GERMINATION DYNAMICS OF EUROPEAN RICE VARIETIES UNDER SALINITY STRESS

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Salinity is one of the major abiotic factors that decrease the productivity of rice worldwide. Rice is mainly transplanted crop in the tropical region; therefore, rice plants can avoid the salinity at the seedling stage. However, in most of the European countries, direct sowing is the sole method, thus rice must grow under stressful condition already from germination. In the present study, rice varieties were analysed for salt stress tolerance during germination. Seeds of ten rice varieties were tested and kept under six salt stress levels (0, 30, 60, 90, 120,150 mM NaCl). Seed germination percentage, dormancy time, median germination time, germination rate, plumule and radicle length were used for the comparison. The results showed that due to the increasing salt stress, germination was not delayed and decreased in all cases. In case of 'DunghanShali' the plumule and radicle length increased significantly until 90 mM and 120 mM, respectively. Three more varieties ('M488', 'Risabell' and 'Unggi-9') could maintain growth potential of radicle until 90mM. 'DunghanShali' was found the most tolerant rice variety, while the most sensitive was 'Dular'. Seven varieties have reached higher germination percentage values than the international standard.

Keywords: germination, mean germination time (MGT), NaCl, rice, salt stress.

INTRODUCTION

Plants in field condition are usually exposed to multitude of biotic and abiotic stresses that limit their growth and productivity (Pereira, 2016). Soil salinity is considered as one of the major factors that reduce the growth of crop plants in many regions over the world.

In rice (Oryza sativa L.), the most crucial developmental phases are seedling and flowering stages in salinity stress(Singh et al., 2004), but germination is insensitive to salt (Heenan et al., 1988). In tropical regions, rice is mainly transplanted, the nurseries can alleviate the effects of salinity both in germination and seedling stages (Singh et al., 2004). Hence the salinity tolerance of flowering stage is more important than tolerance of germination phase. However, in most of the European countries, direct sowing is the sole method, that's why salinity may have an effect on growth already from germination phase. Rice breeding activities in Hungary were started in the 1930s. In that times 'Dunghan Shali' was found as the most prosperous rice with excellent early germination vigour (Takagi et al., 2013) and it's growth potential might be the key mechanism to avoid the toxic effect of salinity (Kumaret al., 2013).

In some studies, rice was reported as a relatively salt tolerant plant species during the germination phase. Germination percentage (GP) of genotypes were not affected significantly even under 16.3 dS m⁻¹ (Heenan *et al.*, 1988). Bangladeshi

genotypes show that GP, germination speed and vigour of two varieties ('BRRI dhan40'and'BINA dhan7') were increased due to the increasing salt concentration (16 g/L NaCl =274 mM) (Islam *et al.*, 2012). In contrast, GP, germination speed and germination energy of NERICA rice varieties were reported as sensitive reaction even in case of 'Pokkali' (Ologundudu *et al.*, 2014). Balkan *et al.* (2015) did not found significant decrease of germination rate up to 4 *d*S m⁻¹. Ghoneim *et al.* (2015) demonstrated that the increasing salt concentration has negative effect on GP, germination rate (GR), length of seedlings and fresh and dry weight of seedlings of two Pakistani and Egyptian varieties. This effect was also reported with other Iraqi varieties earlier (Abbas *et al.*, 2013).

In our study, effects of different salt concentrations were investigated on the germination dynamics of different rice genotypes. Our hypothesis is those Hungarian rice varieties which were selected on salt affected soils in the past has comparable salt tolerance with international standard. Since these local rice varieties are still very popular among the rice producers, especially in salt affected soils. These varieties had unclear salt tolerance, thus our aim was to test these entries under salinity conditions and make a comparison with international genotypes.

MATERIALS AND METHODS

The plant materials were chosen from the Rice Variety Collection maintained by NAIK ÖVKI Galambos Rice Research Station (Szarvas, Hungary). Five Hungarian japonica varieties ('DunghanShali', 'Risabell', 'M488', 'Janka', 'Dáma'), two Italian japonica ('Nembo', 'Sprint'), one Greek ('IE5593'), one Korean ('Unggi-9') and one indica ('Dular') were chosen to test salt tolerance. In our study, altogether 7200 seeds were investigated during the germination phase (10 varieties, 6 treatments and 3 replications). Germination test was carried out in Petri dishes between two layers of filter paper in a Binder Climatic Chamber (KBWF 240). We used pre-treating of seeds for two days at 50°C to break seed dormancy (Gregorio et al., 1997), because Nasim et al. (2000) reported that indica rice varieties had higher dormancy than japonica. The surface sterilized seeds were germinated in salt solution in climate chamber with 12 hour day/night cycle with 30°C and 25°C, and 80% relative humidity, respectively. The following concentrations of NaCl were used under the experiment: 0 mM (0.2 dS m⁻¹), 30 mM (3.3 dS m⁻¹), 60 mM (6.3 dS m⁻¹), 90 mM (9 dS m⁻¹), 120 mM (11.8 dS m⁻¹) and 150 mM (14.5 dS m⁻¹), respectively. The salinizations were carried out with distilled water and NaCl. The filter paper and NaCl solution was changed in every day to maintain the above mentioned concentration.

During our experiment, a seed was considered as germinated when radicle was observed as 1mm long. The longest primary root was measured by tape line. The number of germinated seeds was registered every six hours for four days.

In the present study, six parameters were used to describe germination dynamics of the selected rice varieties:

- Germination Percentage (GP): number of germinated seeds on the fourth day/total number of seeds *100
- Dormancy Time (DT): duration of seed dormancy, number of days to the beginning of germination process
- Median germination time (MGT): time for 50% of germination (Ranal, 1999):
- Germination rate: R 50=1/MGT (Labouriau, 1983)
- Measurement of radicle and plumule length (RL, PL): radicle and plumule length of seedlings were measured after 7 days of seed sowing.

Basic mathematical analyses were performed using of Microsoft Excel. Data were statistically analysed by "IBM SPSS 22" software. One way ANOVA with Tukey-method was used to test differences among treatment means at 5% level of probability. The significance of genotype, treatment and genotype x treatment interaction effects were tested by two ways ANOVA. Pearson correlation was calculated among the germination parameters to estimate the relation among different traits.

RESULTS

Based on our data, the germination dynamics of rice shows Gompertz distribution in control condition in an average of ten varieties. However, under high salinity level (150 mM) this distribution becomes linear. At this salinity level the correlation is highly accurate ($R^2 = 0.954$) (Fig. 1).

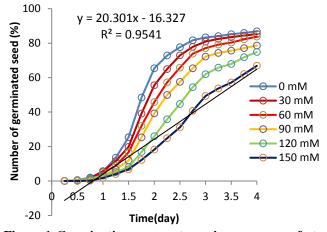


Figure 1. Germination percentage in average of ten varieties at six salinity level

According to Pearson correlation salinity has a medium influence on DT, GP, MGT and R50 in average of ten varieties (Table 1). However, there is strong negative correlation between increasing salinity level and plumule and radicle length.

 Table 1. The Pearson correlation matrix among the studied trait at germination phase.

	DT	GP	MGT	R50	PL	RL	Konc.
DT	1.000						
GP	-0.818	1.000					
MGT	0.912	-0.921	1.000				
R50	-0.876	0.704	-0.813	1.000			
PL	0.256	-0.085	0.242	-0.307	1.000		
RL	-0.016	0.065	0.003	-0.100	0.704	1.000	
Konc.	0.421	-0.425	0.443	-0.464	-0.707	-0.517	1.000

Based on analyses of variance (Table 3) we found significant effect of salinity, genotype and salinity*genotype interaction on every germination parameters.

The germination parameters under salinity stress could be seen in Table 2. Expect of 'Dunghan Shali' all varieties had longer DT than their control. We found the shortest DT in case of 'Dunghan Shali' at 30 mM (0.25 day) and only just 150 mM was significantly higher than the lowest one. The longest DT was in case of 'Dular' in every concentration of salt.

The GP of three varieties ('M488', 'Janka' and 'DunghanShali') was not decreased significantly in spite of salty environment. These varieties reached 80% in every salt

	Salt								Salt						
Variety	level	DT	GP	MGT	GR	PL	RL	Variety	level	DT	GP	MGT	GR	PL	RL
Dáma	Control	0.92±0.14a	95.00±2.50a	1.72±0.06a	0.58±0.02a	3.46±0.15a	7.48±1.04a	Dular	Control	1.42±0.14a	67.50±5.77a	2.90±0.69a	0.34±0.03a	2.74±0.60a	6.31±1.31a
	30 mM	1.00±0.00a	94.17±5.77a	1.87±0.05ab	0.53±0.02a	3.26±0.30a	5.52±1.28b		30 mM	1.75±0.00a	72.50±9.01a	3.10±0.85a	0.32±0.03ab	3.44±0.53b	7.09±0.65a
	60 mM	1.00±0.25a	86.7±2.89ab	2.1±0.13b	0.48±0.03b	2.46±0.22b	4.08±1.15c		60 mM	1.92±0.52a	42.50±5.3a	4.60±0.00ab	0.22±0.04ab	2.04±0.78c	4.13±1.40b
	90 mM	1.33±0.14a	84.2±6.29ab	2.53±0.05c	0.40±0.01c	2.19±0.34c	5.33±0.77b		90 mM	2.08±0.29a	22.5±6.29ab	5.20±0.80bc	0.19±0.01b	1.17±0.37d	3.50±0.90b
	120 mM	1.42±0.14b	78.33±2.89b	2.78±0.21c	0.36±0.03c	1.62±0.24d	5.28±0.41b		120 mM	2.92±0.14b	30.00±2.50b	5.80±0.14cd	0.17±0.00b	0.78±0.22d	3.61±1.12b
	150 mM	1.25±0.25a	65.83±3.82c	3.67±0.10d	0.27±0.01d	1.02±0.12e	2.57±0.50d		150 mM	3.08±0.29b	17.50±1.44b	8.30±0.18d	0.12±0.00b	0.43±0.16d	1.81±0.77c
Risabell	Control	0.83±0.14a	95.8±1.44ab	1.57±0.04a	0.64±0.02a	3.35±0.34a	6.06±0.67a	Nembo	Control	0.75±0.00a	96.67±3.82a	1.67±0.13a	0.60±0.04a	2.27±0.14a	4.17±0.69a
	30 mM	0.92±0.29ab	97.50±4.33a	1.69±0.03ab	0.59±0.01ab	2.82±0.25b	6.56±0.93a		30 mM	0.75±0.25a	90.83±2.89a	1.77±0.18a	0.57±0.06a	2.00±0.15b	3.20±0.44b
	60 mM	0.83±0.14a	97.50±2.50a	1.81±0.11ab	0.55±0.03b	2.64±0.18b	7.81±0.86b		60 mM	0.92±0.14a	90.83±1.44a	2.00±0.25ab	0.50±0.06ab	1.61±0.09c	3.30±0.58b
	90 mM	0.92±0.14ab	88.3±1.44bc	1.91±0.14bc	0.53±0.04b	2.12±0.19c	6.54±0.87a		90 mM	0.75±0.00a	87.50±5.00b	2.15±0.38bc	0.47±0.08ab	1.54±0.07c	3.54±0.51b
	120 mM		88.3±3.82bc		0.46±0.03c	1.61±0.34d	5.26±0.59c				85.00±2.50b		0.37±0.05b	1.25±0.14d	2.54±0.49c
			82.50±2.50c		0.40±0.02c	1.41±0.20d	3.44±0.62d			1.50±0.25b	66.67±1.44b		0.30±0.02b	0.69±0.03e	1.50±0.13d
M488	Control	0.75±0.25a		1.70±0.15a		3.94±0.34a	5.73±1.16ab	Sprint			89.17±3.82a		0.49±0.02a	3.48±0.30a	4.92±0.65a
		0.92±0.14a		1.88±0.08ab			6.28±0.97a			1.25±0.00a		2.40±0.16ab		2.55±0.08c	2.13±0.34d
		0.75±0.00a	89.17±3.82a		0.52±0.01b	3.28±0.31b	6.19±0.89ab			1.42±0.14a		2.51±0.15bc		3.15±0.17b	4.51±0.39ab
		0.83±0.14a	83.33±1.44a		0.52±0.01b	2.12±0.24c	5.93±1.22ab				70.83±2.89b		0.35±0.01b	2.09±0.22d	4.17±0.39bc
		1.17±0.38a	81.67±3.82a		0.42±0.01c	2.31±0.24c	5.30±0.74b				60.8±3.82bc		0.27±0.04c	1.73±0.19e	3.68±0.36c
		1.00±0.25a	79.17±10.1a		0.41±0.02c	1.44±0.20d	3.08±0.89c				47.50±6.61c		0.24±0.01c	$0.80\pm0.16f$	2.16±0.24d
Janka		0.92±0.14a	89.17±5.20a		0.60±0.01a	3.28±0.25a	6.69±0.51a				94.17±1.44a		0.52±0.03a	2.47±0.21a	4.82±0.55b
		1.08±0.14a		1.72±0.02ab		3.26±0.36a	6.28±0.85ab			1.00±0.25a	94.17±1.44a		0.51±0.02a	2.32±0.17a	6.27±0.39a
		0.92±0.29a	82.50±8.66a		0.52±0.02b	2.84±0.30b	5.35±1.36c			1.00±0.25a	96.67±1.44a		0.48±0.05a	2.01±0.09b	5.08±0.75b
		1.25±0.25a	85.83±3.82a		0.52±0.02b	2.29±0.27c	5.46±1.03bc			1.33±0.29a		2.30±0.25ab			
		1.42±0.14a	84.17±1.44a		0.46±0.02c	2.09±0.18c	5.97±0.9abc			1.33±0.14a		2.58±0.11bc		1.15±0.07d	5.02±0.55b
			81.67±2.89a		0.40±0.02d	2.21±0.26c	4.39±0.73d			1.50±0.00a	85.00±6.6ab		0.36±0.02b	0.77±0.08e	3.13±0.19c
D.Shali			87.50±5.00a			4.21±0.27a	4.07±0.62a	IE 5593		1.08±0.29a	95.83±2.89a		0.52±0.05a	3.06±0.14a	5.03±0.67a
			95.83±5.20a			5.29±0.48b	7.77±1.15b			1.33±0.14a	95.00±5.00a		0.47±0.05ab		3.21±0.37d
			95.00±2.50a				9.23±1.37c			1.25±0.25a		2.25±0.19ab			4.49±0.46ab
			92.50±4.33a				8.27±1.11bc			1.42±0.14a		2.46±0.37ab			4.20±0.44c
			89.17±5.20a				4.24±0.98a			1.50±0.00a		2.80±0.18bc		1.60±0.12c	4.42±0.43bc
	150 mM	0.75±0.25b	86.67±5.20a	1.39±0.11b	0.72±0.06b	2.54±0.29e	3.77±0.49a		150 mM	2.08±0.29b	69.17±1.77b	3.13±0.05c	0.32±0.00b	0.92±0.14d	2.21±0.13e

 Table 2. Germination parameters of ten rice varieties under six salt concentration. The letters shows significant differences to the control.

Table 3. Analyses of variance of germination parameters. SS - Sum of squares, df - degree of freedom, MS - Mean squares, F ratio and Sig. - p value.

DT						GP						
Source	SS	df	MS	F	Sig.	Source	SS	df	MS	F	Sig.	
Genotype	34.667	9	3.85	94.82	0.00	Genotype	73793.368	9	8199.26	260.35	0.00	
Salt	9.418	5	1.88	46.37	0.00	Salt	8677.674	5	1735.53	55.11	0.00	
Genotype * Salt	5.127	45	0.11	2.80	0.00	Genotype * Salt	4422.674	45	98.28	3.12	0.00	
Error	4.875	120	0.04			Error	3779.167	120	31.49			
]	E50					R	50				
Genotype	430.221	9	47.80	628.92	0.00	Genotype	4.578	9	0.51	368.22	0.00	
Salt	53.039	5	10.61	139.56	0.00	Salt	0.979	5	0.20	141.70	0.00	
Genotype * Salt	45.164	45	1.00	13.20	0.00	Genotype * Salt	0.095	45	0.00	1.52	0.04	
Error	9.045	119	0.08			Error	0.163	118	0.00			
		PL					R	L				
Genotype	449.365	9	49.93	557.37	0.00	Genotype	550.375	9	61.15	79.47	0.00	
Salt	588.460	5	117.69	1313.81	0.00	Salt	1097.596	5	219.52	285.26	0.00	
Genotype * Salt	100.865	45	2.24	25.02	0.00	Genotype * Salt	868.072	45	19.29	25.07	0.00	
Error	89.133	995	0.09			Error	765.689	995	0.77			

concentration. The other varieties had decreasing tendency. The best performing varieties under highest salinity stress were 'Dunghan Shali' (86.67%), 'Unggi-9' (85.00%) and 'Risabell' (82.50%) and the lowest ones were 'Sprint' (47.50%) and 'Dular' (17.50%).

The MGT increased with increasing salt stress level in an averages of all varieties. The rate of increase MGT was lowest in 'Dunghan Shali'. 'Dunghan Shali' needs 1.39 days to reach 50% of the seeds germination at 150 mM. In contrast 'Dular' needs 8.30 days.

The highest GR was recorded at 'Dunghan Shali' in every salt

concentration and the lowest at 'Dular'. The two-threefold differences between them were significant. The other varieties' values were between 'Dunghan Shali'and 'Dular', and they had similar rate of germination.

The comparison of PL shows different reactions to salinity. Most sensitive varieties, were 'Risabell', 'M488', 'Nembo' and 'Sprint'. These varieties had significant PL reduction already from 30 mM.PL of 'Dáma', 'Janka', 'Dular', 'Unggi-9' and 'IE5593' decreased from 60mM. In contrast, in case of 'Dunghan Shali', increase of PL was observed up to 90 mM. At 150 mM the maximum PL were observed in case of 'Dunghan Shali' (2.54 cm), while the minimum value was measured in 'Dular' (0.43 cm). The reaction of RL also depends on the variety. Most strongly reactions were found in case of 'Dáma', 'Nembo', 'Sprint' and 'IE5593'. 'Janka' and 'Dular' had a medium decrease resulting from the salinity, and the most tolerant varieties were 'Risabell', 'M488', 'Dunghan Shali' and 'Unggi-9' because they could maintain radicle growth despite higher salinity levels (90-120 mM). The highest value of RL was observed in 'Janka' (4.23 cm) at 150 mM while the lowest data of RL was measured in 'Nembo' (1.50 cm).

DISCUSSION

Even though rice is a tolerant crop under germination phase to salinity (Heenan et al., 1988), GP, PL and RL under varying degree of salt stress are good salt tolerance indicators at initial stages (Reddy et al., 2014). There are two important parameters that determine salinity tolerance of different varieties, threshold of damage and slope (Hoang et al., 2016). In this paper we defined the damage threshold as 90% and 50% of control (Bertazzini, 2014). Based on germination rate, the damage threshold of the varieties were 3.3 to 7.58 dS m^{-1} (Table 4) Sensitive varieties reached 50% damage at 14-16 dS m^{-1} , however, the tolerant ones were above 18-20 dS m⁻¹ ¹(Bertazzini, 2014). Henceforth 'Dular' and 'Dáma' are sensitive; 'Nembo' and 'Sprint' have a medium reaction to salinity. 'Risabell', 'Janka', 'Unggi-9' and 'IE5593' are tolerant; while 'M488' and 'Dunghan Shali' are highly tolerant ones (Table 4). The average threshold of rice was 4.95 dS m⁻¹ (Table 4) These results confirm the experiment of Asch and Wopereis (2001), who did not experience higher decrease than 90% of GR, up to salinity levels of 4 dS m⁻¹, however above 6 dS m⁻¹the GR reduced to less than 50%.

 Table 4. The calculation of 10% and 50% damage of germination rate based on linear regression.

_	Damage threshold $(d\vec{S} m^{-1})$					
_	10%	50%				
Dáma	3.48	14.31				
Unggi-9	5.26	23.08				
Dular	3.30	12.96				
IE 5593	5.41	19.70				
Sprint	5.05	17.74				
Nembo	4.79	16.22				
M 488	4.87	24.15				
D.Shali	7.58	29.76				
Risabell	4.51	20.20				
Janka	5.29	22.44				
Average	4.95	20.06				

Diaguna *et al.* (2017) reported that the GP values were 67-71.3% in tolerant genotypes and 27.3-31.3% in sensitive genotypes at 4000 ppm (4g/l=68 mM) salinity level.

Furthermore, Hakim *et al.*(2010) reported 60.5% at 12 dS m⁻¹, while Ologundudu *et al.*(2014) found 68.42% at 15 dS m⁻¹. These results were confirmed by Senanayake *et al.*(2017), who examined 36 Pokkali accessions under 12 dS m⁻¹salinity level and they found that the highest GP was 71.5%. In contrast, seven varieties ('Dunghan Shali', 'Risabell', 'M488', 'Janka', 'Nembo', 'IE5593', 'Unggi-9') showed 80-90% at the same salinity levels (120mM=11.8 dS m⁻¹).

In our study, the most sensitive variety was 'Dular'. It's germination parameters start to decrease 60 mM (6.3 dS m⁻¹). In contrast, Narale et al. (1969) published the threshold on 8.9 dS m⁻¹. Most tolerant rice variety was the 'Dunghan Shali'. It had the shortest DT at the highest salinity level (0.75 day=18)hours). This value was four times lower than in case of the most sensitive variety ('Dular'). The GP of this variety was the highest (86.67%), higher than the earlier reported values of 'Pokkali' (Diaguna et al., 2017; Senanayake, 2017; Hakim et al., 2010;). Moreover, half of the seeds were germinated less than 1.39 days (MGT). The value of GR was also the highest (0.72) among the tested genotype. Balkan et al (2015) found that PL and RL of tolerant rice variety were not significantly affected by salinity levels up to 8 dS m⁻¹. On the contrary, we found an increasing stimulating effect of salinity on both PL and RL until 90 mM in 'Dunghan Shali'. Similar tendency was observed in RL of 'Risabell' and 'M488'. It seems these varieties are able to absorb water despite of osmotic stress, and salty conditions are favourable for the germination. According to Abe et al. (2013) the relative expression levels of GA20ox1 in Dunghan Shali higher than control varieties. OsGA20ox1 gene is working under the germination and this gene ensures high endogenous gibberellin levels. High gibberellin might be the main mechanism which provides the early salt tolerance of genotype.

Conclusion: This study aimed to compare the reactions of different rice varieties under salinity stress. Hungarian varieties have a great salinity tolerance. This is evidenced by the germination percentage, damage threshold and radicle length too. In case of seven varieties, the germination percentage was higher than the earlier reported Pokkali's data. Based on damage threshold two rice varieties named 'Dunghan Shali' and 'M488' had higher salinity tolerance than Bertazzini reported. 'Dunghan Shali' PL and RL was elevated under the salinity stress than the control. Development of 'Dunghan Shali' was the most vigorous under salinity stress, and it's rapid growth potential might be the key mechanism to avoid the toxic effect of salinity.

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