Effect of salinity on rice (*Oryza sativa* L.) in seedling stage

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Abstract: Salinity is an important limiting factor that decreases the yield of rice (Oryza sativa L.) significantly. The breeding of new varieties for salt tolerance can be the most efficient way of adaptation. Germination, early seedling and reproductive stages are critical growth phases. A hydroponics screen (Yoshida medium set to 12dS/m) developed by the International Rice Research Institute was used to test Hungarian ('Dáma', 'Dunghan Shali', 'Janka', 'M 488', 'Risabell') and international ('Nembo', 'Sprint', 'IE-5593', 'Dular', 'Unggi-9') rice varieties. Parameters of germination, biomass production and special plant physiological characters were investigated. In the early seedling stage (3-4 leaves), biomass production of 'Dunghan Shali' (3.62 g per 20 plants) and 'Dular' (3.32 g per 20 plants) was measured as highest, while 'Nembo' and 'Sprint' produced only 1.53g and 1.44g per 20 plants, respectively. The chlorophyll content of the varieties was observed between 0.71 g/1g fresh weight ('Unggi-9') and 0.41 g/1g fresh weight ('Sprint').

Keywords: salt stress, rice, biomass, chlorophyll

Introduction

Plant growth responses to salinity vary with plant life cycle. Critical stages are germination, seedling phase and flowering (Flowers 2004). Criteria for evaluating and screening salinity tolerance in crop plants vary depending on the level and duration of salt stress and the plant developmental stage (Shannon 1985). In general, tolerance to salt stress is assessed in terms of biomass production or yield compared to non-stress conditions. In conditions of low to moderate salinity, the production capacity of the genotype is often the most pertinent measure, whereas survival ability is often used at relatively high salinity levels (Epstein *et al.* 1980).

Abiotic stress tolerance, especially salinity stress, is a complex trait, because of variation in sensitivity at various stages during the life cycle. Rice is comparatively tolerant to salt stress during germination, active tillering (vegetative growth) and maturity. It has the most sensitive reactions during seedling (3-4 leaf) and reproductive stages (booting). Screening at early growth stage (2–4 weeks) is more advantageous than at flowering. This is due to the fact that it is (1) quick, (2) seedlings take up less space and (3) tolerant seedlings may be recovered for seed production. Therefore (4) seedling tests are more efficient in terms of time and costs.

The major inhibitory effect of salinity on plant growth and yield has been attributed to: 1) osmotic effect 2) ion toxicity 3) nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders. The aim of the present study is to provide information on the effect of salinity on pigment concentration and biomass production.

Materials and methods

After our previous study, when effects of salt stress at germination phase were examined (Székely *et al.* 2016), physiological parameters under high salinity during vegetative stage were investigated. Therefore, we used the salt tolerance screening manual of the International Rice Research Institute (Los Banos, The Philippines). The method is simple to use. Ten rice varieties (nine *japonica* varieties ('Dunghan Shali', 'Risabell', 'M 488', 'Janka', 'Dáma', 'Nembo', 'Sprint', 'Unggi-9' and 'IE 5593') and one *indica* ('Dular') were chosen to test salt tolerance. The varieties were grown on Yoshida solution in greenhouse for two weeks. Salt stress was induced by the addition of NaCl to 8 dS/m conductivity (EC) for one week. Final EC was set to 12 dS/m. 20 plants of every variety were used in one replication.

The number of parallels was four. Pigment concentration was estimated on four individual plants after two-week period of stress imposition. A median segment was collected on each youngest leaf. We determined biomass production and content of photosynthetic pigments (chlorophyll a and b, anthocyanins and carotenoids).

According to Krishnan (1996), the best method to extract total chlorophyll is incubation with 80 % acetone; therefore we used Lichtenthaler's method (1987).

The extraction of chlorophyll was made in two phases. We added 1.5 ml 100 % acetone to 25mg piece of leaf and incubated for 24 hours. Then samples were centrifuged (15000 rpm), the supernatant was put aside and 1.5 ml 80 % acetone was added again for 24 hours. At the end of experiment, the extract liquid was made up to a total volume of 6 ml with 80 % acetone.

The absorbance was measured by photometer (Hach DR 4000) at four wavelengths: 470, 534, 643 and 661 nm. The pigment content was calculated by the following formulas:

Anthocyanin = 0.0821*A534 - 0.00687*A643 - 0.002423*A661

Chlorophyll b = 0.02255*A643 - 0.00439*A534 - 0.004488*A661

Chlorophyll a = 0.01261*A661- 0.001023*A534 - 0.00022*A643

Carotenoids = (A470 – 17.1*(Chl a + Chl b) – 9.479*Anthocyanin) / 119.26

Results and discussion

Morphological parameters such as shoot fresh weight and shoot dry weight are correlated with crop salt tolerance at early growth stages and can be used as an indicator for salt tolerance (Noreen and Ashraf 2008).

The results of salt tolerance screening of rice varieties at the seedling stage are shown on the Table 1. 'Dunghan Shali' and 'Dular' had the highest dry weight regarding shoot development (3.62 g and 3,32 g/20 plants), while 'Nembo' and 'Sprint' produced only 1.53 g and 1.44 g per 20 plants, respectively.

The difference is twice as much. In case of root formation 'Dular' developed the biggest root system while 'Nembo' and 'Sprint' were the most undeveloped ones. The difference is quadruplicate.

This is very interesting, because in our previous study (Székely *et al.* 2016) we confirmed that 'Dular' is the most sensitive variety at germination phase under salinity. Dry matter content was observed in a range of 24.15 % ('Janka') and 29.95 % ('Dáma').

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Table 1. Biomass and pigment content of ten rice varieties under saline condition. Biomass was calculated as an average of 20 plants (g). Fw – fresh weight, DW – dry weight. Anthocyanins, Chlorophyll and Carotenoids are given in µg/g fresh weight

		FW	DW	Dry matter	Anthocyanin	Chlorophyll a+b
		Av± SD	Av± SD	Av± SD	Av± SD	Av± SD
D.Shali	shoot	14.90±3.12	3.62±0.36	24.61±2.73	293.82±31.36	1004.12±55.59
	root	9.60±0.60	0.79±0.10	8.17±0.57		
M-488	shoot	7.89±0.31	2.00±0.09	25.34±0.09	178.06±10.31	1126.83±65.38
	root	5.28±0.43	0.47±0.02	8.92±1.11		
Janka	shoot	9.96±0.57	2.41±0.42	24.15±2.87	165.69±11.35	859.74±3.21
	root	7.79±2.20	0.70±0.13	9.17±0.92		
Dáma	shoot	8.39±0.97	2.50±0.02	29.95±3.19	192.15±5.06	1201.75±112.33
	root	6.89±0.30	0.65±0.02	9.46±0.64		
Risabell	shoot	7.25±0.97	1.84±0.31	25.38±0.86	295.86±37.28	1031.67±76.31
	root	4.24±1.10	0.43±0.09	10.12±0.44		
Dular	shoot	13.74±0.15	3.32±0.18	24.17±1.02	352.14±17.90	1226.58±92.39
	root	12.42±0.08	1.29±0.00	10.35±0.10		
Sprint	shoot	5.37±0.72	1.45±0.13	27.00±1.21	300.87±46.12	820.86±33.88
	root	3.10±0.32	0.32±0.03	10.32±0.02		
IE-5593	shoot	11.47±1.19	2.79±0.12	24.37±1.46	435.25±20.05	948.99±30.04
	root	8.15±1.30	0.74±0.11	9.26±2.82		
Nembo	shoot	5.20±0.10	1.53±0.07	29.50±0.86	425.96±40.46	975.37±83.45
	root	3.65±0.59	0.36±0.04	9.78±0.51		
Unggi-9	shoot	8.09±0.74	2.20±0.13	27.22±0.86	326.57±21.89	1426.34±84.90
	root	5.12±0.79	0.50±0.08	9.78±0.14		

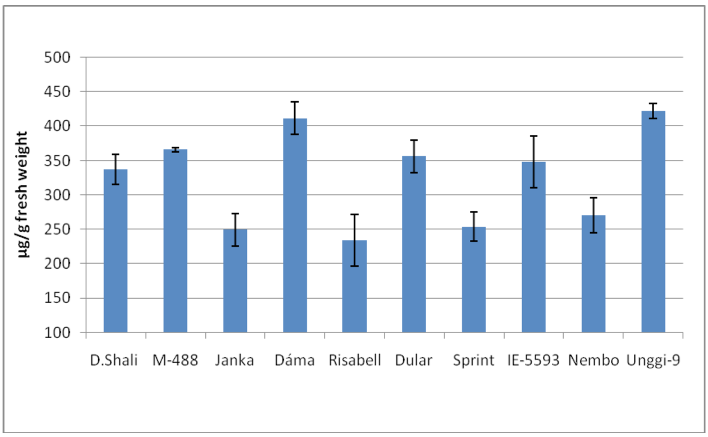

Figure 1: The carotenoid content of rice varieties under salt stress conditions during the early vegetative stage (µg/g fresh weight).

Anthocyanins and carotenoids are parts of antioxidant system of plants. The data showed a large range of pigment concentrations. High level of anthocyanins was observed 'IE-5593' (435.25 µg/g fresh weight), while 'Janka' has just 165.69 µg/g fresh weight. Comparison of different genotypes shows that 'Unggi-9' had highest total chlorophyll content and 'Sprint' and 'Janka' had the lowest value. The maximum of carotenoids under saline condition was recorded in 'Unggi-9' and 'Dáma' as 422.27µg/g and 411.59µg/g, respectively (Figure 1). Minimums were calculated in 'Risabell', 'Janka' and 'Sprint'.

Conclusions

This paper has focused on growth parameters and pigment content of rice seedlings under salt stress conditions and shows that tolerant rice varieties had higher pigment content, while the sensitive ones had lower pigment content. 'Dular' was characterized as the most tolerant variety at seedling stage; 'Sprint' and 'Nembo' were the most sensitive ones. 'Dular', 'Dunghan Shali' and 'IE-5593' produced the highest biomass (shoot and root). 'Dular' had high level of anthocyanin, total chlorophyll and carotenoid content, while 'Dunghan Shali' had only average pigment content. High level of anthocyanin was measured in 'IE-5593' and 'Nembo'. Further testing requires identifying the background of high pigment contents of 'Unggi-9' (high chlorophyll and carotenoid).

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