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Research Article

Development and validation of a membership function-based evaluation system for salinity screening in rice

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Abstract

Salinity is a major abiotic stress that affects plant growth and development in rice. In this study, 37 genotypes along with 2 checks were screened for salinity at the seedling stage using hydroponics. Analysis of variance revealed a significant difference among genotypes for the recorded traits. Most studies have evaluated the salt tolerance based on the independent mean performance of traits. However, this study aimed to utilize membership function values which account for cumulative tolerance from all traits. Based on average MFV, three genotypes were classified as highly tolerant, four genotypes were classified as tolerant, 25 genotypes were classified as moderately tolerant, six genotypes were classified as susceptible, one genotype as highly susceptible. Simple linear regression analysis indicated total fresh weight is the reliable trait for salinity screening in rice at the seedling stage. Further, a multiple regression-based mathematical model was developed, which identified that a membership function-based evaluation system can be utilized for salinity tolerance grading. The identified highly tolerant and tolerant genotypes can be utilized for the improvement of salt tolerance at the seedling stage in rice.

Keywords: Rice, Salinity, Membership function values, Regression

INTRODUCTION

Global warming is a significant threat to world agricultural output, and it is having adverse effects on staple food crop production including rice (Ali *et al.*, 2017). In 2021-22, global rice production reached 525.96 million tonnes, from a rice area of 165.25 million hectares (Hemalatha *et al.*, 2023). To meet the food needs of a projected population of 9 billion by 2050, rice production must increase by 160 million tonnes (Muthu *et al.*, 2020; Behera *et al.*, 2023). The increase in abiotic stresses, a consequence of climate change, is expected to have a profound impact on rice production (Wassmann *et al.*,

2009; Raza *et al.*, 2019). This will have a ripple effect on the socioeconomic fabric of farming communities, as many people rely on rice as a staple food. Over the years, abiotic stresses have severely constrained rice production, especially salinity (Dar *et al.*, 2021; Sultana *et al.*, 2022). Throughout the world, nearly 950 million ha of arable land which includes 250 million ha of irrigated land are affected by salinity (Raja *et al.*, 2022; Avdan *et al.*, 2022). Rice, a salt-sensitive glycophyte, exhibits varied salt tolerance at different growth stages, with the seedling and reproductive stages being the most

sensitive, resulting in a global yield loss of up to 50%. Salinity exerts its deleterious effects on rice through osmotic stress, ion toxicity, and nutrient imbalance (Khare *et al.*, 2020; Ijaz *et al.*, 2023). At the seedling stage, salinity-induced osmotic stress causes impaired root and shoot growth, reduced leaf size, and ultimately premature plant death (Krishnamurthy *et al.*, 2016). Salinity tolerance is a polygenic and complex trait, rendering genotype screening imperative for the identification of salt-tolerant donors (Muthuramalingam *et al.*, 2022). The hydroponics system enables a robust screening of rice genotypes within a carefully regulated environment, effectively bypassing the complexities of stress factors linked to soil and environmental conditions (Gregorio, 1997). The differential responses of diverse genotypes to salinity at the seedling stage proved to be effective in identifying salt-tolerant donor plants. In previous studies, the common approach has typically relied on SES scores or independent trait-based evaluations. However, this study adopted a comprehensive evaluation system based on membership function values (MFV) to integrate multiple traits for screening. This has been successfully implemented in crops such as sweet sorghum, brassica, wheat, and sunflower (Ding *et al.*, 2018; Wu *et al.*, 2019; Choudhary *et al.*, 2021). Therefore, this study aims to screen the 37 rice genotypes along with two checks for salt tolerance at the seedling stage using MFV and to determine the most reliable trait for screening. In addition, multiple regression-based evaluation model was developed to assess the MFV based classification system.

MATERIALS AND METHODS

The salt tolerance of 37 rice genotypes and two checks (Pokkali and IR, 29) was assessed utilizing a completely randomized block design with three replications. To break dormancy, the seeds underwent a 4-day hot air treatment at 55°C, followed by surface sterilization with 4% sodium hypochlorite for 5 minutes. After washing twice with distilled water, the seeds were placed in petri dishes (80 seeds/petri dish) with moistened germination paper. The pregerminated seedlings were then transferred to hydroponics structures containing Yoshida nutrient solution, and it was replaced weekly. The pH of the nutrient solution was closely monitored daily to maintain a constant nutrient supply within the range of 5.1 to 5.5. Seedlings were grown under normal conditions in the nutrient solution for 14 days, after which salinity stress was induced at 6 dS/m for 7 days, followed by an increase to 12 dS/m. In the control treatment, the plants were cultivated in a nutrient solution (1.4 dS/m) without any salt added.

Morphological Traits: Following 14 days of salinization, eight morphological traits were recorded, including shoot length (SL) (cm), root length (RL) (cm), shoot fresh weight (SFW) (g), root fresh weight (RFW) (g), shoot dry weight (SDW) (g), root dry weight (RDW) (g), total fresh weight

(TFW) (g) and total dry weight (TDW) (g). For dry weight determination, root and shoot tissues were subjected to oven drying at 60°C for 3 days.

Salinity tolerance index: Salt tolerance index (STI) values were calculated for each trait using the following formula:

$$\text{Salinity tolerance index (STI)} = \frac{\text{Trait value of NaCl treated plant}}{\text{Trait value of control plant}}$$

Membership Function Values: The salt tolerance of rice genotypes was then assessed using the fuzzy comprehensive evaluation method employing membership function values (MFV) derived from the STI of each trait, calculated through the formula:

$$X_i = \frac{X - X_{\min}}{X_{\max} - X_{\min}}$$

Where,

X_i = Membership function value of each trait for individual genotype

X = Value of STI for individual genotype

X_{\min} = Minimum STI value recorded in all genotypes

X_{\max} = Maximum STI value recorded in all genotypes

Assessment of salinity tolerance: Chen (2012) employed a refined method to categorize salt tolerance in rice genotypes by utilizing the average Membership Function Value (MFV) and Standard Deviation (SD) values, leading to the classification of these genotypes into five distinct classes.

Average MFV Values	Saline Tolerant Class
$X_i \geq \bar{X} + 1.64 \text{ SD}$	Highly saline tolerant
$\bar{X} + 1 \text{ SD} \leq X_i \leq \bar{X} - 1.64 \text{ SD}$	Saline tolerant
$\bar{X} - 1 \text{ SD} \leq X_i \leq \bar{X} + 1 \text{ SD}$	Moderately saline tolerant
$\bar{X} - 1.64 \text{ SD} \leq X_i \leq \bar{X} - 1 \text{ SD}$	Saline susceptible
$X_i \leq \bar{X} - 1.64 \text{ SD}$	Highly saline susceptible

Developing a model to assess salt tolerance in rice: To test whether the MFV based evaluation method was useful in predicting the salt tolerance of the rice genotypes, a multiple regression-

$$Y = \beta_{SL} * STI_{SL} + \beta_{RL} * STI_{RL} + \beta_{SFW} * STI_{SFW} + \beta_{RFW} * STI_{RFW} + \beta_{SDW} * STI_{SDW} + \beta_{RDW} * STI_{RDW} + \beta_{TFW} * STI_{TFW} + \beta_{TDW} * STI_{TDW} + \mu$$

Where, Y – Salt tolerance of genotype, μ - random error term and β - unstandardized coefficient

Statistical analysis: Analysis of variance was performed using R software, STI and MFV values were calculated using Microsoft Excel. Multiple regression was analyzed using SPSS. Linear regression was calculated using Minitab.

RESULTS AND DISCUSSION

Effect of Salinity on Morphological Traits : Salinity-induced significant growth reductions were observed

for all recorded traits (**Table 1**). However, the degree of reduction varied among genotypes, with tolerance genotypes showed significantly less reduction than susceptible genotypes (Banumathy *et al.*, 2018; Amaravel *et al.*, 2019; Janagiraman *et al.*, 2003). Under salinity, STI of shoot length ranged from 0.33 to 0.66, with the highest values observed in Pokkali, Vytilla10, Jothi, and Vytilla8 (0.66, 0.62, 0.62, and 0.60, respectively). Conversely, the lowest STI values were recorded in IR29, CO55,

Table 1. STI values of 8 traits for 37 genotypes and two checks

Genotypes	SL STI	RL STI	SFW STI	RFW STI	SDW STI	RDW STI	TFW STI	TDW STI
Aathur samba	0.48	0.64	0.46	0.4	0.35	0.31	0.43	0.33
ADT 55	0.35	0.38	0.44	0.58	0.2	0.21	0.5	0.21
CO 52	0.46	0.51	0.4	0.47	0.29	0.33	0.43	0.31
CO 54	0.34	0.57	0.28	0.34	0.2	0.25	0.3	0.22
CO 55	0.33	0.47	0.38	0.48	0.25	0.24	0.43	0.25
CR1009 Sub1	0.44	0.65	0.24	0.22	0.11	0.13	0.23	0.12
FR13a	0.39	0.48	0.41	0.36	0.28	0.28	0.39	0.28
Ghandhasala	0.4	0.69	0.55	0.49	0.34	0.31	0.52	0.33
Godavarisamba	0.46	0.37	0.37	0.08	0.3	0.09	0.25	0.21
GodumaraiSamba	0.55	0.42	0.41	0.38	0.24	0.31	0.4	0.26
IG 2	0.45	0.49	0.31	0.23	0.22	0.12	0.27	0.18
IG 10	0.4	0.57	0.52	0.59	0.31	0.35	0.55	0.33
IG 11	0.52	0.79	0.47	0.84	0.35	0.47	0.57	0.39
IG 12	0.52	0.61	0.51	0.57	0.25	0.23	0.54	0.24
IG 13	0.46	0.77	0.36	0.53	0.2	0.26	0.42	0.23
IG 15	0.39	0.53	0.45	0.4	0.2	0.22	0.42	0.21
IG 44	0.44	0.74	0.32	0.44	0.21	0.25	0.37	0.22
Illupaiipoosamba	0.45	0.66	0.31	0.5	0.24	0.24	0.38	0.24
IR 29	0.26	0.3	0.21	0.18	0.06	0.1	0.2	0.08
IR 64	0.34	0.33	0.28	0.43	0.16	0.21	0.35	0.18
Jeevan Samba	0.45	0.39	0.34	0.33	0.29	0.29	0.34	0.29
Jothi	0.62	0.71	0.58	0.72	0.36	0.42	0.63	0.39
Kalanamak	0.55	0.4	0.32	0.46	0.22	0.23	0.37	0.22
Karhigai samba	0.4	0.47	0.42	0.59	0.28	0.24	0.49	0.26
Katta samba	0.51	0.72	0.51	0.43	0.23	0.26	0.47	0.24
Kaviya samba	0.42	0.55	0.31	0.43	0.19	0.24	0.36	0.21
Kurangu samba	0.47	0.72	0.4	0.54	0.3	0.34	0.46	0.31
Manipur chocko	0.45	0.47	0.28	0.42	0.21	0.23	0.34	0.22
Mysore Malli	0.34	0.22	0.26	0.38	0.16	0.3	0.31	0.23
Paiyur 1	0.41	0.47	0.54	0.55	0.32	0.28	0.54	0.3
Pokkali	0.66	0.77	0.65	0.81	0.5	0.47	0.71	0.49
RPHP 44	0.55	0.7	0.6	0.65	0.39	0.35	0.62	0.38
TKM 13	0.48	0.65	0.21	0.2	0.15	0.11	0.21	0.13
TRY 3	0.5	0.63	0.56	0.72	0.36	0.35	0.63	0.36
Vadakathikar	0.45	0.45	0.55	0.48	0.29	0.33	0.51	0.31
Varigarudansamba	0.4	0.59	0.48	0.26	0.31	0.19	0.39	0.25
VYTILLA 4	0.43	0.49	0.53	0.58	0.26	0.26	0.56	0.26
VYTILLA 8	0.61	0.81	0.61	0.69	0.36	0.45	0.65	0.4
VYTILLA10	0.62	0.8	0.62	0.68	0.41	0.53	0.65	0.46

Mysore malli, and IR64 (0.26, 0.33, 0.34, and 0.34, respectively). Larger STI values indicated less reduction in growth parameters compared to control and vice versa. Shoot length gets severely reduced which may be due to the direct or indirect impact of salinity on cellular proliferation and expansion (Munns, 2002; Ravikiran, 2018) and decreased growth hormones (Mazher *et al.*, 2007). Similar results for shoot length reduction were reported by Arif *et al.* (2018), Ravikiran *et al.* (2018) and Banumathy *et al.* (2021).

Root length STI values recorded a range from 0.22 to 0.81. Vytilla8, Vytilla10, IG11, and Pokkali exhibited the highest STI values (0.81, 0.80, 0.79, and 0.77, respectively), while Mysore malli, IR29, IR64, and Godavari samba had the lowest STI values (0.22, 0.30, 0.33, and 0.37, respectively). The results might be due to increased salinity levels caused a rise in the accumulation of H_2O_2 which in turn retarded root growth (Demiral and Türkan, 2005). Salinity affects primary root growth by affecting cell proliferation in the root apical meristem through modification in ethylene biosynthesis pathway (Qin, 2019). The reduction in root length under salinity was also observed by Arif *et al.* (2018), Ravikiran *et al.* (2018), Rasel *et al.* (2020) and Banumathy *et al.* (2021).

Pokkali, Vytilla10, Vytilla8 and RPHP44 recorded high STI values for shoot fresh weight and shoot dry weight whereas IR29, TKM13 and CR1009sub1 observed lower STI values for both these traits. For RFW, genotypes viz., IG11(0.84), Pokkali (0.81) and Jothi (0.72) observed high STI values. Low RFW STI values were recorded in Godavari Samba (0.08), IR29 (0.18) and TKM13

(0.15). In case of RDW, V10 (0.53), Pokkali (0.47) and IG11(0.47) were recorded high STI values. However, low STI values of RDW were recorded in Godavari samba (0.09), IR29(0.10) and TKM13 (0.11). The traits, TFW and TDW recorded high STI values in Pokkali (0.71,0.49), Vytilla 10 (0.65,0.46) and Vytilla8 (0.65,0.40) whereas IR29 (0.21,0.08), TKM13 (0.21,0.13) and CR1009sub1 (0.23,0.12) recorded lower STI values. This reduction in fresh and dry weight was due to the accumulation of sodium and chloride ions in the plant tissues, which can lead to cell wall solidification, damage to cell ultrastructure, and poor metabolic activities (Pauk and Jansco *et al.*, 2017). Reduced root dry weight may be due to insufficient nutrition availability to roots as well as the harmful effects of salt and chloride ions (Rasel *et al.*, 2020). This was observed by Chunthaburee *et al.* (2016), Pongprayoon *et al.* (2018), Rasel *et al.* (2020) and Rasel *et al.* (2021).

Salt tolerance evaluation: To classify the salt tolerance of 39 rice genotypes, the MFV for each trait of each genotype and average MFV were calculated (Table 2). The mean and SD of average MFV were 0.49 and 0.20 respectively. The range of average MFV varied between 0.04 to 0.97. Based on average MFV, 37 rice genotypes and 2 checks were graded into five categories for salt tolerance (Fig. 1). Three genotypes (Pokkali, Vytilla 10, and Vytilla 8) were grouped into highly salt tolerant ($\bar{X} \geq 0.82$) category, 4 genotypes (Jothi, IG11, RPHP44, and TRY3,) under tolerant ($0.69 \leq \bar{X} < 0.82$) category, 25 genotypes under moderately tolerant ($0.28 \leq \bar{X} < 0.69$) category, 6 genotypes (CR1009sub1, TKM13, Mysoremalli, IR64, Godavari Samba and IG2) under susceptible ($0.15 \leq \bar{X} < 0.28$) category and one genotype (IR29) as highly susceptible ($\bar{X} < 0.15$). Salinity

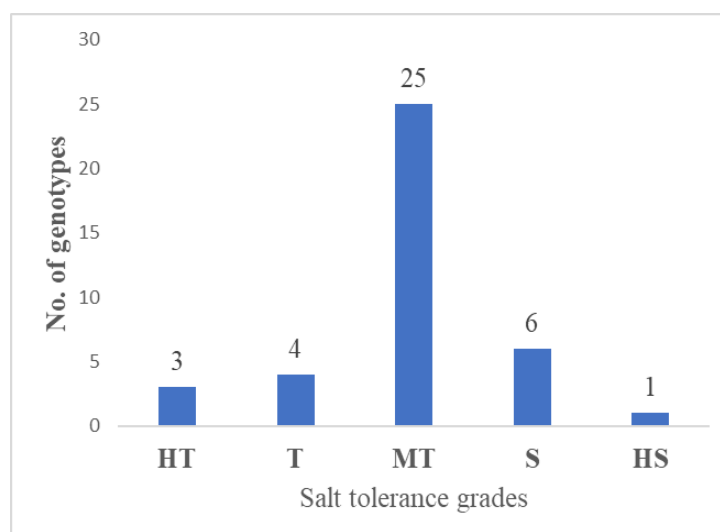


Fig. 1. Distribution of 37 genotypes along with 2 checks in five salt tolerance grades

HT-Highly Tolerant; T -Tolerant; MST, Moderately Tolerant; S-Susceptible; HS: highly salt-sensitive.

Table 2. MFV, Average MFV and Y values of 8 traits for 37 genotypes and two checks

Genotypes	SL MFV	RL MFV	SFW MFV	RFW MFV	SDW MFV	RDW MFV	TFW MFV	TDW MFV	AVG MFV	Y
Aathur samba	0.53	0.71	0.57	0.42	0.66	0.50	0.45	0.62	0.56(MT)	0.56
ADT 55	0.22	0.26	0.52	0.66	0.32	0.27	0.59	0.31	0.40(MT)	0.40
CO 52	0.49	0.49	0.44	0.52	0.51	0.55	0.46	0.56	0.50(MT)	0.50
CO 54	0.20	0.59	0.16	0.34	0.31	0.36	0.21	0.34	0.31(MT)	0.31
CO 55	0.17	0.42	0.39	0.52	0.43	0.35	0.44	0.41	0.39(MT)	0.39
CR1009 Sub1	0.44	0.73	0.07	0.19	0.12	0.09	0.06	0.10	0.22(S)	0.22
FR13a	0.30	0.43	0.46	0.37	0.50	0.44	0.37	0.50	0.42(MT)	0.42
Ghandhasala	0.34	0.79	0.78	0.54	0.64	0.49	0.62	0.61	0.60(MT)	0.60
Godavarisamba	0.48	0.25	0.37	0.00	0.54	0.00	0.11	0.32	0.26(S)	0.26
GodumaraiSamba	0.72	0.34	0.46	0.40	0.40	0.49	0.39	0.46	0.46(MT)	0.46
IG 2	0.47	0.46	0.22	0.19	0.37	0.07	0.14	0.25	0.27(S)	0.27
IG 10	0.34	0.59	0.70	0.66	0.57	0.59	0.68	0.61	0.59(MT)	0.59
IG 11	0.65	0.95	0.59	1.00	0.66	0.86	0.72	0.77	0.78(T)	0.78
IG 12	0.64	0.66	0.69	0.64	0.42	0.32	0.66	0.40	0.55(MT)	0.55
IG 13	0.50	0.92	0.34	0.58	0.32	0.38	0.44	0.37	0.48(MT)	0.48
IG 15	0.30	0.52	0.54	0.42	0.32	0.29	0.44	0.32	0.39(MT)	0.39
IG 44	0.45	0.88	0.25	0.48	0.33	0.35	0.33	0.36	0.43(MT)	0.43
Illupaipoosamba	0.46	0.74	0.24	0.55	0.42	0.34	0.36	0.40	0.44(MT)	0.44
IR 29	0.00	0.13	0.00	0.14	0.00	0.02	0.00	0.00	0.04(HS)	0.04
IR 64	0.19	0.19	0.17	0.46	0.22	0.28	0.29	0.25	0.26(S)	0.26
Jeevan Samba	0.45	0.28	0.31	0.32	0.52	0.44	0.27	0.51	0.39(MT)	0.39
Jothi	0.88	0.82	0.84	0.84	0.69	0.76	0.85	0.76	0.81(T)	0.81
Kalanamak	0.70	0.30	0.25	0.50	0.36	0.32	0.34	0.36	0.39(MT)	0.39
Karhigai samba	0.34	0.42	0.47	0.67	0.49	0.33	0.56	0.44	0.47(MT)	0.47
Katta samba	0.62	0.83	0.67	0.47	0.39	0.38	0.53	0.41	0.54(MT)	0.54
Kaviya samba	0.40	0.56	0.22	0.46	0.30	0.33	0.31	0.34	0.36(MT)	0.36
Kurangu samba	0.52	0.84	0.44	0.60	0.53	0.56	0.50	0.58	0.57(MT)	0.57
Manipur chocko	0.46	0.43	0.15	0.45	0.34	0.31	0.27	0.34	0.34(MT)	0.34
Mysore Malli	0.19	0.00	0.12	0.40	0.23	0.47	0.22	0.36	0.25(S)	0.25
Paiyur 1	0.37	0.42	0.74	0.62	0.59	0.42	0.67	0.55	0.55(MT)	0.55
Pokkali	1.00	0.93	1.00	0.95	1.00	0.87	1.00	1.00	0.97(HT)	0.97
RPHP 44	0.72	0.81	0.89	0.75	0.76	0.60	0.82	0.74	0.76(T)	0.76
TKM 13	0.54	0.72	0.01	0.16	0.20	0.05	0.02	0.14	0.23(S)	0.23
TRY 3	0.59	0.70	0.80	0.83	0.69	0.59	0.84	0.69	0.72(T)	0.72
Vadakathikar	0.48	0.38	0.77	0.53	0.52	0.55	0.62	0.56	0.55(MT)	0.55
Varigarudansamba	0.33	0.63	0.62	0.23	0.56	0.23	0.37	0.43	0.43(MT)	0.43
VYTILLA 4	0.40	0.46	0.74	0.66	0.44	0.37	0.70	0.44	0.53(MT)	0.53
VYTILLA 8	0.86	1.00	0.91	0.80	0.69	0.82	0.87	0.79	0.84(HT)	0.84
VYTILLA10	0.89	0.98	0.93	0.79	0.80	1.00	0.88	0.94	0.90(HT)	0.90

had deleterious effects on all morphological traits (Munns and Tester *et al.*, 2008; Pongprayoon *et al.*, 2018). In the present study, similar effects on morphological traits were observed. This may be due to the accumulation of excess salt in the older leaves of plants, which eventually leads to leaf senescence (Arif *et al.*, 2018). This reduction in

the number of photosynthetically active leaves decreased the rate of photosynthesis, which in turn limited the supply of carbohydrates and growth hormones to the meristematic tissues, ultimately slowing down plant growth (Munns and Tester, 2008). Salinity-induced growth reduction varies between tolerant and susceptible genotypes. The

above-classified highly tolerant and tolerant genotypes have less reduction in growth parameters compared to highly susceptible and susceptible genotypes. Similar findings were reported by Hariadi *et al.* (2015) Chunthaburee *et al.* (2016), Pongprayoon *et al.* (2018), Arif *et al.* (2018), Rasel *et al.* (2020) and Rasel *et al.* (2021). This is probably due to the genotypes capability of adopting tolerance mechanism through physiological and biochemical changes (Rasel *et al.*, 2020). The tolerance mechanisms include salt exclusion, ion compartmentation and partitioning of Na in shoots, vacuoles and in older parts (Chunthaburee *et al.*, 2016).

Evaluation of reliable salt tolerance morphological traits in rice genotypes: To identify the most reliable trait of salt tolerance, a linear model was fitted between the mean of

each trait and the mean of MFV (Fig. 2). The R^2 between the average MFV and the mean of TFW was the highest (87.41%) followed TDW (85.96%), SFW (75.31%), RFW (75.31), SDW (71.75) and RDW (70.35). The R^2 between the mean MFVs and the mean of SL (53.08%) and RL (47.72%) were lower. The results indicated TFW can be used as a reliable morphological trait for salt tolerance in rice at the seedling stage.

Evaluation of a mathematical model for salt tolerance in rice genotypes: To evaluate the predictive performance of the membership function-based evaluation model, the Y values of the 37 rice genotypes and two checks were calculated. The average difference between Y and mean MFV was only 0.00026, with a maximum and minimum difference of 0.00038 and 0.00016. This narrow difference

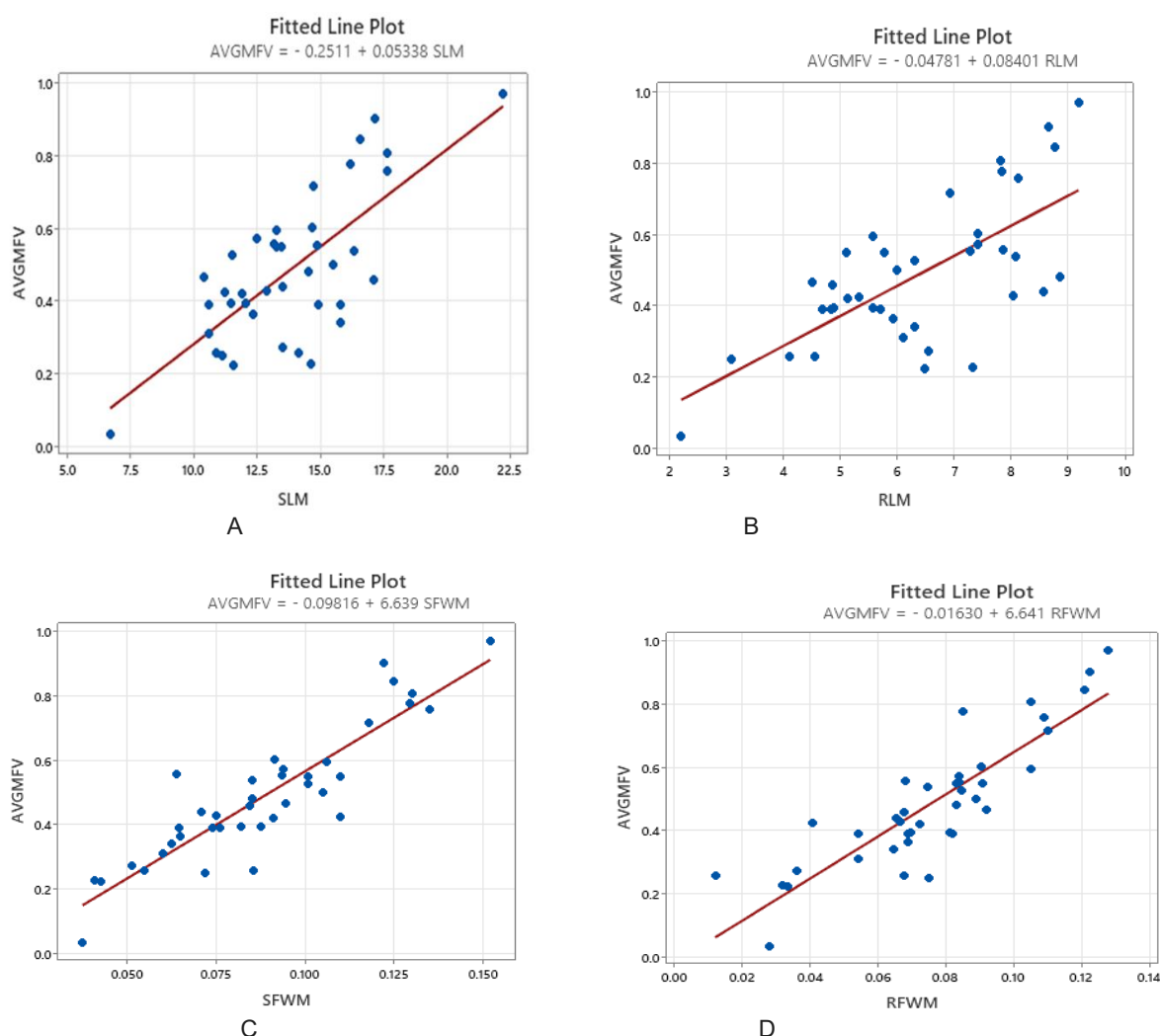


Fig. 2. The linear regression fit between the average of each trait and the average MFV of an individual rice genotype

(A) Is between average MFV and average of SL (B) Is between average MFV and average of RL (C) Is between average MFV and average of SFW (D) Is between average MFV and average of RFW

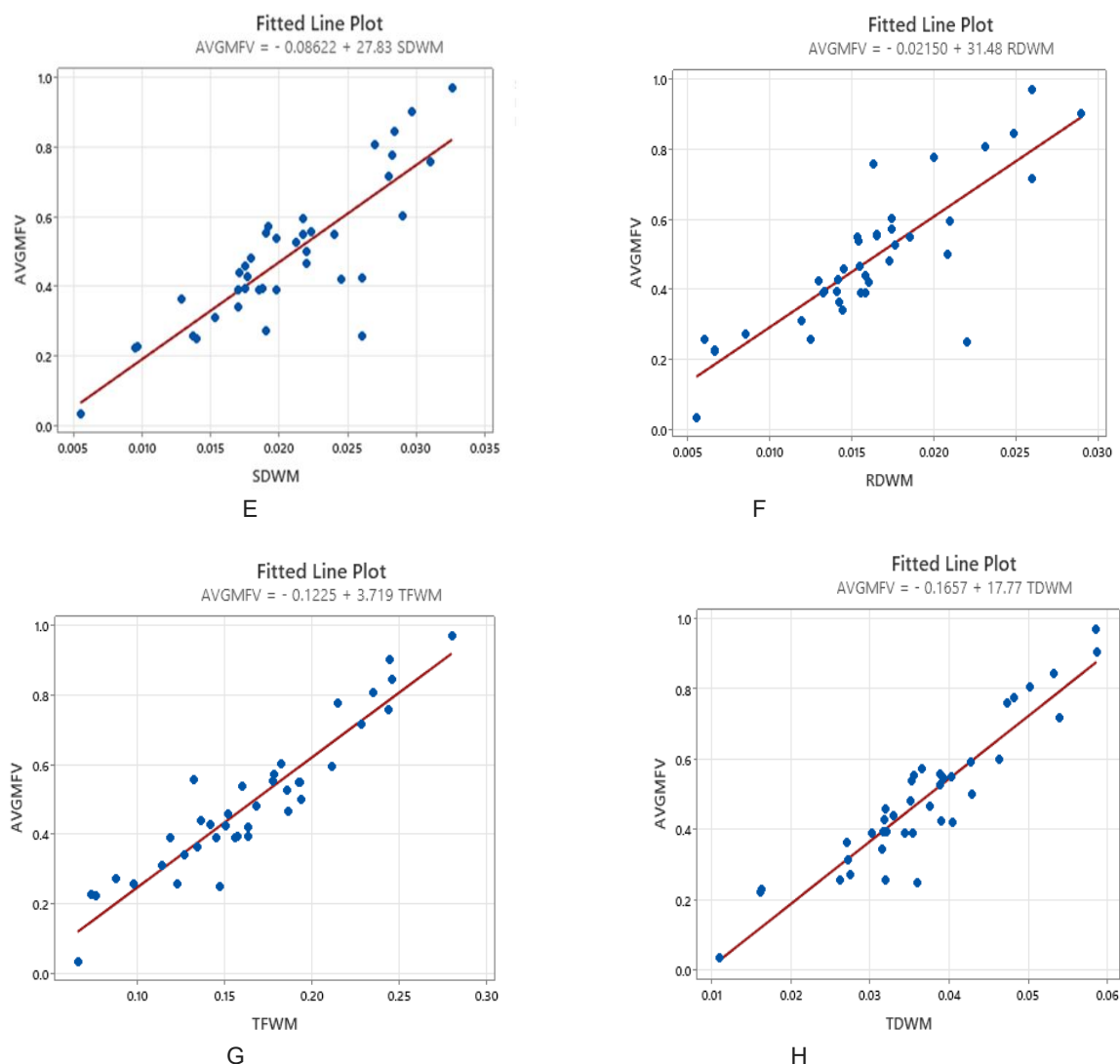


Fig. 2. The linear regression fit between the average of each trait and the average MFV of an individual rice genotype

(E) Is between average MFV and average of SDW (F) Is between average MFV and average RDW (G) Is between average MFV and average of TFW (G) Is between average MFV and average of TDW

between Y and mean MFV values demonstrates the reliability of the MFV-based method for predicting salt tolerance in rice genotypes. Similar results were given by Ding *et al.* (2018) and Wu *et al.* (2019).

A membership function value (MFV)-based salt tolerance grading system was shown to effectively classify rice genotypes for salt tolerance. Total fresh weight (TFW) was identified as a reliable trait for salt tolerance screening at the seedling stage. The identified highly tolerant and tolerant genotypes can be used in breeding programmes for improving seedling stage salt tolerance in rice.

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