MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

Hungarian University of Agriculture and Life Sciences
Doctoral School of Plant Science

STUDY OF THE EFFECTS OF SALT STRESS AND LOW TEMPERATURE ON THE DEVELOPMENT OF RICE (ORYZA SATIVA L.)

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Árpád Székely

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Doctoral school:

Name: Doctoral School of Plant Science

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Head of Doctoral School: Lajos Helyes PhD

Professor, Doctor of the Hungarian Academy of Sciences (MTA)

MATE, Institute of Horticulture

Supervisor: Csaba Lantos PhD

Head of Biotechnology Laboratory

Cereal Research Non-profit Ltd.

Signature of approval by the supervisor and the head of the doctoral school:

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.

....	....
Head of Doctoral school:	Supervisor

Background and objectives of the work

Rice breeding in Hungary started in the 1930s, with the testing of 103 foreign rice varieties. The result of this work was the 'Dunghan Shali' variety, whose popularity continued unabated until the 1950s. It was at this time that the susceptibility of the variety to the fungal disease *Piricularia oryzae* (*Magnaporthe oryzae*) was discovered. This led to further breeding and the appearance of new varieties in public cultivation. Breeding has always tried to use the most modern tools of the time to produce better varieties with more competitive traits in a shorter time. In the 1960s and 1970s, this technology was induced mutagenesis, of which 'Nucleoryza' is the best example. The success of ‘Nucleoryza’ is reflected in the fact that in 1981, the variety was grown on 40 percent of the cultivation rice area. Subsequently, the first biotechnologically-derived rice variety, 'Dama', was produced in Hungary by haploid somaclone breeding, which was granted state recognition in 1992 and later patent protection. For 20 years between 1992 and 2013, 'Dama' was the most widely cultivated rice variety in Hungary. In addition to the examples mentioned above, many varieties have been produced whose success extends beyond our borders. The 'Abel' variety has a very short growing season, the 'Sandora' variety, known as 'HSC 55', is the cold tolerant variety and is known as one of the 20 most cold tolerant varieties in the world, and has been used in the breeding programmes of several countries (Turkey, Australia) and is still used in Chile for cultivation. The former line 'Rotundus' showed salt tolerance close to the *indica* group.

Hungary is located on the northern border of European rice production area, so most of the biotic stressors that are normally found in warmer climates are not present in our country. The only one is the aforementioned fungal disease. The current cultivability is mainly determined by two main abiotic factors. Low temperatures are mainly prevalent at the beginning of the growing season, during germination and the early vegetative phase, and around flowering. The second factor is the presence of salinity in the soil. Although its importance is minor compared to tropical and coastal areas, it is expected to increase in the future. Current rice production in our country is almost exclusively saline affected areas. Furthermore, less good quality irrigation water may be available in the future, which could lead to a further increase in secondary salinization. Thirdly, the low to medium salinity that currently characterises rice-growing areas, due to application of direct seeding, may have an inhibiting effect from germination onwards, in contrast to the planting method in tropical areas, which avoids early developmental susceptibility.

Because of these factors, my research focuses on the study of cold stress at early development and the effects of high salt concentration from germination to ripening phase. The main objectives were:

I. Salt tolerance studies:

1) To determine experimentally the salt tolerance parameters of genotypes belonging to the *japonica* and *indica* groups.

2) A comparative study of the salt tolerance of rice cultivars selected in a marginal habitat in Hungary with other *japonica* cultivars.

3) Determination of the adverse effects of salt stress at three developmental stages (germination, 3-4 leaf seedling and reproductive stage).

4) Identification of short and long term stress responses, mainly based on the concentration of sodium and potassium in different plant parts (root, flag leaf, grain).

5) To determine whether the tolerance at early developmental stage has an effect on the parameters determined at later developmental stage.

II. Testing cold tolerance:

1) Determination of the germination capacity of the rice variety collection at low temperature and comparison of the local varieties with the international standard ones.

2) Determination of cold tolerance of our variety collection at germination (3-4 leaves) by testing low temperature induced chlorophyll degradation.

Material and Methods

Salt tolerance tests

Plant material

The plant material for the experiments was provided by the rice variety collection maintained by the Galambos Rice Experimental Station, MATE, KÖTI-ÖVKI. Seven *japonica* cultivars ('Dunghan Shali', 'Risabell', 'M 488', 'Janka', 'Dáma', 'Nembo' and 'Sprint') and one *indica* ('Dular') were selected for testing (Table 1).

Table 1: Variety group and salt tolerance of the varieties used in the salt tolerance studies, with source works

Variety	Origin	Variety group	Salt tolerance	Other	Source
D. Shali	Hungary	*japonica_1*	n.d	High vigor	Abe et al. 2012
Dular	India	*indica*	n.d	-	
Dáma	Hungary	*japonica_1*	n.d	DH variety	Heszky et al. 1996
Janka	Hungary	*japonica_1*	n.d	Under cultivation	
M 488	Hungary	*japonica_1*	n.d	Under cultivation	-
Nembo	Italy	*japonica_2*	sensitive	Under cultivation	Frouin et al. 2018
Risabell	Hungary	*japonica_1*	n.d	-	
Sprint	Italy	*japonica_2*	tolerant	-	Frouin et al. 2018

Salt tolerance at germination

In our study we used 6 treatments and 3 replicates. The germination test was carried out in a climatic chamber (Binder Climatic/Photostability Test Chamber KBWF 240, Germany). Pre-treatment of the seeds at 50°C for two days was used to break the dormancy of the seeds (Gregorio et al. 1997). The seeds were separated into filled and unfilled seeds in 10% NaCl solution and then the surface of the healthy seeds was disinfected with 5% NaClO solution for 10 min. Germination was carried out in Petri dishes using 2 pieces of filter paper. The climate chamber was set at 30°C during the day and 25°C at night in 12 h cycles. The saline treatment was carried out with distilled water and NaCl at the following concentrations: 0 mM, 30 mM, 60 mM, 90 mM, 120 mM and 150 mM. The pH, electrical conductivity (EC) and temperature of the prepared solutions are given in Table 2.

Table 2: Physical parameters of the solutions used as treatments: conductivity of the solution (EC), pH, and temperature scale (T).

	EC (*d*S/m)	pH	T (°C)
0mM	0.19	6.76	21.9
30mM	3.33	6.83	21.5
60mM	6.26	6.58	21.2
90mM	9.08	6.51	21.9
120mM	11.82	6.49	21.8
150mM	14.52	6.51	21.8

The following parameters were used to describe the germination dynamics of the selected rice varieties:

- Germination percentage (GP): number of seeds germinated on the fourth day/total number of seeds *100
- Length of dormancy (DT): the dormancy period of the seeds, the number of days until germination starts
- Median germination time (MGT): time required for 50% of germination (Ranal 1999): $MGT = ti + (N/2-ni) - (tj-ti) / (nj-ni)$
- Germination rate: R 50=1/MGT (Labouriau 1983)
- Threshold for inhibition of germination rate: IC10,50=R 50*0,9; 0,5. The concentration at which R50 is reduced by 10% and 50% compared to the control (Bertazzini et al. 2018).
- Root and shoot length measurements (RL, CL): shoots were measured on day 7 using a ruler.

Seedling test

The seedling salinity tolerance test was carried out according to the IRRI method (Gregorio et al. 1997). Plants were grown in Yoshida nutrient solution (Yoshida et al. 1976) until 4 leaves, after which the nutrient solution was changed to 12 *d*S/m NaCl. Evaluation was performed on the 10th day after treatment. In the study, 17 parameters were used to describe the physiological changes in the selected rice cultivars: biomass parameters: shoot (SDW), root (RDW) and total (TDW) biomass dry weight. Membrane stability index (MSI) was used to assess leaf membrane damage, and root damage was assessed by root viability test (RV). Pigment content was determined according to the method of Sims and Gamon (2002), while sodium and potassium content was measured using Thermo Scientific Solaar M6 atomic absorption spectrophotometer.

Salt tolerance during the reproductive phase

The degree of salt tolerance during the reproductive phase was determined by a greenhouse experiment in a pot experiment. In preparing the experiment, the method of Gregorio et al. 1997 was followed to investigate the long (S1) and short (S2) term stress responses of the cultivars. In the experiment we used 90 litre plastic boxes filled with 50 kg of untreated soil, which is representative of the quality of rice fields in Hungary today. This soil was used as a control to simulate the field performance of the varieties. Salt treatment was carried out with 20 litres of 12 dS/m NaCl water. The first treatment (S1) was initiated at 4 leaf stage for continuous salt stress and the second (S2) during reproductive stage (BBCH 37-43) Water level was maintained at 1 cm above the soil surface during the growing season (Gregorio et al. 1997). Soil electrical conductivity was monitored weekly in 1:5 soil : water samples. The conductivity of the soil extract (EC25) measured at 25°C was used to express the salinity of the soil (Bado et al, 2016). During the experiment, vegetative and yield-related parameters were determined, as well as the sodium and potassium concentrations and the ratios calculated from them, which are most commonly used to determine the degree of the tolerance.

Cold tolerance tests

Plant Material

For the preliminary experiment, 7 cultivars were selected ('Dunghan Shali', 'Dular', 'IRAT 109', 'Janka', 'Nembo', 'Sandora', 'Sprint') to represent a wide range of responses to cold stress. In particular, the following selection criteria were considered: cold tolerance/sensitivity, country of origin and subspecies *(indica, japonica, tropical japonica*).

After the preliminary experiment, the variety collection of 164 genotypes from MATE-ÖVKI was tested for low temperature germination and chlorophyll degradation.

Germination testing

In the pre-experiment, different temperatures were set at 10°C, 12°C, 13°C and 15°C, and during the experiment, 40 sterilized seeds were germinated in Petri dishes between two filter papers in 3 replicates for 28 days in a Lovibond TC 256 G type refrigerated thermostat with 0.1°C accuracy. Adequate humidity was provided by distilled water. The median germination time (MGT) was used for comparison.

After the preliminary experiment, the genotypes were germinated at 13°C under the same conditions. The grains underwent similar pre-treatment as mentioned for the salt tolerance and the comparison was based on similar parameters: median germination time (MGT), germination percentage (GP), germination index (GI): GI = (N14+N21/2)/40 x 100 (Cruz and Milach 2004) and root and shoot length measurements (R%, C%).

Seedling test

Cold tolerance was monitored by changes in chlorophyll content during a natural cooling period under field conditions over four years. A Konika Minolta SPAD 502 instrument (in 2018, 2019 and 2020) and a CID Bio-Science leaf spectrometer (in 2021) were used for the measurements. During the cold tolerance studies, 60-60 grains per genotype were sown in a 1 m row. To match the natural cooling period to the plant population with the appropriate sensitivity, three different sowing dates were used: an early (10 April), a normal (end of April) and a late (early-mid May) sowing date. For each genotype, 10 biological replicates were used. The experiment lasted for 10 days, with time zero being the beginning of the natural cooling period and the start of the experiment. The first measurement was on day 5, immediately after the cooling period, and another five days on day 10. Using a leaf spectrometer, the chlorophyll content was calculated using the following formula following Parry et al. (2014):

CPHLT – Chlorophyll TOTAL (μg/cm^2): CPHLT = (8,2*A663) + (20,2*A645)

The varieties were classified into five categories according to the degree of chlorophyll change (Table 3). To avoid possible measurement uncertainty, a change of 1 unit was not considered a significant difference. The resulting responses and distributions were compared with the values of the cold tolerant cultivars ('M202', 'Sandora') used in our experiments and proven in the literature.

Table 3: Categories based on changes in relative chlorophyll content (SPAD, 2018, 2019, 2020) and chlorophyll content (µg/cm2; 2021) to separate reactions of each genotype

Category	Change	Explanation
1.	>+1	Chlorophyll content increase by at least 1 unit
2.	+1-(-1)	No significant change
3.	-1-(-2)	Chlorophyll content reduced by 1-2 units
4.	-3-(-5)	Chlorophyll content reduced by 3-5 units
5.	>-5	Chlorophyll content reduced by >5 units

Statistical evaluation

The effects of the treatments (salt, cold) were tested using the MANOVA. After detecting a significant treatment effect, the interaction effects of genotype, treatment and genotype x treatment were tested by two-factor analysis of variance, followed by Tukey or Games-Howell postHoc test. Correlation between parameters was calculated using Pearson's method. Significant differences in cold tolerance at seedling stage compared to the control time were determined by Dunnett's test. To further compare treatments, cultivars and years studied, principal component analysis was used after standardization of the data.

Results and discussion

The germination parameters (DT, MGT, GP, GR) clearly showed the detrimental effects of high salt concentrations on the average of the varieties. However, significant inhibition occurred only at Na^+ concentrations of 120 mM. However, after germination, the tested varieties showed different responses to different salt concentrations (Figure 1).

'M 488' and 'Janka' showed concentration-independent root growth, while in 'Risabell' and 'Dunghan Shali' increasing salt concentration increased root length. In addition, the shoots of the latter responded similarly (Figure 1). Previously published data showed that increasing Na^+ concentration clearly caused a decrease in coleoptile and radicle length (Girma et al. 2016).

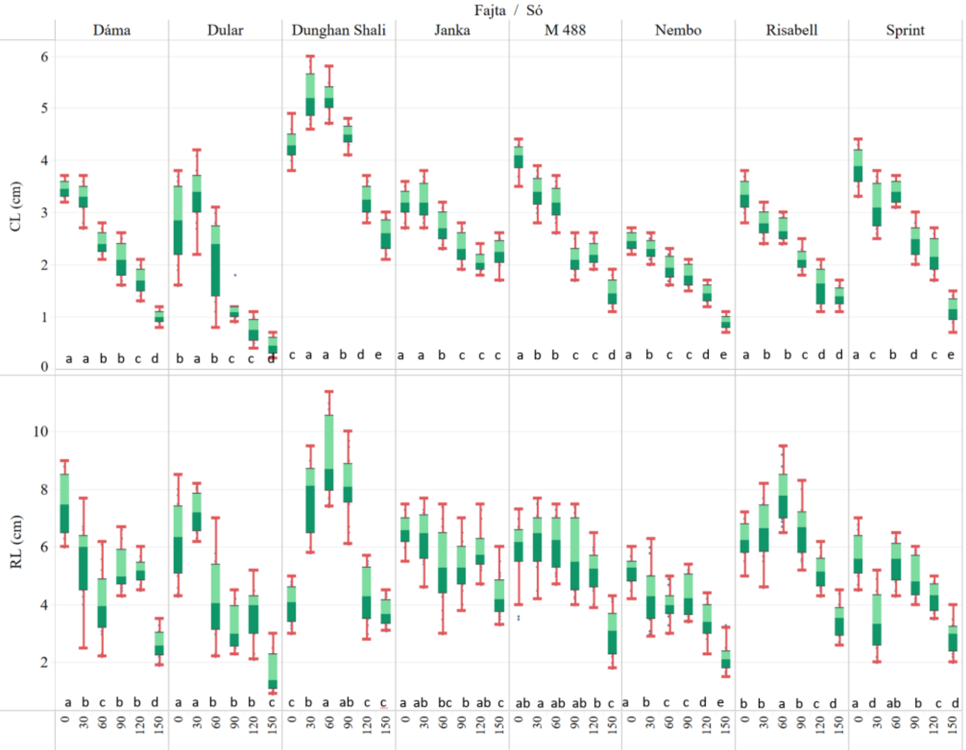

Figure 1: Coleoptile (CL) and radicle (RL) length (cm) of different cultivars under an increasing salt concentration (0mM-150mM). Different letters indicate significant differences between concentrations.

At germination age, significant effects of 12 *d*S/m salt on both biomass production and root and shoot injury were observed, both between cultivars and between cultivar groups. Based on sodium and potassium concentrations (Table 4), we found that there was no difference in root Na^+ concentration (0.99-1.37) as much as in root K content (0.38-0.71). Pires et al. (2015) determined an average of 56 genotypes after 12 *d*S/m treatment to have a potassium concentration of 0.3 mmol/g, which is half the average of our experimental measurements (0.6 ± 0.06 mmol/g). The highest potassium uptake was observed for the 'Risabell' variety (K/Na selectivity, Table 4). However, potassium concentration was less variable in the shoot. Based on our tests, the highest Na/K ratio was detected in 'Nembo' and 'Sprint', while the lowest was detected in 'Dunghan Shali'. The sodium concentrations of 'Nembo' and 'Sprint' (2.77 and 1.96 mmol/g, respectively) are also outstanding because Pires et al. (2015), under the same salt stress, did not find sodium concentrations higher than 1.8 mmol/g in sensitive varieties.

Table 4: Concentrations of sodium and potassium (mmol/g) in shoot and root and the relative values of the parameters calculated from them. Small letters indicate significant differences between varieties, while capital letters indicate significant differences between groups of varieties $p < 0.05$.

	Shoot				Root				
Genotype	Na	K	Na/K	K/Na selectivity	Na	K	Na/K	K/Na selectivity	SNa/RNa
Dáma	1,53b	0,77a	1,99b	30,65bc	1,06ab	0,65cde	1,64ab	36,07bc	1,44
Dular	1,71bc	0,88a	1,96b	30,66bc	1,37c	0,38a	3,60c	16,43a	1,25
D. Shali	1,17a	0,89a	1,31a	45,29d	1,17bc	0,67de	1,76ab	33,73bc	1,00
Janka	1,49b	0,77a	1,93b	52,05bc	0,96a	0,58bcd	1,65ab	35,96bc	1,55
M 488	1,74bc	0,82a	2,13bc	27,78bc	1,06ab	0,55b	1,91b	31,11b	1,64
Nembo	2,77d	0,75a	3,68d	16,09a	1,09ab	0,64cde	1,70ab	34,88bc	2,54
Risabell	1,46b	0,80a	1,82ab	32,61c	0,99ab	0,71e	1,39a	42,83c	1,47
Sprint	1,96c	0,73a	2,69c	22,02ab	1,17b	0,56bc	2,07b	28,53b	1,68
Japonica_1	1,48A	0,81A	1,84A	37,68B	1,05A	0,63B	1,67A	35,94B	1,42
Japonica_2	2,37B	0,74A	3,18B	19,06A	1,13A	0,60B	1,89A	31,71B	2,11
Indica	1,71A	0,88A	1,96A	30,66AB	1,37B	0,38A	3,60B	16,43A	1,25

We also investigated whether the Na$^+$ and K$^+$ concentrations measured in the shoot were influenced by any root parameters. We found that an increase in root viability resulted in decreased shoot Na$^+$ concentration (Figure 2).

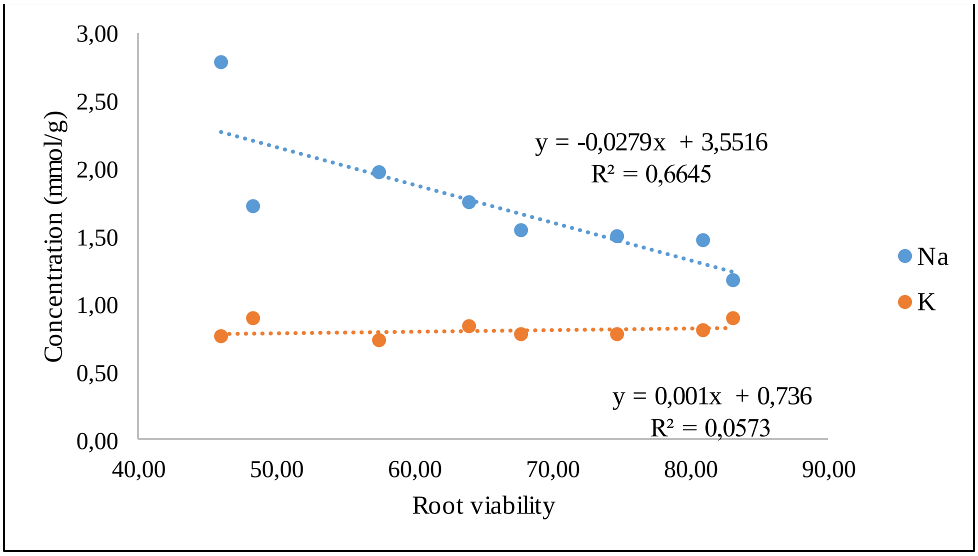

Figure 2: Leaf Na$^+$ and K$^+$ concentration as a function of root viability

In the greenhouse culture dish experiment, the root sodium concentration increased significantly (396.55 µmol/g; $p < 0.001$) under S2 treatment, as confirmed by a previous study (Pires et al. 2015). However, this level decreased under the longer-term stress (S1) (Table 12). Potassium levels decreased with the short-term treatment except for 'Dáma', which increased again to control levels in 'Janka', 'Risabell' and 'Sprint' due to long-term adaptation (Table 5).

Table 5: Amount of root Na and K in µmol/g in two treatments (S1 and S2) by cultivar and cultivar group. S1 shows the values measured under continuous salt stress, S2 shows the values in the reproreduction phase. Lower case letters indicate sig-significant differences between varieties and upper case letters between variety groups, while * indicates differences between treatment and control (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$).

	Na (µmol/g)			K (µmol/g)		
Variety	Control	S1	S2	Control	S1	S2
Dáma	227,93ab	212,70cd	287,74a	168,16abc	142,58d	122,33b*
Dular	289,40bcd	234,88d*	483,25d***	145,10ab	108,82bc**	166,07c*
D. Shali	235,10ab	205,89cd*	366,25ab***	141,82ab	124,30cd*	91,05a**
Janka	192,98a	144,63ab*	418,88bcd***	183,89c	81,97b**	165,09c
M 488	226,33ab	135,49a**	315,35a***	135,04a	48,08a**	79,2a**
Nembo	338,84d	244,16d*	471,08cd*	309,08d	183,76e*	163,43c**
Risabell	270,77bc	195,01c*	396,48bc**	154,99abc	92,84b***	158,91c
Sprint	304,05cd	176,16bc*	448,16cd**	172,76bc	107,54bc**	161,64c
Mean	255,37	198,89**	396,55***	173,98	112,00***	139,41*
Japonica_1	227,19A	182,36A*	353,16A***	158,89A	100,35A***	122,58A*
Japonica_2	321,45B	216,96A**	457,33B***	240,92B	145,65A*	162,53B
Indica	289,40B	234,89B*	483,25B***	145,10A	108,83A**	166,07B*

Both stress treatments (S1 and S2) increased the average sodium content of flag leaves to twice the control level (Table 6). In addition to increased Na^+, K^+ concentration also increased significantly. The S2 treatment did not increase flag leaf Na concentration in only two cultivars 'M 488' and 'Dular', although they had significant levels in the roots. In the case of 'M 488', the extremely high Na^+ concentrations, independent of the treatment, should be highlighted. A value of 234-272 µmol/g was typically observed in the third and fourth leaves, as measured by Ahmadizadeh et al. (2016). The 'Dáma' cultivar shows tolerance equivalent to the *indica* group based on sodium and potassium concentrations and their distribution among plant organs. In addition, the varieties 'Janka', 'M 488' and 'Risabell' are able to maintain high K^+ concentrations. This contradicts the studies of Lee et al. (2003), who found that *japonica* do not have high K uptake, so that their concentration would not rise above the control under stress.

Table 6: Concentrations of Na^+ and K^+ in flag leaf in µmol/g in two treatments (S1 and S2) by cultivar and cultivar group. S1 shows values measured under continuous salt stress, S2 shows the effect of stress applied during the reproductive phase. Lower case letters indicate significant differences between breeds and upper case letters between breed groups, while * indicates differences between treatment and control (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$).

	Na (µmol/g)			K (µmol/g)		
Variety	Control	S1	S2	Control	S1	S2
Dáma	21,03c	29,73ab	86,56b**	411,42de	433,25cd	404,73bc
Dular	11,59abc	14,81a	11,75a	267,52a	288,41a	347,57ab**
D.Shali	17,31abc	39,60ab*	83,3b**	434,10e	344,76ab**	520,21d*
Janka	19,53bc	89,60b**	90,91b**	452,17e	520,97e**	481,72cd
M 488	271,86e	250,54c	233,8c	362,79bc	454,39de*	428,39c
Nembo	6,71ab	54,37ab**	22,00a**	317,73b	280,48a*	316,71a
Risabell	11,44abc	93,95b***	55,25a**	370,16cd	503,32de**	517,4d**
Sprint	4,41a	41,13ab*	25,00a*	375,45cd	367,26bc	414,15bc
Mean	43,20	87,09***	76,07**	374,40	393,19***	417,9***
Japonica_1	68,23AB	112,25B	109,96C	409,23C	447,37B	470,49B*
Japonica_2	5,79A	47,75B***	23,49B**	346,59B	315,19A	365,43A
Indica	11,59B	14,81A	11,75A	267,52A	288,41A	347,57A**

In the cold tolerance tests, the entire variety collection of MATE-ÖVKI was tested for low temperature stress. After the principal component analysis of germination parameters, the varieties can be classified into three main groups. Group I. consists of varieties with no or very slow germination and high MGT. Within this group, a gradient is observed along the component II., where the negative part contains varieties with no germination at all and the positive part contains very slow germinating lines. Group II already contains varieties with low and medium MGT, also along a gradient. The negative part of component I. is characterised by medium MGT and low FGP, while the positive part is also characterised by medium MGT with high FGP. Group III. includes the varieties with the most favourable values for all three parameters.

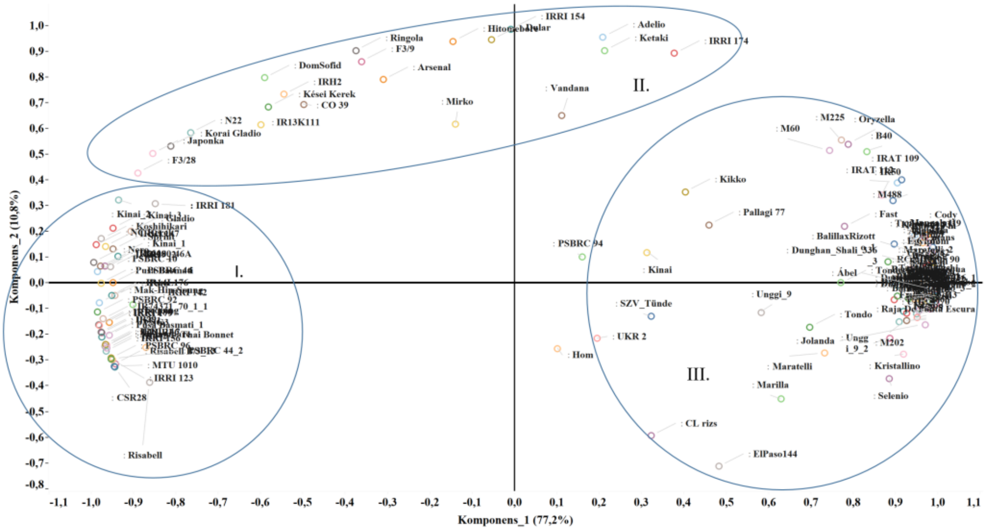

Figure 3: Distribution of varieties by germination parameters (MGT, FGP, GI) using principal component analysis.

Significant differences between varieties groups were detected (Table 7). However, there are no significant differences in several parameters between *temperate japonica* and *tropical japonica* due to large variation, although for example the length of coleoptiles shows almost a twofold difference. However, a significant difference was found for the germination index. Cruz and Milach (2004) also found this parameter to be the most reliable of the germination parameters.

Table 7: Germination parameters for the five different groups of varieties. N is the number of genotypes that can be assigned to a group based on the source data. Different letters indicate significant differences between groups. MGT = median germination time, FGP = final germination percentage, GI = germination index, C% = % of > 5mm coleoptiles, R% = % of > 5mm radicles.

Variety group	N	MGT	FGP	GI	C%	R%
aromatic	3	22,73c	52,22a	20,69a	8,56a	2,72a
aus	2	19,20ab	86,67b	40,83ab	1,83a	2,67a
indica	37	20,73ab	56,27ab	39,78ab	9,60a	3,33a
temp. japonica	94	12,86a	85,34ab	74,96c	57,39b	52,04b
trop.japonica	12	17,23ab	77,08ab	54,51ab	24,96ab	14,54ab

Four-year data on chlorophyll degradation during early vegetative development suggest that the *japonica* group has a stronger cold tolerance than the *indica* group. This is also supported by the scientific literature (Mackill and Lei 1997). Cold tolerant *indica* line has so far only been published by Biswas (Biswas et al. 2017). The *tropical japonica* group showed weaker cold tolerance compared to the *temperate japonica* group in two years (2019 and 2021), although a significant difference was only detected at the more advanced stage of stress, day 10 (Table 8). Our observation partially supports the results of Mackill and Lei (1997), who found that *tropical japonica* genotypes take values between *indica* and *temperate japonica* genotypes, but are not significantly different. Based on our results, this claim can be accepted with the addition that under more severe stress, however, the two groups are already significantly different. Within the *indica* group, we found that the 'Tequing' and 'CO 39' cultivars showed the highest cold tolerance, with the chlorophyll reduction measured on day 5 being able to increase to pre-treatment levels during regeneration. Although 'Tequing' is usually reported as a negative control, it is a sensitive genotype (Zhi-Hong et al. 2005). In addition, 'CO 39' has achieved the performance of the standards ('M202' and 'Sandora'), making it the only *indica* group genotype in our collection that has shown significant cold tolerance.

Table 8. Changes in average relative chlorophyll content (SPAD, 2018, 2019) and chlorophyll content (μg/cm^2; 2021) compared to control by variety group in 2018, 2019 and 2021. Different letters indicate significant differences between variety groups.

	2018			2019			2021		
	Control	5. day	10. day	Control	5. day	10. day	Control	5. day	10. day
aromatic	34,85ab	31,85ab	33,00ab	23,70a	18,50ab	18,10a	19,85a	16,35a	15,85a
aus	32,60a	29,00a	31,80a	21,20a	17,30a	17,10a	18,35a	1,80ab	15,85a
indica	34,91ab	31,24a	33,59abc	20,83a	16,95a	17,76a	18,86a	17,04ab	15,78a
temp.japonica	35,25ab	36,29bc	37,98bc	24,04a	22,02b	24,72c	22,54a	21,79b	21,14b
trop.japonica	36,83b	36,70c	38,37c	22,69a	19,29ab	21,38b	20,05a	18,18ab	16,47a

Conclusions and suggestions

My results confirmed that varieties bred in Hungary generally have good cold and salt tolerance. The performance of several local varieties/genotypes significantly exceeded international standards. Since there are only few examples of international use of rice produced in our country (Sandora), it is recommended that these lines be used in further breeding programmes. Nowadays, due to climate change, there is a growing interest in gene sources that can contribute to abiotic stress tolerance. This research programme is intended to contribute to this need and could provide a basis for further breeding programmes and genetic studies to further investigate the stress tolerance of these lines.

The genotypes used in this thesis were tested in several developmental stages for their responses to stress effects induced by high salt concentration and low temperature. Our studies showed that the root growth at germination of two cultivars currently in cultivation, 'M 488' and 'Janka', remained persistent up to Na^+ concentrations of 90 mM. A positive effect was also shown for the cultivars 'Risabell' and 'Dunghan Shali'. Root growth of these two varieties was more pronounced in the presence of Na^+. In 'Dunghan Shali', this growth vigour was also present in the shoot. During vegetative development, the cultivars bred in Hungary (*japonica_1*) were visually observed to have lighter leaves. Experiments confirmed that this means lower chlorophyll, carotenoid and anthocyanin content. Under stress, the levels of carotenoids and anthocyanins, which also function as antioxidants, increase significantly. However, in *japonica_2* and *indica*, stress does not alter anthocyanin levels, while carotenoids are slightly increased. These results demonstrate the rapid response of the non-enzymatic antioxidant system of *japonica_1*. It would be worthwhile to further investigate the levels of other related substances, ascorbic acid, and glutathione.

Testing at the seedling stage showed that root parameters, especially root mass and potassium concentration, have a significant effect on later developmental status. Potassium concentrations in 'Janka', 'M 488' and 'Risabell' are able to maintain high levels in the flag leaf, even under severe salt stress. According to the literature, the *japonica* group does not have high K^+ uptake under stress. This is confirmed by the majority of the varieties, as potassium concentrations do not recover to control levels after S2 treatment. In the case of 'Janka' and 'M 488', the measured high K levels are achieved by remobilization of root K content. However, in the case of 'Risabell', it is fully recovered upon application of long-term stress.

The rapid K mobilization of 'M 488' is achieved even under control conditions, and extremely high Na^+ concentrations were measured under high Na^+ concentration. A value of 234-272 μmol/g is typically observed in the third and fourth leaves. The rapid transport of Na is unhindered in this cultivar, as the Na^+ concentration in the grains is also extremely high. Despite the high Na^+ concentration, no physiological damage was observed in the control, despite the fact that almost identical Na^+ concentrations were observed. The existence of a more pronounced compartmentalization could be determined with certainty by examining the expression of four vacuolar NHX genes (OsNHX1, OsNHX2, OsNHX3 and OsNHX5).

In addition, we have identified genotypes with outstanding low-temperature germination capacity. This trait is of particular importance in temperate rice production, where direct seeding is the used technique. Based on literature data, the cold tolerance of *tropical japonica* lines is not significantly different from *temperate japonica*. However, four years of measurements show that this is indeed the case under moderate stress, but that the difference is statistically confirmed in the case of a significant temperature drop. What makes it difficult to detect the difference between groups is that there are sometimes significant differences between varieties, especially in the temperate japonica lines. For this group, it is observed that the long grain shape genotypes ('Risabell'; 'Ringola'; 'Oryzella' and 'Risabell B-3/13') show poorer performance at germination. However, the most cold tolerant of the *indica* lines was the round grain 'CO 39'. There seems to be a strong relationship between grain length and cold tolerance. Thus, in the future, it will be particularly important to test cold tolerance when producing long-grain lines.

New scientific results

1) 1) Two cultivars ('Janka', 'M 488') showed stable root growth up to 120mM despite increasing salt concentrations, and two others ('Risabell' and 'Dunghan Shali') showed stronger root growth than the control up to 90mM.
2) 2) The chlorophyll content of the *japonica_1* group is significantly lower than the other two groups under continuous salt stress. The anthocyanin and carotenoid contents are also lower under non-stress conditions, their concentrations increase under salt stress, with anthocyanin levels reaching the same level as *indica*.
3) 3) Root parameters measured at seedling age, especially root mass and K concentration, are significantly correlated with biomass during the reproductive phase and subsequent yield in the S1 treatment.
4) 4) The 'Dáma' variety is unique within the *japonica* group in showing salt tolerance equivalent to *indica*. A variety with outstanding tolerance is rare among *japonicas,* as certain tolerance mechanisms are absent in the group.
5) The 'Risabell' variety shows high potassium concentrations in both root and flag leaf, which cannot be explained by remobilisation alone.
6) The cold tolerance of the *tropical japonica* group at germination and under more severe cold stress at seedling stage is weaker than that of the *temperate japonica* group, as expected from the literature.
7) The *indica* group commonly described as cold-sensitive, only one genotype was previously identified as cold tolerant. Based on our studies, we have identified 'CO 39' as cold tolerant. This genotype achieved the performance of the standards ('M202' and 'Sandora') in all years, both at germination and at seedling age.

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