



RESEARCH ARTICLE

Screening of rice genotypes for salt tolerance by physiological and biochemical characters

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ABSTRACT

Crop production is unexpectedly hampered by different abiotic stresses. Salinity is one of the leading stresses, which snappishly hampers plant developmental progression. Local rice landraces exhibit noticeable salt tolerance as well as high yield. However, research is scarce about the physio-biochemical responses of local landraces and modern cultivar under saline conditions. Therefore, the present experiment was designed to reveal the physio-biochemical responses of local landraces and modern cultivar under salinity stress. Five landraces (Jotai, Icheburogolghor, Morishal, Chapail, Kumro buro) and two modern cultivars (BR23 and BRRI dhan41) were subjected to 0, 20, 40, 60 and 80 mM NaCl treatment. The effects of salt stress on morphological parameters, proline contents, and activities of antioxidant enzymes were assessed. Salt stress reduces the morphological parameters of all tested cultivars. The Morishal and BRRI dhan41 exhibited higher growth of plant and physiological parameters than other cultivars under the highest salinity. The catalase (CAT) and ascorbate peroxidase (APX), exhibited a significant increase whereas peroxidase (POX) activity significantly declined in all the cultivars under salinity stress. Morishal and BRRI dhan41 showed the highest proline content under the maximum saline condition. These results suggest that the high tolerant landrace and modern cultivars were Morishal and BRRI dhan41 respectively. These results also suggest that Morishal and BRRI dhan41 exhibited high tolerance to salinity by enhancing proline content and antioxidant enzyme activities.

Introduction

World population is increasing day by day and it is foreseen that the increasing trend of the population going to be approximately 960 crores by 2050 (1). For the expanding population, it is urgent to enhance crop production by about 44 million MT/year using limited arable lands. Moreover, multiple stresses such as salinity, drought, cold, heat, UV and so on increasing tirelessly. Among the environmental constraints, salinization of soil is the vital stress factor that hinders crop production. Thus, it will be a massive challenge for the future generation to increase crop production by minimizing environmental constraints.

Due to climatic variability, the coastal regions of Bangladesh has been continuously facing different environmental stresses. Among the stresses in the coastal areas, soil salinity appeared as a major threat that causes at least 30-50% yield loss of different crops and a 15% decrease in rice yield (2) depending on the

level of salinization (3). Approximately, 27% of the world's land and almost half of the moistened land are affected by salinity (4, 5). Nowadays, the soil salinity is appeared as a threat to agricultural systems because it unpleasantly affects plant physiological as well developmental processes (6). Salt stress hampers plant progress and limits yield by interfering photosynthetic rate (7) and accumulation of toxic such as Na⁺ and Cl⁻ ions (8). Several studies reported soil salinity decreased chlorophyll content (9), increased ROS (10), nutritional disorder (11) and ionic imbalance in plants (12), which finally contributed to significant yield loss (13).

Among the different staple food, rice is the main food for more than half of the world's population (14, 15), and it is a major crop in Bangladesh that covers 80% of the total cultivable area (16). Rice production is still gaining attention due to the increasing trend of populations. Therefore, the enhancement of rice production is necessary to feed up the increasing trends of the population in Bangladesh. Moreover, a densely-

populated country like Bangladesh has no scope to annex agricultural lands to cultivate rice to make sure sufficient production. Coastal areas having saline soil in the south-part of Bangladesh could be an auspicious source of arable lands which nearly remain fallow. Besides, climatic fluctuations enhance the extent of salinization of soil in Bangladesh. In this context, the development of salt-tolerant cultivar to maintain optimal crop production can be a way-out to mitigate the salinity crisis in the coastal regions (17).

Several reports indicated multiple plant responses and recommended different approaches to address the hindrances of soil salinization. Possible strategies include growing salt-tolerant rice genotypes (18), supplementation of a compatible solute such as proline (19), organic amendments with K and Zn fertilization (20), and modifying antioxidant enzymatic activities (21). As the rice is grown as a major crop in the south-part seaside areas. So the development and/or selection of salt-tolerant rice cultivars might be the best way to enhance rice production in the coastal regions of Bangladesh. In this regard, landrace may be an appropriate contender as it has some distinctive genes that have aided them to thrive in saline areas (17).

Therefore, screening or selecting the best-suited cultivars and exploring their differential responses in saline soil is essential to maintain sustainable rice production in seaside areas of Bangladesh. Salt-tolerant crop cultivars should be identified based on physio-biochemical responses to salt stress. Therefore, the present work was aimed to explore the physio-biochemical responses of mostly cultivated rice cultivars in coastal regions of Bangladesh for sustainable rice production.

Materials and Methods

Seed materials

Five local landraces (Jotai, Icheburogolghor, Morishal, Chapail and Kumro buro) were collected from the saline coastal areas of Batiaghata Upazilla, Khulna. These local landraces were selected due to their distinct phenotype, response to salt stress and yield attributes as well as they are widely cultivated in the coastal regions and two high yielding variety were collected from the Bangladesh Rice Research Institute for physiological and biochemical characterization.

Investigational layout and salt treatments

The present study was performed in a net-house (with an average temperature of 24 °C day length 12 h and RH 60%) of the Soil Science department, Bangladesh Agricultural University (24° 45'8.39" N 90° 24'6.59" E). Homogenously mixed and air-dried soil was collected from the field laboratory of the Soil Science department, BAU. The properties of collected soil were silt loam and exchangeable Na and K were 0.383 meq/100 gm and 0.082 meq/100 gm, respectively. Many studies reported that 2 to 12 dS/m salinity levels negatively affect rice growth and yield (17, 18). Moreover, the coastal regions of Bangladesh affect by different ranges of salinity such as very

slight (2-4 dS/m), slight (4-8 dS/m) and so on (22). Based on these reports, we selected the 0, 20, 40, 60 and 80 mM NaCl doses of salinity and the EC values of soil were 1.00, 2.25, 5.60, 6.58 and 7.50 dS/m for 0, 20, 40, 60 and 80 mM NaCl treatments respectively.

A plastic pot (15 L) was used for the experiment and pot was filled-up with 8 kg soil and 5 L water to ensure sufficient space to maintain flooded conditions. Seedlings of rice (30 days old; active tillering stage) were exposed to 0, 20, 40, 60 and 80 mM NaCl. For 20, 40 and 60 mM NaCl treatments, 15.24, 30.47 and 45.71 gm pure NaCl were dissolved in 1000 mL water and then added to the pots, respectively. For control, no NaCl was added to the soils. The experiment was set with 4 replications and designed in a randomized complete block design. For data collection, plants were up-rooted at two-weeks after salt application and morphological parameters like plant height, shoot and root FW and DW and the number of tillers hill⁻¹ were recorded.

Measurement of proline contents

Proline contents were measured following the standard procedure (23). The 0.5 gm of fresh leaves were homogenized in 3% sulfosalicylic acid and centrifuged at 5000 rpm for 15 min. After centrifugation, 2 ml of supernatant was collected and incubated in acid ninhydrin and glacial acetic acid for 1 hr at 100 °C. After cooling the reaction mixture was extracted with toluene and at 520 nm the absorbance was recorded.

Measurement of antioxidant enzyme activities

CAT activity

The CAT activity was measured following the standard procedure (21). The 0.5 gm leaf sample was homogenized in a reaction mixture (50 mM Tris-HCl buffer, 0.25 mM EDTA, and 20 mM H₂O₂). Then, the homogenate was centrifuged and the supernatant was collected for measurement of CAT activity.

APX activity

The APX activity was determined following the standard procedure (24). The 0.5 gm leaf sample was homogenized in a reaction mixture (50 mM KH₂PO₄ buffer, 0.1 mM EDTA, 0.1 mM H₂O₂ and 0.5 mM AsA). Then, the homogenate was centrifuged and the supernatant was collected for measurement of APX activity.

POX activity

The POX activity was measured following the standard procedure (24). The 0.5 gm leaf sample was homogenized in a reaction mixture (50 mM KH₂PO₄ buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM H₂O₂ and 10 mM guaiacol). Then, the homogenate was centrifuged and the supernatant was collected for measurement of POX activity.

Data analysis

The collected data were analyzed statistically using "Duncan's Multiple Range Test" to classify significant (5% level of probability) differences among the treatments. Data were analyzed using GenStat 18th edition for Windows statistical software.

Results

Growth parameters (root and shoot)

The presented results showed that salinity stress decreases the growth of root and shoot of the rice cultivars (Table 1 and 2, $P \leq 0.05$). At the highest salt stress (80 mM NaCl), Morishal exhibited the lowest reduction in fresh weight (FW) (9%) and dry (43%) weight (DW) of shoot compared to control. Similar to Morishal, Jotai and Kumro buro also exhibited higher FW, 27.2 and 26.5 and DW, 2.4 and 2.5 g/pot respectively, of the shoot at the highest salt stress compared to control (Table 1). The reduction in root FW of Kumro buro was 50% and in Morishal 16%, which was the highest and lowest reduction, respectively. However, at 80 mM NaCl, Morishal decreases in root DW by 54% whereas other cultivars exhibited at least 60% decrease in root dry weight (Table 2).

condition. Under highest stress condition, Icheburogolghor and BRRI dhan41 exhibited 92% and 54%, respectively, reduction in proline content compared with control. At a high saline level, the maximum proline content was observed in BRRI dhan41 (1.34 mM) and the minimum proline content was in Icheburogolghor (0.22 mM). With increasing salt concentration, intercellular proline content drastically decreased in all the rice cultivars except Morishal and BRRI dhan41.

Antioxidant enzymes, CAT, POX and APX activities

Activities of antioxidant enzymes were notably influenced by salt stress (Fig. 2). The actions of CAT and APX in all cultivars enhanced with the rising salt concentration (Fig. 2A and 2C). The landraces, Jotai, Morishal and Kumro buro showed significantly higher CAT activity 0.117, 0.09, 0.09 mmol/min/gm FW,

Table 1. Shoot fresh and dry weight of seven rice cultivars in response to different levels of soil salinity (0, 20, 40, 60 and 80 mM NaCl). Values are means of four replicates and means with the different letters in a column are significantly different ($P \leq 0.05$).

Treatments	Shoot fresh weight (g/pot)						
	Jotai	Icheburogolghor	Morishal	Chapail	Kumro buro	BR 23	BRRI dhan 41
0mM NaCl	31.54 a	29.59 a	28.50 a	31.70 a	30.70 a	28.70 a	28.59 a
20mM NaCl	29.54 b	27.27 ab	26.39 b	31.30 a	30.02 ab	28.29 ab	28.30 a
40mM NaCl	28.60 bc	25.84 bc	26.01 b	28.70 b	29.29 ab	26.50 b	28.18 a
60mM NaCl	28.53 bc	25.09 bc	25.96 b	26.29 c	28.10 bc	23.20 c	25.29 b
80mM NaCl	27.20 c	23.29 c	25.98 b	23.50 d	26.50 c	18.70 d	21.20 c
SE (\pm)	0.599	0.771	0.502	0.513	0.690	0.550	0.547
Treatments	Shoot dry weight (g/pot)						
	Jotai	Icheburogolghor	Morishal	Chapail	Kumro buro	BR 23	BRRI dhan 41
0mM NaCl	4.100 a	4.420 a	3.920 a	4.250 a	4.880 a	3.800 a	4.950 a
20mM NaCl	3.800 a	4.100 ab	3.450 ab	3.290 b	3.720 b	3.290 b	3.290 b
40mM NaCl	3.290 b	3.820 b	3.150 bc	3.150 bc	3.450 b	2.920 c	3.150 bc
60mM NaCl	2.790 c	3.290 c	2.900 c	2.900 c	2.910 c	2.710 c	2.900 c
80mM NaCl	2.42 c	2.910 c	2.250 d	2.420 d	2.520 c	2.210 d	2.420 d
SE (\pm)	0.138	0.138	0.154	0.105	0.095	0.105	0.095

Plant height and number of tillers hill⁻¹

Similar to other growth characters, plant height and the number of tillers hill⁻¹ decrease under salinity stress (Table 3, $P \leq 0.05$). Jotai and Icheburogolghor were exhibited the tallest plant (50.44 cm) and the shortest plants were in BR23 (38.85 cm) under the highest salinity. Besides, the maximum number of tillers per hill⁻¹ 8 was observed in BR23 and the minimum number of tillers per hill⁻¹ 3 were in Icheburogolghor under the highest salinity level.

Proline content

Tested rice cultivars showed significant differences in proline contents in response to salinity levels (Fig. 1). Among the rice cultivars, Kumro buro showed the highest intercellular proline content (3.0 mM) under non-stress condition whereas proline content drastically decreased when exposed to different levels of salt stresses. At 80 mM salt stress, Kumro buro exhibited 86% reduction of proline content compared to the control. Icheburogolghor and BRRI dhan41 genotypes also showed higher levels of intracellular proline 2.8 and 2.9 mM, respectively at the non-stress

respectively at the maximum salinity level (80 mM NaCl) whereas BRRI dhan41 exhibited the highest APX activity 57.14 $\mu\text{mol}/\text{min}/\text{gm}$ FW at 80 mM NaCl compared to other rice cultivars. The actions of POX significantly reduced in all rice cultivars except Chapail with increasing salinity (Fig. 2B). Similar to APX, the POX was also highest in BRRI dhan41 at the maximum salinity level (80 mM NaCl).

Discussion

Many studies showed that salt stress decreased the morphological parameters of different crops (17, 25, 26). In this study, the shoot (Table 1) and the root (Table 2) growth, as well as the plant height and number of tillers hill⁻¹ (Table 3), were reduced in all cultivars under salinization. Similar results were reported in hybrid rice and maize under salinity stress (27, 28). A greater decrease in shoot and root biomass accumulation was observed in Kumro buro than Morishal and BRRI dhan41 at 80 mM NaCl level. This result may indicate a better salt resistance in BRRI dhan41 and Morishal

Table 2. Root fresh and dry weight of seven rice cultivars in response to different levels of soil salinity (0, 20, 40, 60 and 80 mM NaCl). Values are means of four replicates and means with the different letters in a column are significantly different ($P \leq 0.05$).

Treatments	Root fresh weight (g/pot)						
	Jotai	Icheburogolghor	Morishal	Chapail	Kumro buro	BR 23	BRRi dhan 41
0mM NaCl	7.800 a	7.790 a	7.350 a	8.500 a	8.880 a	8.900 a	7.200 a
20mM NaCl	7.100 b	7.250 b	7.120 ab	8.350 a	8.160 ab	7.250 b	7.000 a
40mM NaCl	6.100 c	6.920 bc	6.830 ab	8.100 a	7.290 b	7.050 b	6.920 a
60mM NaCl	5.350 d	6.790 c	6.560 bc	7.200 b	5.320 c	6.820 b	6.820 a
80mM NaCl	4.980 d	6.300 d	6.150 c	6.900 b	4.520 c	6.850 b	5.950 b
SE (\pm)	0.115	0.124	0.194	0.171	0.321	0.303	0.129

Treatments	Root dry weight (g/pot)						
	Jotai	Icheburogolghor	Morishal	Chapail	Kumroburo	BR 23	BRRi dhan 41
0mM NaCl	1.400 a	1.220 a	1.310 a	1.210 a	1.330 a	1.150 a	1.520 a
20mM NaCl	0.900 b	0.800 b	1.150 a	0.920 b	0.690 b	0.920 b	1.090 b
40mM NaCl	0.790 bc	0.780 b	0.850 b	0.870 b	0.670 b	0.780 b	0.910 c
60mM NaCl	0.720 c	0.590 c	0.720 bc	0.790 b	0.540 c	0.620 c	0.820 c
80mM NaCl	0.500 d	0.520 c	0.590 c	0.500 c	0.410 d	0.420 d	0.700 d
SE (\pm)	0.044	0.033	0.057	0.038	0.028	0.048	0.035

Table 3. Plant height and number of tillers per hill of seven rice cultivars in response to different levels of soil salinity (0, 20, 40, 60 and 80 mM NaCl). Values are means of four replicates and means with the different letters in a column are significantly different ($P \leq 0.05$).

Treatments	Plant height (cm)						
	Jotai	Icheburogolghor	Morishal	Chapail	Kumro buro	BR 23	BRRi dhan 41
0mM NaCl	60.50 a	59.83 a	52.48 a	62.06 a	56.22 a	48.66 a	58.47 a
20mM NaCl	57.60 a	57.60 ab	46.55 b	52.33 b	48.40 b	47.48 a	56.22 a
40mM NaCl	56.42 ab	56.42 ab	42.33 c	50.89 b	47.61 bc	41.72 b	56.34 a
60mM NaCl	54.33 ab	54.33 bc	42.48 c	48.33 b	46.58 bc	41.66 b	54.75 ab
80mM NaCl	50.44 b	50.44 c	41.63 c	47.14 b	42.47 c	38.85 b	50.33 b
SE (\pm)	1.78	1.28	1.11	1.82	1.56	1.37	1.41

Treatments	Number of tillers per hill						
	Jotai	Icheburogolghor	Morishal	Chapail	Kumro buro	BR 23	BRRi dhan 41
0mM NaCl	8.00 a	8.00 a	8.00 a	7.00 a	7.00 a	8.00 a	7.00 a
20mM NaCl	7.00 ab	6.00 b	8.00 a	7.00 a	6.00 b	8.00 a	7.00 a
40mM NaCl	6.00 bc	5.00 bc	7.00 b	5.66 b	5.00 c	8.00 a	6.00 b
60mM NaCl	5.00 cd	4.00 cd	6.00 c	5.00 b	6.00 b	7.00 b	5.00 c
80mM NaCl	4.00 d	3.33 d	5.00 d	5.00 b	4.00 d	6.00 c	4.00 d
SE (\pm)	0.415	0.363	0.256	0.347	0.158	0.211	0.194

compared to Kumro buro, which might be attributed to being more tolerant to salt stress.

Intracellular proline contents gradually decreased in cultivars with the rising of salinization (Fig. 1). Among the cultivars, BRRi dhan41 contains a high level of proline at 80 mM NaCl. High proline contents in BRRi dhan41 indicate that cultivar BRRi dhan41 may be more salinity tolerant to other cultivars. In the case of soybean similar result was found and that result showed proline content was enhanced in soybean with the rising of salinization (29). Salt-sensitive rice cultivar KDML105 and Thai jasmine rice showed an increasing pattern of proline content under salt stress (30, 31).

Antioxidant enzymes are the key regulator for the mitigation of abiotic stresses in crop production (19, 32). Antioxidant enzymatic activities contributed to increasing salt-tolerant of rice cultivars exposed to the different levels of salt stress (Fig. 2). Our results showed the actions of CAT and APX significantly enhanced under salt stress (Fig. 2A and 2C), whereas the actions of POX reduced under salt stress (Fig. 2B). Similarly many researchers reported that the actions of APX synchronized with the actions of CAT and POX in rice and cotton during salinity stress (33, 34). In this present study, BRRi dhan41 and Morishal exhibited higher CAT, POX and APX activities compared to other cultivars under salt stress. It is important to mention that BRRi dhan41 and Morishal also exhibited significantly higher

proline contents at the highest salt level. These results indicate and suggest that the higher antioxidant enzyme activities coupled with higher intercellular proline content might play a key role in salinity tolerance to these cultivars.

It has been reported that genotypes of rice varied in capacity to withstand the salinization when grown in a range of soil salinity (18). In this study, Morishal exhibited the lowest percent decrease in dry biomass accumulation at the highest salt stress compared to control. It is essential to mention that the rice cultivars used in this study demonstrated differential responses to withstand soil salinity. The findings were compatible with the study (18), which revealed rice cultivars fluctuate significantly in their capacity to withstand soil salinity. Variation in the ability to withstand salinity investigated in this work may have a potential for genetic selection for higher salt tolerance as well as may assist in determining the best-suited rice cultivars for coastal areas of Bangladesh.

Conclusion

It is concluded that salt stress negatively affected rice cultivars with a significant decrease in their physiological and biochemical aspects. Among the tested rice cultivars, Morishal and BRRi dhan41 exhibited high tolerance to salinity by enhancing

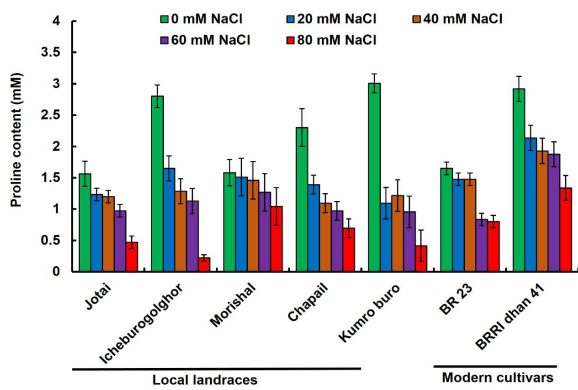


Fig. 1. Proline content of landraces and modern cultivars in response to different levels of soil salinity (0, 20, 40, 60 and 80 mM NaCl). Values are means \pm SE of four replicates.

intracellular proline content and antioxidant enzyme activities. Furthermore, morphological data such as yield and biochemical data chlorophyll content and SOD activity should be performed to more validation of our results.

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Authors' contributions

Conceptualization, Methodology and Supervision: MAH; Formal analysis and investigation: UB & MGK; Writing - original draft preparation: UB & MSR; Writing - review and editing: YM, MAH & MSR.

Conflict of interests

The authors declare no conflict of interest.

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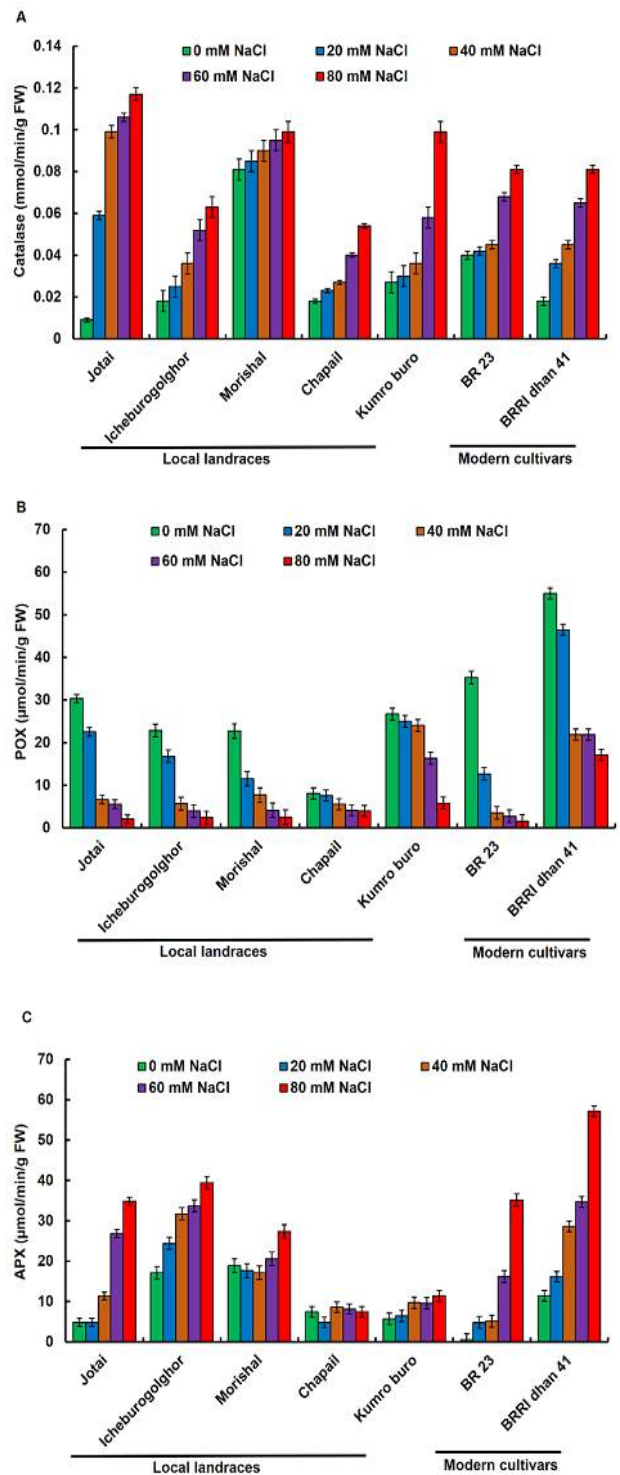


Fig. 2. Antioxidant enzymatic activities (catalase, CAT (A); peroxidase, POX (B); ascorbate peroxidase, APX (C)) of landraces and modern cultivars in response to different levels of soil salinity (0, 20, 40, 60 and 80 mM NaCl). Values are means \pm SE of four replicates.

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