further selection was conducted during the breeding season, focusing on early vegetative vigor, cold tolerance, resistance to sheath blight, earliness, plant habitus, stem strength, and grain and panicle type. As a result, six DH lines received positive evaluations: Tünde 14.VII, 1009/1/17, 1009/1/12, Karola 11.I., Karola 11.I/3., and

Karola 11.I/1. These lines are expected to be effectively utilized in variety registration processes or as parent partners in further crosses, based on previous experiences and earlier determinations by Simonné Kiss (2001).

In the following years, our research-breeding work continued, utilizing DH lines derived from new and different crossing combinations. Similar arrangements were used to assess the performance of ten DH2-3 lines in a two-year, four-replication experiment conducted in 2019 and 2020. Upon analyzing the experiment results, significant year-to-year effects were observed only in milling quality, specifically in the quantities of whole and polished white rice. These datasets were analyzed separately, while the data from other traits were combined for the two years.

Based on these analyses, six DH lines were selected as genotypes worthy of further selection, or for potential use as parent genotypes in our breeding program. Analyzing the plant height parameter revealed statistically significant differences among DH lines, yet, except for Köröstáj 17.II. 1, all genotypes remained within the desired size range for practical farming (50-75 cm). This size range is crucial due to the risk of mechanical harvesting and lodging; smaller plants can lead to harvesting losses on uneven ground, while taller plants increase the risk of lodging and decrease the harvest index (HI).

Excessive biomass development and increased susceptibility to sheath blight are undesirable traits. Among the studied genotypes, the HI values for the 1009 combinations were lower (36.3%, 41.7%, and 42.8%) compared to the average value of control varieties (52.1%). For other genotypes, this value ranged between 47.9% and 52.0%. Results from grain yields indicated that nutrient supply during the experiments was moderate, with yields per hectare approaching operational averages. The highest yield averages among DH lines were observed in Köröstáj 14.III. 2, Köröstáj 17.II. 1, Tünde 16.II. 2, Tünde 25.I.1, and Tünde 16.II. 1 lines. Among them, Tünde 16.II. 1 achieved the highest yield, reaching 111.6% compared to the average yield of control varieties.

As a result of individual and small-plot selections conducted in previous generations, several genotypes showed low infection levels, notably Tünde 16.II.1, which also exhibited a high thousand-grain weight value (33.9g). Despite rice's numerous applications, one of its most critical quality attributes is tied to milling processing. For rice varieties, high yields of both brown and polished white rice are fundamental criteria. Tünde 16.II. 1 also exhibited the highest value in hulled brown rice yield, with a significant difference compared to control varieties.

The quantity of polished white rice is divided into two main fractions by the processing industry, with whole grains being more valuable and sought after by consumers, thereby influencing the economic efficiency of production. As shown in our results, the yield of polished whole grains depends heavily on genotype and is also strongly influenced by annual climatic variations. Temperature fluctuations during maturation and precipitation levels primarily influence the formation of microcracks that can lead to grain breakage during processing. In our case, the 2019 whole grain values were significantly lower than those measured in 2020, likely due to higher temperatures in September and lower precipitation, which aided the maturation process.

Overall, the DH line Tünde 16.II. 1 emerged as the most promising for future breeding and research tasks. DH lines have demonstrated the ability to produce homogeneous breeding materials within one generation, which, after pre-selection and propagation, can be directly used in comparative experiments for variety selection. DH lines were consistent, and their data dispersion did not differ from that of nationally recognized control rice varieties in various parameters.

DH lines in the aerobic conditions

Aerobic rice cultivation is a specialized method in rice production where the rice plants are grown for the majority of the growing season (at least 80%) without anaerobic conditions. The fundamental characteristics of this technology include intensive nutrient management and crop protection.

In aerobic cultivation, the suppressive effect of flooding on weeds and its role as a temperature-regulating buffer are not utilized. Therefore, rapid initial growth and good cold tolerance are particularly important, as the plants are more likely to experience water deficit if there are issues with irrigation technology. Thus, ensuring safe production and reducing water demand are key breeding priorities, emphasizing drought tolerance and efficient water use.

During the integration of DH lines into our aerobic breeding program, we paid special attention to studying how these lines respond to water deficit (through instrumental and phenological analyses), enhancing crop security under aerobic conditions, and determining water requirements across different lines (measured via evapotranspiration and gravimetric lysimeters).

This paper presents some results of these investigations and the selection of DH lines under aerobic rice cultivation conditions.

Based on these findings, we decided to create hybrid plants and segregating populations through controlled crossbreeding using genotypes that best fit our future breeding and research goals. This aims to select early-maturing and drought-tolerant lines that can be effectively utilized under domestic conditions. Among several successful crosses, the combinations Marilla/IRAT 109 (1080) and Dáma/IRAT 109 (1087) showed the greatest promise and diversity under aerobic cultivation conditions. Bug collection from early progeny generations of these crosses allowed us to isolate and cultivate ports in Szeged, leading to the development of DH lines studied in both field and laboratory settings.

DH_1 lines in aerobic and conventional cultivation

Based on our earlier experiments, we set up micro-plot trials under aerobic conditions at the MATE ÖVKI Liziméter Station, as well as traditional flooded cultivation at the MATE ÖVKI Galambosi Rice Experiment Station. Our aim was to compare the performance of new DH lines under these two different cultivation methods.

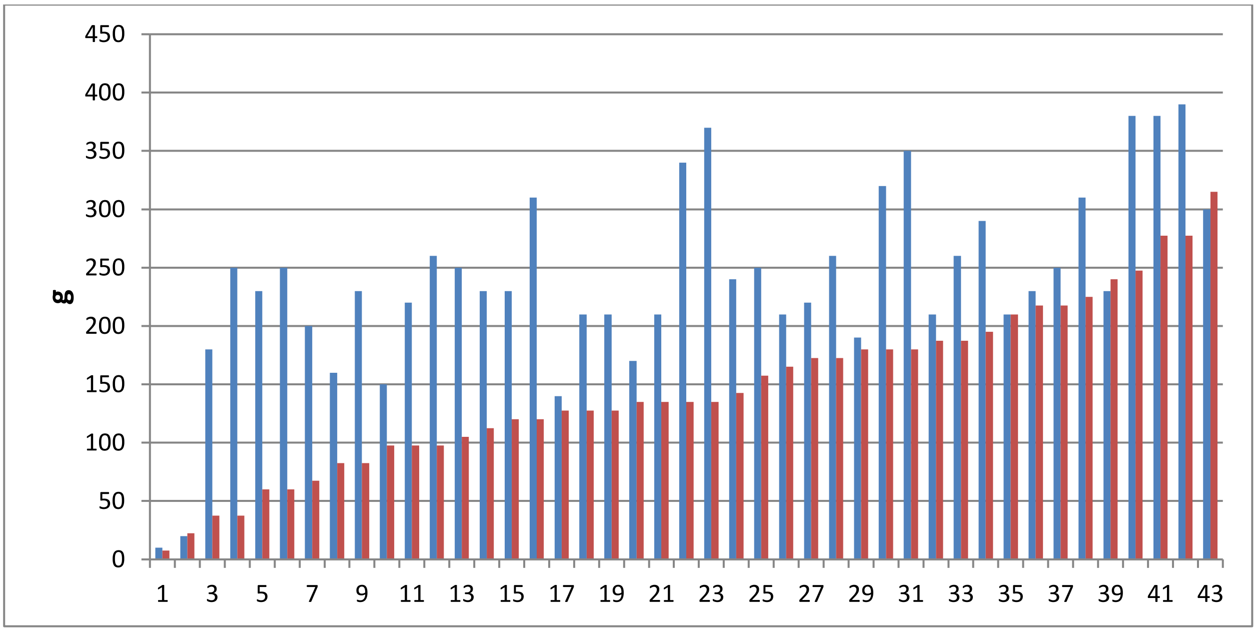

Figure 2 Yield of DH_1 lines (g/plot) in aerobic (red) and conventional (blue) cultivation (Szarvas, 2017)

A selection of experiments during the breeding season focused on assessing the detrimental effects of water scarcity and low temperatures, using the general selection guidelines provided by the International Rice Research Institute.

Significant variations were observed in the yield results of the tested lines (Figure 4). Comparing the yield results of the various DH lines, under aerobic cultivation, yields ranged from 8 g to 315 g of grain yield, whereas in traditional flooded conditions, yields varied between 10 g and 390 g. Generally, the majority of tested lines produced higher grain yields under flooded conditions. However, lines Tünde 19.III. and Tünde 14.I. did not significantly decrease in yield under aerobic conditions.

Different DH1 lines were also compared to standard varieties under non-flooded conditions. Based on yield results, Ábel and Auguszta varieties performed well. Among the offspring of the most valuable crossbreeding combinations, DH lines Tünde 19. II/1 (380 g/plot) and Karola 11. I (390 g/plot) were the most successful. These lines also performed exceptionally well on flooded rice fields with yields of 660 g/plot and 640 g/plot, respectively.

Based on our preliminary findings, combining these two different water supply methods can lead to more efficient selection of successful genotypes in aerobic cultivation, while also ensuring potential high yields under flooded conditions. This is particularly important in practical applications, as the number of maintained varieties in domestic rice cultivation needs to be sustainable given its scale.

Evapotranspiration of DH lines in large weighing lysimeters

A cultivation of rice involves ensuring an adequate supply of irrigation water, which is critical in Hungary due to insufficient natural rainfall for normal growth in most years. To gather more precise data on the water requirements of aerobic rice under Hungarian conditions and determine the key development stages when water demand peaks, we conducted experiments using a variety of rice (SZV Tünde 2021, a nationally recognized rice variety) and pre-selected DH lines in large-scale precision lysimeters at the MATE ÖVKI Lysimeter Station.

The initial evapotranspiration measurements were conducted with the SZV Tünde variety in 2020. The plants' water needs were met through natural precipitation and irrigation, which was continuous and uniform throughout. No signs of water stress were observed on the plants during the growing season (IRRI, 2013). At the end of the growing season, we measured a grain yield of 506.7 g/m2 and above-ground biomass of 1140.4 g/m2. The water use efficiency (WUE) was calculated at 0.65 g/l, which is considered low based on literature values (Tabbal et al., 2002).

The total irrigation water used during the growing season was 315.6 mm, also considered low compared to literature values (Avila et al., 2015; Pimentel et al., 2004). Throughout the growing season, we continuously monitored changes in the ETc values. The growing season can be divided into three main stages based on rice development. The initial stage lasted from seeding to the end of tillering (DAS 52), during which the ETc ranged between 2.04 and 3.86 mm/day until June 25.

The middle stage represented the period of most intensive biomass development and transpiration, lasting until September 5 (DAS 124). During this period, the 5-day average ETc ranged from 3.57 to 7.90 mm/day. The highest ETc values were recorded during the flowering period in mid-August, emphasizing the critical importance of adequate water supply matching the plants' water needs during this phase.

Higher ETc values were attributed to unrestricted water supply and frequent irrigation to maintain uniform water availability. Towards the end of the growing season, during grain ripening, the plants' water requirements gradually decreased due to the maturation process and changing weather conditions. During this phase, average ETc ranged between 0.90 and 4.26 mm/day.

Overall, the total ETc during the growing season was 648.3 mm, which aligns with values reported by other researchers. According to Tabbal et al. (2002), modern short-duration rice varieties typically require 600-700 mm of ET in dry seasons. Based on our preliminary tests, we planted the highly drought-tolerant IRAT 109 genotype from the Ivory Coast and the drought-sensitive Hungarian varieties Marilla and Dáma in 2022. In three lysimeters (Dáma, IRAT 109, and Marilla), we provided less than optimal water supply (816.1 mm, 820.3 mm, and 823.1 mm of irrigation and precipitation), resulting in visible symptoms of water stress on the sensitive plants (leaf curling, reduced growth). As a physiological control, all three rice varieties were also planted in a fourth lysimeter where optimal growth conditions were maintained (989.3 mm of irrigation and precipitation).

Based on our results, in the lysimeter containing all three rice varieties with abundant water supply, the total ET value was 1022.4 mm between June 25 and September 14. Under reduced water supply, all three rice varieties exhibited lower ETc values compared to this figure. Particularly noteworthy was the minimal decrease in ET observed for the IRAT 109 variety (973.7 mm), while the Dáma and Marilla varieties transpired almost equally at 904.7 mm and 904.6 mm, respectively. This finding supports Blum's conclusion that efficient utilization of available water under identical conditions is of great importance (Blum, 2009).

Plant coefficients (Kc) in different developmental stages

A practical challenge for researchers and even more so for producers is the availability of direct measurements of crop water use represented by evapotranspiration (ET). To more accurately estimate current ET values under specific cultivation conditions (weather, management practices, variety selection), knowledge of crop coefficients (Kc) related to different growth stages is essential. A precise description of Kc contributes to calculating the current water demand of plants using easily accessible and determinable ETo models (Raes, 2012). Ultimately, this can aid in adjusting irrigation water application according to plant water requirements and thereby reducing water consumption. This is particularly crucial for rice production, whether under traditional flooded conditions, periodic flooding, or aerobic cultivation.

To determine Kc values, we utilized results from direct ET measurements conducted in 2020. The methodology followed for calculating crop coefficients specific to different growth stages was based on Allen et al. (1998).

During the initial growth stage (until DAS 52), the Kcini values ranged between 0.57 and 1.19, with an average value of 0.82. In practical terms, this means that during the initial growth stage, rice requires less water for stress-free cultivation compared to the amount determined by reference evapotranspiration (ETo). This is a natural occurrence because the leaf area of developing plants is less than that used to determine ETo for reference grass. These values align with data reported by Allen et al. (1998).

In the intensive growth stage (up to DAS 124), higher Kc values were observed. The Kcmid ranged from 1.11 to 1.80, with an average value of 1.40 during this growth stage. The high biomass and leaf area, coupled with high temperatures, increased the water consumption of the plants, resulting in significantly higher ETc values compared to reference evapotranspiration during this period.

Towards the end of the growing season, as the plants approached the end of their life cycle and daily average temperatures decreased, the ETc values also decreased significantly. Consequently, the Kcend values ranged between 0.57 and 0.88, with an average Kcend value of 0.77.

We will continue our work by processing data from subsequent years to further refine these values according to the conditions of temperate zone aerobic rice cultivation.

Drought tolerance of DH lines under aerobic conditions

During the 2022 and 2023 growing seasons, DH lines originating from two crossing combinations were examined in microplot experiments at the MATE ÖVKI Lizimeter Field. Seeds from 1080 DHs (Marilla x IRAT109) were planted in 60 plots, while seeds from 1087 DHs (Dáma x IRAT109) were planted in 230 plots. In 2022, uneven emergence and insect damage prevented a detailed examination of the entire population. However, in 2023, we were able to evaluate the full range of DH lines under aerobic cultivation conditions in field reactions.

Throughout the growing seasons, field assessments (IRRI SES drought sensitivity) and measurements (Testo 885 thermal camera, CI-710s leaf spectrometer) were conducted. The average maturity period of the 1087 DHs was longer compared to observed values for the 1080 DHs. We determined the reactions of the different DH lines to induced water scarcity (9 days without irrigation) and an extreme heat period (daily maximum temperatures of 36-38°C).

Based on our surveys, 21.1% of the genotypes examined from the 1087 DHs showed mild symptoms (few or very few slightly drooping leaves) under stress conditions. Furthermore, 31.3% exhibited moderate tolerance, while 47.7% were severely affected by the significant heat and water stress (Figure 5).

For DH lines from the other examined combination, the appearance of stress symptoms was much lower. 63.4% of the plants showed mild symptoms due to water deprivation. Moderate symptoms were observed in 33.2% of the plants. Only 3.4% of the lines exhibited severe symptoms. Therefore, for the DH lines of the 1080 combination, we can consider more significant water deprivation for further selection in the future. Additionally, this latter combination can be more likely used to select genotypes suitable for aerobic rice cultivation in temperate regions.

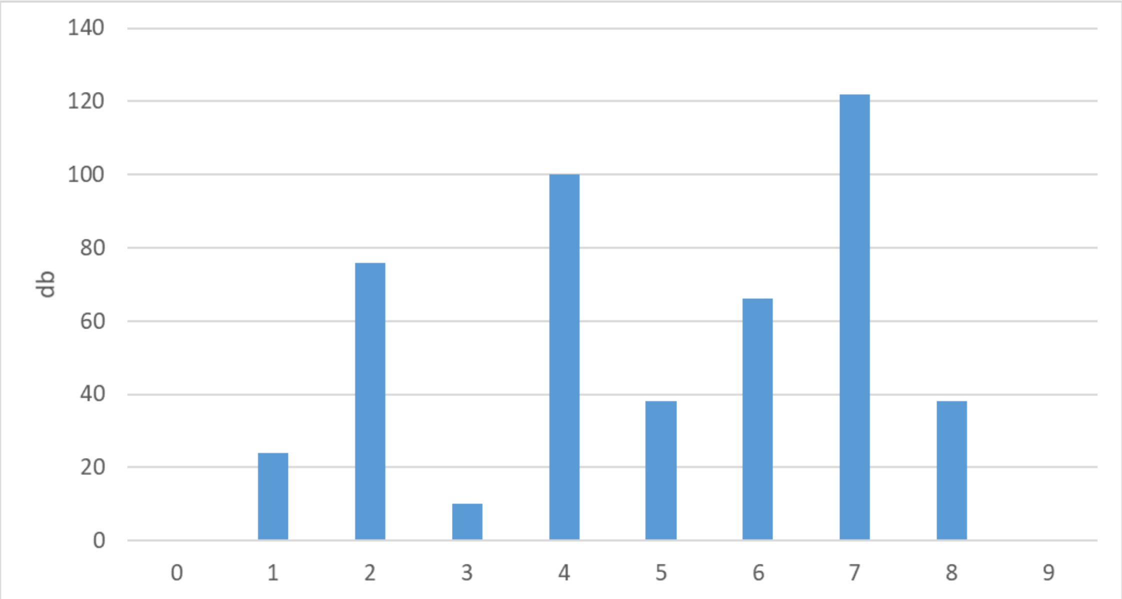

Figure 5 Drought tolerance (IRRI SES) of DH lines 1087 (Dáma x IRAT 109) at the MATE ÖVKI Lysimeter Station under aerobic conditions after a significant drought treatment (Szarvas, 2023)

Leaf spectroscopy for the evaluation of drought tolerance of DH lines in drought prone environments

To enhance more effective selection methods, we characterized 18 of the available 1087 DH breeding lines based on spectral indices during the flowering period. Significant differences were observed particularly in PRI, CRI 1, and CCI indices. Regarding PRI, it was generally observed that the index values increased in most lines due to water deprivation. However, due to high variability, significant differences were found not within treatments but between treatments across DH lines. The smallest average PRI change was recorded in DH line 1087 2 35. The highest CCI values were exhibited by DH line 1087 4_60 under well-watered conditions. Significant differences were identified among DH lines under well-watered conditions in multiple instances. Under water deprivation, notable CCI results were observed in lines 1087 2_22, 1087 2_35, 1087 6_26, and 1087 8_55; however, significant differences were not detected within this treatment. The third index examined was CRI 1, with the highest value measured in line 1087 4_43 under stress-free conditions, while line 1087 2_22 showed the highest value under water-deprived conditions.

CONCLUSIONS

Based on our results, the induction of androgenesis can effectively be used to produce DH lines from both the commonly used japonica genotypes in Hungary and the increasingly important indica genotypes expected in the future, for breeding purposes. These DH lines can be derived from various progeny generations of successful crosses, tailored to meet both breeding and research objectives. If the goal is to create genetically diverse DH lines, such as for developing mapping populations or selecting for recessive traits, it is recommended to initiate in vitro culture from early generations (F1-F2). This approach maximizes time savings in the breeding process and ensures higher genetic variability, albeit with a potentially higher occurrence of unfavorable traits for breeding.

To enhance breeding progress and efficiency in producing favorable DH lines, it is beneficial to conduct preliminary selection in the first and second meiotic generations (F3-F4) for easily selectable traits (e.g., plant habitus, grain type, disease resistance). Laboratory work should commence with selected lines from these generations. This practice typically achieves a 2-3 year acceleration compared to traditional breeding methods.

In later generations (F5-F8), the advantage of the technique lies not primarily in speeding up the process but in stabilizing favorable traits and achieving population equilibrium. This step allows for the creation of candidate lines directly from the best breeding materials in one step.

Our experience shows that DH lines produced in vitro are successfully applicable in both traditional and water-saving aerobic rice cultivation under Hungarian conditions, given appropriate selection methods are available. In the DH0 generation, these methods are limited compared to traditional breeding tools, as only a single plant is available for testing.

To increase the efficiency of DH production in the future, particularly for indica genotypes, it will be essential to improve the success of androgenesis induction. Our research group has already made progress in this area recently (Lantos et al., 2023).

One of the most crucial elements in the breeding process is to apply the most effective selection methods that serve breeding goals optimally. For rice production, ensuring high yields and good food quality is paramount. However, selecting large breeding materials often faces challenges in terms of labor intensity and differing selection criteria, such as for aerobic and traditional cultivation technologies.

In the future, it would be prudent to incorporate marker-assisted selection (MAS) into the MATE rice breeding program to achieve key objectives (e.g., Sub1, SalTol, Pi9), facilitating pre-screening of valuable traits in early DH generations (Das et al., 2017; Jiang et al., 2020). This approach can significantly reduce costs associated with induced selection conditions and increase the number of genotypes tested. Many authors have reported on the effectiveness of MAS in various traits like cold tolerance (Fujino et al., 2019), resistance to bacterial blight (Xiao et al., 2019), amylose content regulation (Yang et al., 2019), drought tolerance enhancement (Anilkumar et al., 2023), and others.

Further opportunities to enhance selection efficiency are provided by remote sensing tools, increasingly equipped with extended camera systems, user-friendly processing software, and the application of artificial intelligence (Adak et al., 2021; Shakoor et al., 2019). Drones, for instance, offer the possibility to simultaneously examine and screen hundreds of breeding materials based on various vegetation indices (Liu et al., 2023), surface temperature of plant canopies (Jang et al., 2020), symptoms of diseases and stress conditions (Lin et al., 2023), as well as plant height or susceptibility to lodging (Su et al., 2022).

In the future, it is crucial to conduct detailed analyses on water requirements associated with different developmental stages for various rice genotypes. Using lysimeter studies, for instance, can provide essential information to significantly reduce water requirements while maintaining production security (Bouman et al., 2006). Combining complex solutions against water stress helps protect plants, including developing water-saving and moisture-retaining production technologies and improving genetic foundations (Nemali and Stephens, 2014). While several international examples exist for measuring rice evapotranspiration (ET) primarily in tropical and subtropical conditions (Tyagi et al., 2000), planned measurements can further validate and refine ET models under temperate region rice cultivation conditions (Raes, 2012).

NEW SCIENTIFIC RESULTS

Our research team has been using modern selection methods to increase the efficiency of Hungarian rice breeding, alongside focusing on the opportunities presented by in vitro androgenesis induction for many years. As a result, we can significantly shorten the breeding process and conduct selection on homogeneous populations. Of course, the efficacy of this method and the achievable time savings depend on the post-crossing generation of the initial plant material and the effectiveness of the selection methods used for testing DH lines. In traditional rice cultivation, selection methods primarily focus on enhancing milling quality and yield, including abiotic stress tolerance (cold, drought, and salt). In aerobic rice cultivation, the emphasis also includes improving drought tolerance. In our experiments, we have achieved the following new and innovative results:

1. To improve the effectiveness of Hungarian rice breeding, we created several combinations through sexual crossing. Our goal was to further enhance tolerance against abiotic (cold, drought, and bacterial blight) stresses while preserving the beneficial traits of previous Hungarian rice varieties (early maturity, cold tolerance, and good quality). To enhance efficiency, we were the first in Hungary to use a vacuum emasculator in rice breeding.
2. We integrated DH lines into the traditional rice breeding process, allowing us to conduct replicated performance comparison trials as early as the DH2 generation under flooded conditions.
3. We used homogenous lines produced via the doubled haploid technique for the first time in Hungarian breeding to directly select the most resistant lines under aerobic rice cultivation conditions. To select the most drought-resistant genotypes effectively, we employed leaf spectrometry and thermal imaging techniques.
4. For the first time in Hungarian climatic conditions, we used large-scale lysimeters to characterize the evapotranspiration of various rice genotypes at different developmental stages under aerobic cultivation conditions. We also determined specific evapotranspiration coefficients associated with each developmental stage of these genotypes. These findings can be used in the future to plan optimal irrigation related to water needs and to create targeted water-deficit conditions for more effective selection methods.

5. As a result of our work, two new rice varieties received state recognition: SZV Szellő in 2020 for its outstanding quality traits and SZV Tünde in 2021 for its high yield potential, both standing out in the domestic variety portfolio.

IMPROTANCE OF THE RESULTS FOR THE AGRICULTURAL PRACTICE

Our experiments have confirmed that DH lines can be effectively utilized in breeding programs. Based on trials conducted with japonica and indica rice varieties, researchers at the Szeged Cereal Research Non-profit Ltd. have developed efficient DH production protocols, which can significantly expedite breeding goals on an international level (Lantos et al., 2023, 2022).

To enhance the efficiency of Hungarian rice breeding, we produced DH plants from different generations of breeding lines. These homogenous DH lines were integrated into the traditional breeding process, allowing us to selectively breed for traits most important to production. In flooded conditions, agronomic traits, high yield, and milling quality were the primary selection parameters. Among the DH lines we examined, several significantly outperformed rice varieties already recognized by the state. Consequently, these lines can be directly utilized as candidate varieties or as parents in new crossing combinations in the near future. The introduction of new varieties is expected to increase market opportunities and competitiveness for domestic producers.

In my research, aimed at achieving breeding goals, I employed scientifically rigorous selection methods. These methods not only help identify genotypes that can be most effectively applied under Hungarian production conditions but also provided detailed insights into our entire plant collection. Armed with this knowledge, we can provide targeted genetic materials to domestic and international partners for breeding and research programs (mapping populations).

While ensuring flooded conditions in Hungary is beneficial not only for production but also for the natural environment (shallow water habitats), the technology of non-flooded rice cultivation presents new opportunities for rice growers. This method allows nearly any irrigable area to be suitable for rice cultivation alongside traditional rice paddies. However, cultivation under aerobic conditions increases the frequency and intensity of abiotic stresses. Without flooding water, drought conditions can develop more rapidly, exacerbating the negative effects of cold periods and soil salinity. Nevertheless, non-flooded technologies can significantly reduce water usage and, specifically for rice, the concentration of heavy metals like arsenic in plants can also be reduced. However, suitable varieties are essential for these methods. Our research helps select genotypes that can be safely used under aerobic conditions. One practical example is the success of our Austrian partner, who has been economically successful for several years near Vienna (ÖsterReis, Gerasdorf bei Wien, Austria) based on early Hungarian rice varieties.

To lay the foundation for further, more effective breeding work, we have generated over a hundred crossing combinations in the past 8 years. Noteworthy among these are combinations involving genotypes such as IRAT 109 and Dáma (1087), as well as IRAT 109 and Marilla (1080), whose offspring include several DH lines identified with similar drought tolerance as IRAT 109 but significantly earlier maturity, making them potentially viable for safe cultivation under Hungarian conditions. These lines are scheduled for detailed greenhouse and field trials in the coming years, complemented by molecular genetic studies.

For the safety of rice cultivation and the protection of natural resources, it is crucial to have detailed data on the water requirements at different growth stages of rice. Using precision field lysimeters initiated by us, evapotranspiration measurements provide a more accurate determination of irrigation needs under Hungarian environmental conditions. Furthermore, these measurements yield additional information on evapotranspiration changes characteristic of different developmental stages of plants and under various environmental stressors.

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