

## ARTICLE

# Evaluation of rice (*Oryza sativa* L.) genotypes grown under combined salinity and submergence stresses based on vegetative stage phenotyping

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**ABSTRACT** Sixteen rice genotypes were screened against combined salinity and submergence stresses to find out potential salt-submergence tolerant genotypes. Rice seedlings were subjected to submergence stress including two salinity levels viz., EC-6 dSm<sup>-1</sup> and EC-8 dSm<sup>-1</sup> for 10 days. Imposition of combined stress considerably decreased the growth parameters in rice seedlings, however *ACM-18*, *ACM-35*, and *RC-251* demonstrated the maximum value for growth attributes such as leaf live (%), root and shoot length, plant fresh and dry biomass. The results of correlation and regression revealed a significant and negative association of leaf lives (%), root length, fresh weight of root and shoot, and root dry weight with mean tolerance score under combined stress indicating their importance as the useful descriptors for the selection. Euclidean clustering was categorized the rice genotypes into three major clusters, i.e., A-susceptible, B-tolerant and moderately tolerant, and C-highly tolerant. Results of cluster analysis showed that the highly tolerant genotypes namely *ACM-18*, *ACM-35*, and *RC-251* were placed in the same cluster. Similar results were further confirmed by principal component analysis having the highly tolerant genotypes in the same group. Combining the morphological and multivariate analysis, *ACM-18*, *ACM-35*, and *RC-251* were selected as promising genotypes for developing high-yielding salt-submergence tolerant rice.

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## Introduction

Agriculture sector is facing different adverse conditions such as salinity, drought, submergence, and extreme temperature stress because of climate change (Shelley et al. 2016; Rahman et al. 2020). Among these, salinity is one of the most brutal environmental constraints that affect plant growth and development negatively. It is estimated that about 1125 million hectares (Mha) is affected by varying degrees of soil salinity which covers approximately 6.5% of the world's total land area (Parihar et al. 2015). Usually, soil salinity is caused by different natural processes or anthropogenic activities such as higher accumulation of soluble salts in the soil surface (Hussain et al. 2017). Moreover, the accumulation of excessive sodium ions with dominant anions of chloride and sulfate commanding high electrical conductivity of above 4 dSm<sup>-1</sup> causes soil salinity (Ali et al. 2013). The higher procurement of

salt in the soil is an intense problem that suppresses crop production in the coast-line areas, particularly in the low-lying developing countries around the world (Nicholls et al. 2007). The threat of soil salinity is also an extensive concern in the southern part of Bangladesh deteriorating the status of soil health and soil fertility leading to low agricultural production (Ahmed and Haider 2014). Approximately 53% of the coastal areas of Bangladesh are invaded by salinity stress which is roughly covering 50% of the country's average (Rahman and Ahsan 2001; Haque 2006). Since crop plants are unable to grow in the saline environment due to an imbalance of biological and biochemical functions, a significant proportion of agricultural land in the coastal belt remains fallow throughout the year (Rasel et al. 2019). Furthermore, soil salinity is frequently associated by water logging/submergence stress which also imbeds their eminent effects on plant growth and development (Yeo 1999; Singh 2015). Now a days, the biggest concern is the occurrence

of combined salinity and water logging stress is increasing throughout the globe because of acute irrigation practice in agricultural production systems, unexpected emergence of saline water tables, and intrusion of seawater in coastal environments (Hatton et al. 2003; Carter et al. 2006). Submergence stress regularly affects about 15 Mha of rainfed lowland areas especially in South and Southeast Asia (Neerja et al. 2007). In Bangladesh context, above one Mha of rice fields in the coastal areas suffer from prolonged partial flooding during the wet season every year (Ismail et al. 2010). This type of severe flooding can lead to complete inundation of the entire plant for up to 2 weeks and usually occurs at the early vegetative stage causing the hindrance of plants normal growth potential (Singh et al. 2014a).

Rice (*Oryza sativa* L.) is consumed as the most important food by more than 3.5 billion people around the world which supplies about 20 percent of the world's total dietary energy supply (Alexandratos and Jelle 2012; CGIAR 2016). Rice is also the dominant crop in the economic system of Bangladesh, and it is taken as the staple food for about 156 million people of the country (Shelley et al. 2016). In Bangladesh, the total land used for rice cultivation is approximately 10.83 Mha leading to an average production of 33.54 million metric tons per year (Kibria et al. 2017). However, currently the rice-based cropping systems are increasingly threatened by the salinity and submergence stress individually or simultaneously causing lower rice production in Bangladesh (Masutomi et al. 2009). On the other hand, existing modern high-yielding rice varieties of Bangladesh are susceptible to combined salt and submergence stress for which they cannot thrive the devastating effect of salinity stress with flash floods (Afrin et al. 2018; Bui et al. 2019). Thus, the development of high-yielding, multiple stress-tolerant varieties against salinity and submergence has a great importance that could provide farmers with the cost-effective options in saline-prone flood-affected areas for achieving food security and sustainable agriculture (Ismail et al. 2013; Singh et al. 2013).

Both water logging and salinity are pernicious to plant growth and yield in different ways (Singh 2017). The prevailing low osmotic potential in soil solution leading salt stress which causes nutritional imbalance, and specific ion effects in plant cells (Evelin et al. 2009). The growth and development of plants are adversely affected by the accumulation of excess salt in soil once the osmotic stress severely declines the uptake of water by roots (Munns and Tester 2008). Plant response to salt stress is very complicated and depends on numerous factors for instance type and concentration of the solutes, growth stage of the plant, the genetic potential of the plant and different environmental factors (Kordrostami et al. 2017). Usually,

rice is relatively salt tolerant during seed germination, active tillering and, maturity stage but very sensitive at the seedling and early vegetative stage and thus, seedling vigor including leaf live (%), root length, shoot length, plant biomass and other growth traits are substantially affected and reduced under salt stress conditions (Singh and Flowers 2010; Hakim et al. 2014; Kumar et al. 2015). Besides these, salinity stress may also lead to membrane disorganization, toxic metabolites generation, photosynthesis inhibition, over-production of reactive oxygen species (ROS), and attenuated nutrient acquisition causing cell death (Chartzoulakis 2005; Sun et al. 2011; Rahman et al. 2020). Likewise salt stress, submergence stress also affects plant early growth stage and thus, reduces plant productivity to a great extent (Yadav et al. 2018). When salt stress is coupled with submergence stress, salinity can cause even greater damage to plants (Jackson and Ram 2003). For many plant species, when salinity and waterlogging happen together,  $\text{Na}^+$  and/or  $\text{Cl}^-$  concentrations dramatically increase in shoots due to the entry of excess toxic ions into oxygen-deficient roots of plants (Barrett-Lennard 2003). Submergence situation for more than seven days can cause in the plants leaf rotting, loss of dry mass and lodging after the flood waterfall down (Goswami et al. 2015). In submergence condition, the availability of free  $\text{O}_2$  around the roots is depleted and thus, hypoxia stress occurs which makes anaerobic metabolism in roots from an aerobic environment with the dramatic reductions of ATP synthesis (Teakle et al. 2006). Because of these changes, plants exhibit altered membrane transport, decreased conductance of stomata and decreased leaf water potentials, enhanced root senescence, reduced root and shoot length, and eventually caused the death of the whole plant (Barrett-Lennard 2003).

To find out multiple stress-tolerant genotypes, a proper screening technique is required with the selection of the appropriate developmental stage of plant (Ghosh et al. 2016). Usually, the seedling stage or vegetative stage of the rice plant is more affected by both salinity and submergence stress (Chunthaburee et al. 2016; Abedin et al. 2019). Thus, screening of rice genotypes for salinity or submergence tolerance at the early growth or vegetative stage can be undertaken using the parameters described as effective tolerant indices like leaf live (%), root length, shoot length, and plant biomass because variations in the genotypes on these stages are controlled by genetic components (Zeng et al. 2007; Ali et al. 2014). However, the combined effect of salinity and submergence stress on the vegetative stage of rice plants has rarely been examined (Rogers et al. 2011; Chen et al. 2013), thereby reflecting further study on rice plant at early growth stages in relation to multiple abiotic stresses like salinity and submergence are recommended (Yan et al. 2013).

**Table 1.** Modified standard evaluation score (SES) of visual salt injury for growth traits at the seedling stage of rice genotypes..

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant (HT)
3	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant (T)
5	Growth severely retarded, most leaves rolled; only a few are elongating	Moderately tolerant (MT)
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible (S)
9	Almost all plants dead or dying	Highly susceptible (HS)

Source: Gregorio et al. (1997)

Therefore, the present study has been undertaken to evaluate rice genotypes to explore their response to combined salinity and submergence stresses by assessing morphological traits at the late vegetative stage along with the classification of rice genotype employing hierarchical clustering and principal component analysis to identify robust salt-submergence tolerant rice genotype(s).

## Materials and Methods

### Plant materials and experimental design

A total of 16 rice genotypes (viz., *ACM-3*, *ACM-4*, *ACM-5*, *ACM-6*, *ACM-8*, *ACM-10*, *ACM-11*, *ACM-15*, *ACM-16*, *ACM-18*, *ACM-23*, *ACM-26*, *ACM-29*, *ACM-35*, *Binadhan-7*, and *RC-251*) collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, Bangladesh and International Rice Research Institute (IRRI), together with two check genotypes viz., *RC-251* (positive check, both salinity and submergence tolerant), and *Binadhan-7* (negative check, both salinity and submergence susceptible) were used as plant materials in the present study. The experiment was conducted following completely randomized design with two factors (salinity and submergence) and three replications (each replication had 5 seedlings). Thus, the treatment combinations were as follows: control treatment,  $T_0$  (distilled water), two stress treatments viz.,  $T_1$  (EC-6 dSm<sup>-1</sup> salinity level with complete submergence), and  $T_2$  (EC-8 dSm<sup>-1</sup> with complete submergence).

### Plant culture and stress imposition

Initially, the uniform size rice seeds were sorted out and surface sterilized with 5% sodium hypochlorite and 0.1% mercuric chloride followed by three times washing with distilled water (dH<sub>2</sub>O). Then, the seeds were kept in the oven at 50 °C for 2 days for breaking dormancy. The oven-treated seeds were soaked overnight with dH<sub>2</sub>O and placed in the Petri dishes on a moist filter paper (50 seeds/petri dish) at room temperature (25 ± 2 °C) to induce germination. The 3-days old pre-germinated seeds were then sown in the seedbed for seedling growth. Seedbed

was prepared by raising soil up to 5-10 cm from the field surfaces with the mixing of 8 kg cow dung properly. Afterwards, 30-days old seedlings were uprooted from the seedbed and transplanted in the previously prepared 15 cm in length and 11 cm in diameter sized perforated cotton fiber pots (3 seedlings in each pot). The pots were filled with silt loam soil having the following characteristics: pH of 6.2, electrical conductivity (EC) of 0.12 dSm<sup>-1</sup>, cation exchange capacity of 8.5%, exchangeable Na<sup>+</sup> of 0.33 milliequivalent per 100 g soil, exchangeable K<sup>+</sup> of 0.15 cmol kg<sup>-1</sup>, total nitrogen of 0.13%, organic carbon of 0.70%, and organic matter of 1.16%. The pots were placed in the trays (130 cm×75 cm×25 cm) filled with tap water (Ca<sup>2+</sup> 3.2 mM, Mg<sup>2+</sup> 0.50 mM, Na<sup>+</sup> 1 mM, K<sup>+</sup> 0.2 mM, HCO<sub>3</sub><sup>-</sup> 5.0 mM, Cl<sup>-</sup> 0.21 mM, SO<sub>4</sub><sup>2-</sup> 0.08 mM, and P <0.002 mM), so that the fiber pots were half-drowned in tap water. After one week of transplanting, approximately one-third of urea (5 g), a full dose of phosphorus (4 g), and a full dose of potassium (3 g) fertilizers were applied on each of the pot at the optimal field application rates. The remaining urea (10 g) was applied later at 30<sup>th</sup> and 50<sup>th</sup> days of crop life span. Plants were kept in these normal water trays for 20 days for seedling establishment. Afterwards, the perforated pots with 50-days old seedlings were transferred from the trays into the large black tanks (16 pots per tank) containing salinized water solution. The salinized water solution was prepared by dissolving crude salt (sodium chloride with some trace minerals like potassium, iron, and zinc) with water. The water level for submergence stress treatment in the tank was maintained at 14 mm from the soil surface by adding tap water artificially. The rice seedlings were exposed to saline and complete submergence stress simultaneously for next 10 days. The EC and pH (6.5) of the salinized solution in the tank was kept constant and measured by an EC-meter (Hanna HI 4321) and pH-meter (Hanna HI 22111), respectively. Afterwards, plants were taken out from the drums and settled in the normal environmental condition until harvesting (day 70). The control plants were also grown in natural conditions without undergoing the salinity and submergence treatment with

**Table 2.** Combined analyses of variances among seven studied traits under in rice genotypes grown under combined salinity (6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) and submergence stress

Sources of variation	df	Mean sum squares						
		LL (%)	SL	RL	SFW	RFW	SDW	RDW
Genotypes (G)	15	5003.1***	2868.5***	345.0***	314.0***	6.32***	10.75***	0.44***
Treatments (T)	2	18095.1***	10489.9***	1354.2***	1321.5***	12.39***	71.04***	1.56***
G x T	30	916.3***	312.5***	20.03***	29.23***	0.55***	1.74***	0.06***
Error	64	36.11	4.18	1.02	1.18	0.01	0.01	0.01***
CV (%)	-	8.40	3.46	6.57	10.28	6.82	4.69	7.41***

\*\*\* indicates significant at 0.001 level of probability, CV coefficient of variation, df degrees of freedom. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg).

well-drained conditions. The modified standard evaluation score (SES) was estimated at the late vegetative stage based on the visual symptoms of salt-submergence injury in 70-days old plants (IRRI, 1997) (Table 1). This scoring discriminated the susceptible genotypes from the tolerant and the moderately tolerant genotypes.

**Data collection of morphological traits under normal and stress conditions**

Different growth parameters were measured from 5 plants in each replication after 70<sup>th</sup> days of the plantation, and afterwards, the average value was taken. The leaf live (%) was measured by dividing the number of leaves live by the total number of leaves multiplied by hundred. The root length was measured from the shoot initiation to the root tip and shoot length was measured from the shoot initiation point to the tip of the plant by using a meter (m) scale. Immediately after harvesting, the shoot samples were separated from the root, and the fresh weight (mg) of root and shoot weight were taken carefully using an electric balance and afterwards, roots and shoots samples were separately enclosed in brown envelopes (20 cm x 10 cm) and kept in an oven at 60 °C for five days. Then, the dry weight (mg) was also measured separately by using an electric balance.

**Statistical analysis**

The data obtained were analyzed by a two-way analysis of variance using Statistical Tool for Agricultural Research (STAR) (IRRI, Los Baños, Laguna, Philippines). The mean values were separated at the p < 0.001 level using the least significant difference (LSD) test and presented indicated by different alphabetical letters in the same column. The heat map and hierarchical clustering was performed by Biovinci considering the percent reduction values of growth traits of different genotypes under combined stress situations. PCA was conducted by using the STAR and the biplots were constructed with the first two PCA components viz., PC1, and PC2 that explained the maxi-

um variations exist among the datasets. The Pearson's correlation coefficients and regression coefficients among the studied traits were also calculated using the STAR and SPSS 22 software package, respectively. The genetic parameters such as phenotypic variance ( $\sigma^2_p$ ), genotypic variance ( $\sigma^2_g$ ), phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), heritability ( $h^2_b$ ), and genetic advance (GA) for all the studied morphological traits were estimated following the method as previously described by Rasel et al. (2018).

**Results**

**Growth characteristics changes in rice seedlings in response to combined salinity and submergence stresses**

The analyses of variance (ANOVA) of different morphological parameters of 16 rice genotypes under combined salinity and submergence stresses indicated that the difference among genotypes for all the traits viz., leaf live (LL %), shoot length (SL), root length (RL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), and shoot dry weight (SDW) were highly significant (p < 0.001) (Table 2). The interaction of salt-submergence stress decreased growth parameters of rice seedlings in all genotypes (Table 5). As three genotypes viz., ACM-8, ACM-10, and Binadhan-7 were completely dead after 10-days of fully submergence stress in salinized water, the phenotypic traits for those genotypes were not measured (Table 4) and the percent reduction of all morphological parameters was maximum of 100% under stressed situations for those genotypes (Table 5). Rice seedlings showed more reduction of growth parameters with the increasing of salinity level in waterlogging condition. However, tolerant genotypes were least affected by imposed stress than susceptible genotypes for different agro-morphological traits. In the present study, the LL (%) was drastically reduced in ACM-5 (T<sub>1</sub> 59.4%, T<sub>2</sub> 64.8%), ACM-6 (T<sub>1</sub> 37.4%, T<sub>2</sub> 53.1%), and ACM-16 (T<sub>1</sub> 34.1%, T<sub>2</sub> 34.6%) whereas minimum



**Table 3.** Ranking of rice genotypes for relative salt tolerance obtained from growth-related traits based on percent reduction.

Genotypes	Treatments	LL	SL	RL	SFW	RFW	SDW	RDW	Sum	MTS	Tolerance degree
ACM-3	T <sub>1</sub>	1.00	3.00	3.00	3.00	1.00	1.00	5.00	2.42	4.56	MT
	T <sub>2</sub>	1.00	5.00	9.00	5.00	9.00	9.00	9.00	6.71		
ACM-4	T <sub>1</sub>	1.00	1.00	3.00	7.00	1.00	9.00	3.00	3.57	3.78	T
	T <sub>2</sub>	1.00	1.00	5.00	9.00	1.00	9.00	9.00	5.00		
ACM-5	T <sub>1</sub>	9.00	3.00	5.00	9.00	5.00	9.00	3.00	6.14	6.57	S
	T <sub>2</sub>	9.00	5.00	5.00	9.00	7.00	9.00	5.00	7.00		
ACM-6	T <sub>1</sub>	5.00	3.00	9.00	3.00	1.00	5.00	1.00	3.86	5.15	MT
	T <sub>2</sub>	7.00	5.00	9.00	9.00	3.00	7.00	5.00	6.43		
ACM-8	T <sub>1</sub>	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	HS
	T <sub>2</sub>	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00		
ACM-10	T <sub>1</sub>	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	HS
	T <sub>2</sub>	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00		
ACM-11	T <sub>1</sub>	1.00	3.00	1.00	1.00	3.00	1.00	1.00	1.57	2.99	T
	T <sub>2</sub>	1.00	3.00	3.00	7.00	3.00	9.00	5.00	4.42		
ACM-15	T <sub>1</sub>	3.00	1.00	5.00	7.00	3.00	9.00	1.00	4.14	4.86	MT
	T <sub>2</sub>	5.00	3.00	7.00	9.00	3.00	9.00	3.00	5.57		
ACM-16	T <sub>1</sub>	5.00	1.00	3.00	9.00	1.00	9.00	3.00	4.43	5.00	MT
	T <sub>2</sub>	5.00	3.00	3.00	9.00	3.00	9.00	7.00	5.57		
ACM-18	T <sub>1</sub>	1.00	1.00	1.00	3.00	1.00	3.00	1.00	1.57	2.42	HT
	T <sub>2</sub>	1.00	1.00	7.00	5.00	3.00	5.00	1.00	3.28		
ACM-23	T <sub>1</sub>	1.00	1.00	1.00	7.00	1.00	7.00	1.00	2.71	3.57	T
	T <sub>2</sub>	3.00	5.00	3.00	9.00	1.00	9.00	1.00	4.43		
ACM-26	T <sub>1</sub>	1.00	3.00	3.00	7.00	1.00	9.00	1.00	3.57	5.85	MT
	T <sub>2</sub>	5.00	7.00	9.00	9.00	9.00	9.00	9.00	8.14		
ACM-29	T <sub>1</sub>	1.00	3.00	5.00	5.00	5.00	7.00	1.00	3.86	5.58	MT
	T <sub>2</sub>	3.00	5.00	7.00	9.00	9.00	9.00	9.00	7.29		
ACM-35	T <sub>1</sub>	1.00	1.00	3.00	3.00	1.00	3.00	1.00	1.85	2.42	HT
	T <sub>2</sub>	1.00	1.00	7.00	5.00	3.00	3.00	1.00	3.00		
Binadhan-7	T <sub>1</sub>	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	HS
	T <sub>2</sub>	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00		
RC-251	T <sub>1</sub>	1.00	1.00	1.00	3.00	1.00	1.00	1.00	1.28	1.99	HT
	T <sub>2</sub>	1.00	3.00	1.00	7.00	1.00	1.00	5.00	2.71		

The treatments T<sub>1</sub> 6 dSm<sup>-1</sup> salinity level with complete submergence, and T<sub>2</sub> 8 dSm<sup>-1</sup> salinity level with complete submergence. Scoring range based on % reduction values of growth traits under stress conditions were count for 10-20%= score 1, 21-31%= score 3, 32-42%= score 5, 43-53%= score 7, and 54-64%= score 9; score 1,3,5,7 and 9 value was given according to IRRI standard protocol (1997), where score 1 denotes highly tolerant (HT), score 3 denotes tolerant (T), score 5 denotes moderately tolerant (MT), score 7 denotes susceptible (S), and score 9 denotes highly susceptible (HS). The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg). MTS denotes mean tolerance score.

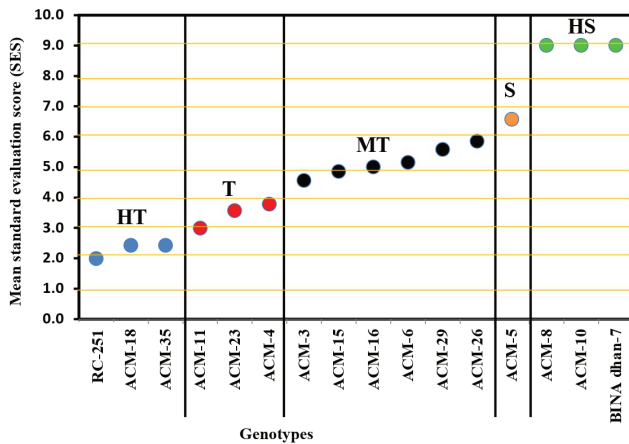
reduction was observed in *RC-251* (T<sub>1</sub> 2.61%, T<sub>2</sub> 7.17%), *ACM-35* (T<sub>1</sub> 0.20%, T<sub>2</sub> 9.60%), and *ACM-3* (T<sub>1</sub> 5.79%, T<sub>2</sub> 8.69%) when exposed to salt-submergence stress (Table 5). The severe curtailment of SL and RL were found in all the genotypes under combined submergence plus salt stress. However, some genotypes namely *RC-251*, *ACM-18*, and *ACM-35* displayed the highest root and shoot growth in salt-submergence stress situations (Table 4). Considering RL, the maximum reduction was found in *ACM-6* (69.6%), *ACM-26* (56.3%), *ACM-3* (55.4%) and the minimum reduction was observed in *ACM-11* (27.6%),

and *ACM-23* (28.5%) like tolerant check *RC-251* (20.8%) at stagnant conditions with 8dSm<sup>-1</sup> salinity level (Table 5). Similarly, the genotypes namely *ACM-35* (19.1%), *ACM-18* (20.3%), and *RC-251* (23.4%) also exhibited the least reduction for SL whereas maximum reduction was recorded in *ACM-26* (45.5%), *ACM-29* (42.8%), and *ACM-5* (40.0%) in combined waterlogging + 8dSm<sup>-1</sup> salt treatments (Table 5). Under salinity-submergence stress, RFW and SFW in rice seedlings showed a differential response and significantly decreased in submerged rice plants with the increasing of salinity. However, some genotypes viz.,

**Table 4.** Performance of rice genotypes considering morphological traits at the late vegetative stage under salt-submergence stress and non-stressed conditions.

Genotypes	Treatment (T)	LL	SL	RL	SFW	RFW	SDW	RDW
ACM-3	T <sub>0</sub>	98.0 ab	79.0 ab	24.6 b-e	12.72 f	3.59 b	2.59 h	1.05 a
	T <sub>1</sub>	92.3 ab	60.0 c-f	18.6 b	10.0 cd	3.06 b	2.12 cd	0.65 cd
	T <sub>2</sub>	89.5 a-c	53.0 b-d	11.0 ef	7.8 c-e	1.34 cd	0.93 ef	0.37 d
ACM-4	T <sub>0</sub>	90.25ab	63.3 fg	25.5 bc	15.2 f	2.06 fg	3.11 fg	0.49 fg
	T <sub>1</sub>	85.0 a-c	54.3 fg	17.0 b	8.07 d-f	1.98 cd	1.29 g	0.38 fg
	T <sub>2</sub>	72.3 a-d	50.6 cd	16.0 bc	6.18 c-f	1.94 b	1.12 c-e	0.16e
ACM-5	T <sub>0</sub>	100 a	71.6 c-e	18.6 h	15.8 de	2.22 ef	2.84 gh	0.51 fg
	T <sub>1</sub>	40.5 f	54.0 g	12.0 c	6.32 ef	1.51 e	1.24 g	0.35 g
	T <sub>2</sub>	35.1 g	43.0 ef	11.6 d-f	5.24 d-g	1.20 d	0.97 de	0.34 d
ACM-6	T <sub>0</sub>	93.7 ab	76.0 a-c	29.0 a	16.5 de	1.90 fg	3.00 fg	0.49 fg
	T <sub>1</sub>	58.6 e	56.1 e-g	11.1 c	11.29 c	1.65 e	1.90 d-f	0.46 ef
	T <sub>2</sub>	43.9 fg	47.8 de	8.81 f	2.63 gh	1.47 cd	1.40 c	0.33 d
ACM-8	T <sub>0</sub>	70.8 c	53.3 ij	8.25 j	2.60 i	1.06 i	1.12 k	0.59 ef
	T <sub>1</sub>	0.00 g	0.00 i	0.00 d	0.00 g	0.00 g	0.00 h	0.00 h
	T <sub>2</sub>	0.00 h	0.00 h	0.00 g	0.00 h	0.00 f	0.00 g	0.00 f
ACM-10	T <sub>0</sub>	81.2 bc	57.3 hi	9.17 j	4.46 hi	1.52 h	1.42 j	0.46 g
	T <sub>1</sub>	0.00 g	0.00 i	0.00d	0.00 g	0.00 g	0.00 h	0.00 h
	T <sub>2</sub>	0.00 h	0.00 h	0.00 g	0.00 h	0.00 f	0.00 g	0.00 f
ACM-11	T <sub>0</sub>	98.8 a	80.1 a	26.5 ab	30.0 a	2.74 d	5.94 a	0.94 c
	T <sub>1</sub>	89.6 a-c	63.4 a-d	22.1 a	25.7 a	1.99 cd	5.30 a	0.83 ab
	T <sub>2</sub>	86.4 a-c	61.9 a	19.1 a	17.0 a	1.89 b	2.44 b	0.59 ab
ACM-15	T <sub>0</sub>	84.5 a-c	74.2 b-d	25.1 b-d	17.4 d	2.52 de	3.93 e	0.83 d
	T <sub>1</sub>	59.6 e	66.5 ab	16.0 b	8.17 de	1.98 cd	1.72e f	0.73 bc
	T <sub>2</sub>	54.2 ef	57.1 d-g	13.5 c-e	4.76 e-g	1.91 b	1.08 de	0.60 a
ACM-16	T <sub>0</sub>	96.3 ab	76.1 a-c	22.5 d-g	23.8 bc	4.18 a	4.97 c	1.19 a
	T <sub>1</sub>	63.4 de	65.0 a-c	16.8 b	9.12 c-e	3.83 a	1.62 f	0.87 a
	T <sub>2</sub>	62.9 de	54.3 bc	15.8 bc	9.09 c	2.89 a	0.94 ef	0.60 cd
ACM-18	T <sub>0</sub>	94.8 ab	78.5 ab	21.5 f-h	21.7 c	1.84 gh	4.39 d	0.58 ef
	T <sub>1</sub>	91.9 ab	67.5 a	18.5 b	14.8 b	1.60 e	3.10 b	0.51 e
	T <sub>2</sub>	80.9 c	62.5 a	11.5 d-f	12.6 b	1.39 cd	2.85 a	0.49 bc
ACM-23	T <sub>0</sub>	97.0 ab	69.16 de	19.8 g-h	16.9 de	1.92 fg	4.12 de	0.63e
	T <sub>1</sub>	79.4 b-d	65.1 a-c	16.1 b	9.13 c-e	1.67 de	2.17 cd	0.56de
	T <sub>2</sub>	70.9b-e	42.5 ef	14.1 cd	4.84 e-g	1.54 c	0.90 ef	0.56 a-c
ACM-26	T <sub>0</sub>	90.7 ab	75.1 a-c	22.1 e-g	22.3 bc	2.18 f	5.73 a	0.79 d
	T <sub>1</sub>	72.4 c-e	55.5 e-g	17.0 b	11.29 c	2.06 c	1.93 de	0.64cd
	T <sub>2</sub>	61.1 d-f	40.9 fg	9.67 f	3.28 fg	0.69 e	0.86 ef	0.16 e
ACM-29	T <sub>0</sub>	93.3 ab	76.3 a-c	24.0 b-f	25.1 b	3.16 c	5.26 b	0.87 c
	T <sub>1</sub>	80.8 bc	57.9 d-g	16.1 b	15.8 b	2.04 c	2.92 b	0.69 cd
	T <sub>2</sub>	65.8 c-e	43.6 ef	11.3 d-f	3.33 fg	0.63 e	0.66 f	0.13 e
ACM-35	T <sub>0</sub>	98.6 a	68.1 ef	24.5 b-e	14.0 ef	1.93 fg	3.27 f	0.50 fg
	T <sub>1</sub>	98.4 a	60.9 b-e	16.0 b	10.7 cd	1.55 e	2.32 c	0.50 e
	T <sub>2</sub>	89.1 a	55.1 bc	12.5 de	8.07 cd	1.47 cd	2.23 b	0.47 c
Binadhan-7	T <sub>0</sub>	100 a	59.1 gh	14.0 i	8.36 g	1.57 h	2.26 i	0.51 fg
	T <sub>1</sub>	0.00 g	0.00 i	0.00 d	0.00 g	0.00 g	0.00 h	0.00 h
	T <sub>2</sub>	0.00 h	0.00 h	0.00 g	0.00 h	0.00 f	0.00 g	0.00 f
RC-251	T <sub>0</sub>	91.9 ab	48.1 j	23.2 c-f	6.45 gh	0.97 i	1.56 j	0.33 h
	T <sub>1</sub>	89.5 a-c	43.3 h	22.9 a	5.01 f	0.92 f	1.31 g	0.31 g
	T <sub>2</sub>	85.3 ab	36.8 g	18.4 ab	3.57 fg	0.81 e	1.24 cd	0.21 e

Different letter within the column denotes the statistical significance difference among the mean values at P < 0.05. The treatments T<sub>0</sub> control, T<sub>1</sub> 6 dSm<sup>-1</sup> salinity level with complete submergence, and T<sub>2</sub> 8 dSm<sup>-1</sup> salinity level with complete submergence. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg).

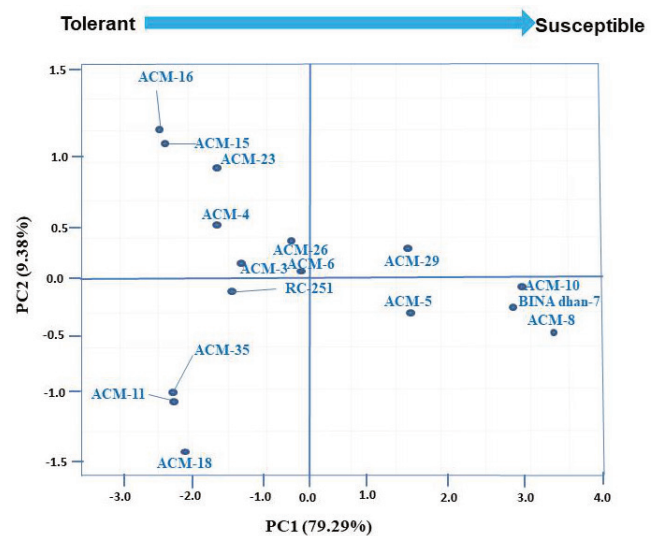


**Figure 1.** Evaluation of standard evaluation score (SES) in rice genotypes grown under combined salinity (6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) and waterlogging stress for 10 days at the late vegetative stage. Scoring range based on % reduction values of growth traits under stress conditions were count for 10-20% = score 1, 21-31% = score 3, 32-42% = score 5, 43-53% = score 7, and 54-64% = score 9; score 1,3,5,7 and 9 value was given according to IRR standard protocol (1997), where score 1 denotes highly tolerant (HT), score 3 denotes tolerant (T), score 5 denotes moderately tolerant (MT), score 7 denotes susceptible (S), and score 9 denotes highly susceptible (HS). Table 3 showed the detailed parameters-wise scoring of rice genotypes under salt-submergence stress.

ACM-35, ACM-4, ACM-23, and ACM-3 minimized the reduction of RFW and SFW demonstrating higher fresh biomass likewise positive tolerant check RC-251 when faced salt-submergence stress. In contrast, susceptible genotypes namely ACM-5, ACM-16, ACM-26, and ACM-29 reflected the maximum reduction of RFW and SFW under combined stressed situations (Table 5). The results of the present investigation also showed the reduction of dry matter of rice plants in all genotypes under combined stressed conditions though tolerant genotypes exhibited less reduction of dry matter in comparison with susceptible genotypes (Table 5). When waterlogging with 8dSm<sup>-1</sup> salt treatment is induced, the drastical reduction of root dry biomass was noted for ACM-29 (84.6%), ACM-26 (80.1%), and ACM-4 (67.5%) whereas ACM-35 (5.96%), ACM-23 (11.1%), and ACM-18 (15.5%) displayed the least reduction of root biomass compared to the other genotypes (Table 5). Similarly, the minimum reduction of shoot dry matter was also reported in ACM-35 (T<sub>1</sub> 29.0%, T<sub>2</sub> 31.9%), and ACM-18 (T<sub>1</sub> 29.5%, T<sub>2</sub> 35.2%) similar to positive check RC-251 (T<sub>1</sub> 15.6%, T<sub>2</sub> 20.3%), whereas ACM-26 (T<sub>1</sub> 66.3%, T<sub>2</sub> 85.0%), ACM-16 (T<sub>1</sub> 67.4%, T<sub>2</sub> 81.1%), and ACM-15 (T<sub>1</sub> 56.3%, T<sub>2</sub> 52.4%) reflected maximum shoot dry matter reduction under combined waterlogging + salt stress conditions (Table 5).

### Categorizing of rice genotypes based on for mean salinity-submergence tolerance scores

Various degrees of phenotypic responses were reported in rice plants under salinity-submergence stress. Rice seedlings grown in the control condition showed normal growth and development whereas under stressed conditions, several impairments of salt-submergence induced injury such as yellowing and drying of leaves, reduction in root and shoot growths, and dying of seedlings were observed in the plants. Based on the morphological changes due to salinity-waterlogging stress, the rice genotypes were clustered into five groups viz., highly tolerant (mean tolerance score ≤2.5), tolerant (mean tolerance score ≤4.5), moderately tolerant (mean tolerance score ≤6.5), susceptible (mean tolerance score ≤8.5) and highly susceptible (mean tolerance score >8.5-9) (Table 3) following IRR standard evaluation system (SES). After 10-days of combined stress induction, some plants were completely decayed and those were depicted as highly susceptible genotype viz., ACM-8, ACM-10, and Binadhan-7 (Fig. 1). The genotypes namely RC-251, ACM-18, and ACM-35 demonstrated the normal growth potential with the lowest mean tolerance score when both stresses is induced



**Figure 2.** Hierarchical clustering and heatmap elucidating the genotype variable relationships. Color scale shows the intensity of the normalized mean values of different parameters. Clustering of rice genotypes considering percent reduction of growth parameters under combined salinity (6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) and submergence stress based on Euclidean distances grouped the genotypes into three clusters. Cluster-A includes BINA dhan-7, ACM-8 and ACM-10; Cluster-B includes ACM-5, ACM-6, ACM-15, ACM-16, ACM-26, ACM-29, ACM-3, and ACM-23; Cluster-C includes ACM-18, ACM-35, RC-251, ACM-4, and ACM-11. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg).

**Table 5.** Percent reduction of growth parameters of 16 rice genotypes at the late vegetative stage under salt-submergence stress condition.

Genotypes	Treatment (T)	LL	SL	RL	SFW	RFW	SDW	RDW
ACM-3	T <sub>1</sub>	5.79	24.0	24.3	20.7	14.7	18.0	37.7
	T <sub>2</sub>	8.69	32.9	55.4	38.4	62.6	63.9	65.0
ACM-4	T <sub>1</sub>	5.82	14.2	33.3	47.1	3.57	58.3	23.6
	T <sub>2</sub>	19.8	20.0	37.2	59.5	5.83	63.9	67.5
ACM-5	T <sub>1</sub>	59.4	24.6	35.7	60.2	32.2	56.5	30.2
	T <sub>2</sub>	64.8	40.0	37.5	67.0	46.0	65.8	32.2
ACM-6	T <sub>1</sub>	37.4	26.1	61.4	31.9	13.1	36.7	5.44
	T <sub>2</sub>	53.1	37.0	69.6	84.1	22.7	53.3	32.6
ACM-8	T <sub>1</sub>	100	100	100	100	100	100	100
	T <sub>2</sub>	100	100	100	100	100	100	100
ACM-10	T <sub>1</sub>	100	100	100	100	100	100	100
	T <sub>2</sub>	100	100	100	100	100	100	100
ACM-11	T <sub>1</sub>	9.35	20.8	16.3	14.2	27.3	10.7	11.3
	T <sub>2</sub>	12.5	22.7	27.6	43.1	31.0	58.8	37.5
ACM-15	T <sub>1</sub>	29.4	10.3	36.1	53.1	21.5	56.3	11.2
	T <sub>2</sub>	35.7	22.9	46.2	72.6	24.3	72.4	27.0
ACM-16	T <sub>1</sub>	34.1	14.6	25.1	61.7	8.22	67.4	26.6
	T <sub>2</sub>	34.6	28.6	29.5	61.8	30.7	81.1	49.5
ACM-18	T <sub>1</sub>	3.03	13.9	13.9	31.8	13.0	29.5	12.6
	T <sub>2</sub>	14.6	20.3	46.5	41.9	24.2	35.2	15.5
ACM-23	T <sub>1</sub>	18.1	5.88	18.4	46.2	13.1	47.3	10.1
	T <sub>2</sub>	26.9	38.4	28.5	71.5	19.7	78.1	11.1
ACM-26	T <sub>1</sub>	20.1	26.1	22.9	49.4	5.21	66.3	18.9
	T <sub>2</sub>	32.6	45.5	56.3	85.3	68.4	85.0	80.1
ACM-29	T <sub>1</sub>	13.4	24.1	32.6	36.9	35.2	44.5	20.3
	T <sub>2</sub>	29.4	42.8	52.7	86.7	79.9	87.4	84.6
ACM-35	T <sub>1</sub>	0.20	11.1	34.5	23.0	19.6	29.0	1.32
	T <sub>2</sub>	9.60	19.1	48.6	42.3	23.6	31.9	5.96
Binadhan-7	T <sub>1</sub>	100	100	100	100	100	100	100
	T <sub>2</sub>	100	100	100	100	100	100	100
RC-251	T <sub>1</sub>	2.61	9.79	1.26	22.3	4.83	15.6	6.06
	T <sub>2</sub>	7.17	23.4	20.8	44.6	15.8	20.3	36.3

The treatments T<sub>1</sub> 6 dSm<sup>-1</sup> salinity level with complete submergence, and T<sub>2</sub> 8 dSm<sup>-1</sup> salinity level with complete submergence. %R<sup>t</sup> means percent reduction calculated as [(control value – salt treatment value)/control value × 100]. Scoring range based on % reduction values of growth traits under stress conditions were count for 10-20%= score 1, 21-31%= score 3, 32-42% = score 5, 43-53% = score 7, and 54-64% = score 9; score 1,3,5,7 and 9 value was given according to IRRI standard protocol (1997), where score 1 denotes highly tolerant (HT), score 3 denotes tolerant (T), score 5 denotes moderately tolerant (MT), score 7 denotes susceptible (S), and score 9 denotes highly susceptible (HS). Table 3 showed the detailed parameters-wise scoring of rice genotypes. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg).

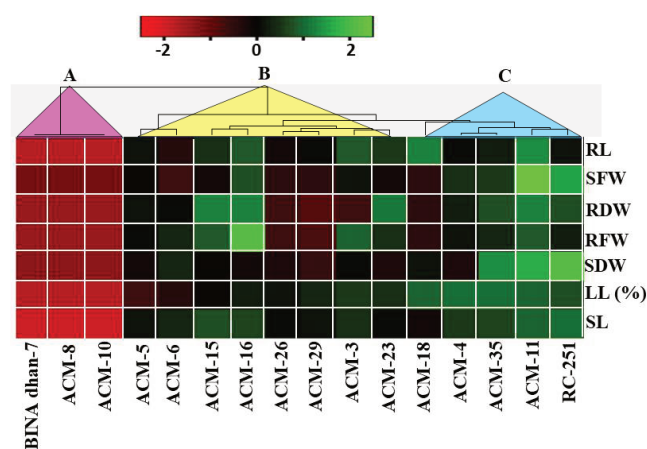
simultaneously and therefore, these were identified as highly tolerant whereas some other genotypes namely *ACM-11*, *ACM-23*, and *ACM-4* showed moderate to little injury symptoms under salt-submergence stress and consequently, these were regarded as tolerant genotypes (Fig. 1). The six genotypes viz., *ACM-3*, *ACM-15*, *ACM-16*, *ACM-6*, *ACM-29*, and *ACM-26* were identified as moderately tolerant as the seedling growth of those genotypes were partially inhibited under salinity-submergence stress conditions. Moreover, *ACM-5* exhibited the greater reduction of growth traits having a higher mean tolerance score (6.57) in stressed conditions and therefore, it

was depicted as a susceptible genotype (Fig. 1; Table 3).

**The results of the cluster analysis**

The heatmap and hierarchical clustering based on the percent reduction of growth traits under combined salinity and waterlogging stress using Euclidean distance grouped the 16 rice genotypes into three main clusters (Cluster-A, -B, and -C) (Fig. 2). The genotypes of the cluster-A are *Binadhan-7*, *ACM-8* and *ACM-10* which demonstrated the maximum reduction of growth traits and highest mean tolerance score (9.00) (Fig. 1; Table 3) under stressed conditions. Therefore, those genotypes





**Figure 3.** Loading plots of principal components 1 (PC1) and principal components 2 (PC2) of the PCA among 15 rice genotypes-based percent reduction of growth traits at the seedling stage under combined salinity (6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) and submergence stresses.

were regarded as highly susceptible genotypes. The distribution pattern of cluster analysis revealed maximum of eight genotypes viz., *ACM-5*, *ACM-6*, *ACM-15*, *ACM-16*, *ACM-26*, *ACM-29*, *ACM-3*, and *ACM-23* in cluster-B which showed differential response to salt-submergence tolerance under combined stress conditions such as *ACM-6*, *ACM-15*, *ACM-16*, *ACM-26*, *ACM-29*, *ACM-3* (moderately tolerant), *ACM-23* (tolerant) and *ACM-5* (susceptible) (Fig. 2). The cluster-C had five genotypes and among them, *ACM-18*, *ACM-35*, and *RC-251* displayed the least reduction of morphological parameters as well as the lowest mean tolerance score ( $\leq 2.5$ ) (Fig. 1; Table 3) under combined stagnant and salinity stress, therefore these genotypes could be depicted as highly tolerant whereas rest two genotypes namely *ACM-4*, and *ACM-11* were identified as tolerant genotypes (Fig. 2) as the heatmap showed that these genotypes had a lower reduction of growth traits as well as lower mean tolerance score ( $\leq 4.5$ ) under salt-submergence stress situations (Fig. 1; Table 3).

#### The results of the principal component analysis (PCA)

Loading plots of principal component 1 (PC1) and principal component 2 (PC2) of the PCA among 16 rice genotypes based on the percent reduction of morphological attributes under combined salinity with waterlogging stress are presented in Fig. 3. In the present investigation, the PC1 from the PCA analysis explained about 79.29% of the total variation present in these genotypes, whereas PC2 describes 9.38% and the two components (PC1 and PC2) together explained 88.67% of data variability. The clustering patterns via PCA revealed that the genotypes viz., *ACM-11*, *ACM-35*, *ACM-18*, and *RC-251* which exhibited the least reduction of growth traits as well as the lowest

mean tolerance score (Fig. 1; Table 3) under salinity-submergence stress, are grouped in the left lower side of the individual plot of PCA (Fig. 3). Therefore, those genotypes were identified as highly tolerant for salt-submergence stress. The genotypes namely *ACM-16*, *ACM-15*, *ACM-4*, *ACM-23*, *ACM-3*, *ACM-26* and *ACM-6* found in the left upper side of the biplot (Fig. 3) which showed lower growth reduction when combined salinity and submergence stress is imposed, and therefore these were regarded as tolerant and moderately tolerant genotypes based on the mean tolerance score (Fig. 1; Table 3). The genotypes grouped in the right lower side of biplot are *ACM-8*, *ACM-10*, *ACM-5*, and *Binadhan-7* (Fig. 3) depicted as susceptible genotypes because these genotypes demonstrated the maximum reduction of growth parameters under stress conditions having higher mean tolerance score (Fig. 1; Table 3). Besides, another genotype *ACM-29* which is grouped in the upper right side of the biplot exhibited lower growth reduction in waterlogging + NaCl treatment's, therefore it was regarded as a tolerant genotype according to the mean tolerance score (Fig. 1; Table 3).

#### Correlation and regression among different morphological attributes

To determine the most desirable traits for combined salt-submergence tolerance, Pearson's correlations were analyzed among different growth parameters of rice plants grown in saline condition with submergence stress (Table 6). The correlation matrix revealed a positive and significant correlation between all the growth traits in rice seedlings in submerged condition with both salinity levels. The highest positive and significant correlation was found between SFW and SDW ( $r = 0.986^{**}$ ,  $P < 0.01$ ), LL and RL ( $r = 0.946^{**}$ ,  $P < 0.01$ ), and RFW and RDW ( $r = 0.895^{**}$ ,  $P < 0.01$ ) at stagnant + 8dSm<sup>-1</sup> salinity level whereas the least significant and positive correlation was observed in RFW with SDW at two different salinity level viz., at 6 dSm<sup>-1</sup> salinity level ( $r = 0.524^{**}$ ,  $P < 0.05$ ), at stagnant 8 dSm<sup>-1</sup> salinity level ( $r = 0.556^{**}$ ,  $P < 0.05$ ), and SFW with RFW ( $r = 0.569^{**}$ ,  $P < 0.05$ ) at 6 dSm<sup>-1</sup> salinity level (Table 6). On the other hand, individual correlation of plants growth related traits, viz., LL ( $r = -0.979^{**}$  &  $-0.818^{**}$ ,  $P < 0.01$ ), RL ( $r = -0.858^{**}$  &  $-0.724^{**}$ ,  $P < 0.01$ ), SL ( $r = -0.951^{**}$  &  $-0.808^{**}$ ,  $P < 0.01$ ), SFW ( $r = -0.734^{*}$  &  $-0.650^{*}$ ,  $P < 0.05$ ), RFW ( $r = -0.613^{**}$  &  $-0.613^{**}$ ,  $P < 0.05$ ), SDW ( $r = -0.768^{**}$  &  $-0.811^{**}$ ,  $P < 0.01$ ), and RDW ( $r = -0.722^{**}$  &  $-0.676^{**}$ ,  $P < 0.01$ ) showed negative and moderately significant correlation with mean tolerance score in both salinity levels with complete submergence situation (Table 6). There was a maximum negative and significant correlation observed between mean tolerance score with RFW ( $r = 0.613^{**}$ ,  $P < 0.01$ ) at waterlogging + 6 dSm<sup>-1</sup> salinity level, RFW ( $r = 0.613^{**}$ ,  $P < 0.01$ ) at 8

**Table 6.** Correlation matrix among the morphological parameters under combined salinity (6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) and submergence stresses.

Traits	Treatment (T)	LL	SL	RL	SFW	RFW	SDW	RDW	MTS
LL (%)	T <sub>1</sub>	1	0.875**	0.946**	0.730**	0.668**	0.748**	0.744**	-0.979**
	T <sub>2</sub>	1	0.873**	0.871**	0.717**	0.637**	0.745**	0.644**	-0.818**
SL	T <sub>1</sub>		1	0.881**	0.741**	0.818**	0.735**	0.891**	-0.858**
	T <sub>2</sub>		1	0.851**	0.777**	0.830**	0.799**	0.815**	-0.724**
RL	T <sub>1</sub>			1	0.726**	0.707**	0.747**	0.801**	-0.951**
	T <sub>2</sub>			1	0.693**	0.779**	0.648**	0.706**	-0.808**
SFW	T <sub>1</sub>				1	0.569*	0.986**	0.794**	-0.734**
	T <sub>2</sub>				1	0.682**	0.844**	0.746**	-0.650**
RFW	T <sub>1</sub>					1	0.524*	0.895**	-0.603*
	T <sub>2</sub>					1	0.556*	0.847**	-0.613*
SDW	T <sub>1</sub>						1	0.773**	-0.768**
	T <sub>2</sub>						1	0.680**	-0.811**
RDW	T <sub>1</sub>							1	-0.722**
	T <sub>2</sub>							1	-0.676**

The treatments T<sub>1</sub> 6 dSm<sup>-1</sup> salinity level with complete submergence, and T<sub>2</sub> 8 dSm<sup>-1</sup> salinity level with complete submergence. MTS denote mean tolerance score. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg). \*\*\* correlation is significant at 1% level of probability, and \* correlation is significant at 5% level of probability.

dSm<sup>-1</sup> salinity level and SDW ( $r = 0.676^{**}$ ,  $P < 0.05$ ) at waterlogging + 8 dSm<sup>-1</sup> salinity level (Table 6). Among the growth traits, LL (%), RL, SL, RDW, and SFW exhibited higher association with the mean tolerance score at higher salinity level (submergence + 8 dSm<sup>-1</sup>) whereas RFW and SDW showed higher correlation with mean tolerance score at lower salinity level (submergence + 6 dSm<sup>-1</sup>) (Table 6).

The multiple correlation coefficient (R) also demonstrated the positive and significant correlation between mean tolerance score (dependent variable) and growth parameters viz., LL (%), RL, SL, RFW, SFW, RDW, and SDW (independent variables) under combined stress of salinity and submergence (Table 7). However, the explained variation of mean tolerance score by independent variables was higher at 6 dSm<sup>-1</sup> salinity level with complete submergence ( $R^2 = 0.959^{**}$ ,  $P < 0.01$ ) in comparison with 8 dSm<sup>-1</sup> with complete submergence ( $R^2 = 0.922^{**}$ ,  $P < 0.01$ ) (Table 7). The multiple regression lines were also analysed for each salinity level with submergence stress

considering the constant value (mean tolerance score) and regression coefficients for morphological parameters such as LL (%), RL, SL, RFW, SFW, RDW, and SDW (Table 8). The results of the regression coefficients revealed that at 6 dSm<sup>-1</sup> salinity with complete submergence conditions, only LL (%) had the negative and significant association with mean tolerance score ( $r = -0.054$ ,  $**P < 0.01$ ) whereas at 8 dSm<sup>-1</sup> salinity with complete submergence, several traits such as LL ( $b = -0.042^*$ ,  $P < 0.05$ ), RL ( $b = -0.170^*$ ,  $P < 0.05$ ), RFW ( $b = -1.049^*$ ,  $P < 0.05$ ), and RDW ( $b = -2.768^{**}$ ,  $P < 0.001$ ) exhibited a significant and negative association with the mean tolerance score (Table 8). Besides, SL, and SFW had positive-significant association with the mean tolerance score at 8 dSm<sup>-1</sup> salinity with complete waterlogging situations. However, RDW showed the non-significant association with the mean tolerance score in both salinity levels with waterlogging conditions (Table 8).

**Table 7.** Multiple correlation coefficients (R) between mean tolerance score (dependent variable) and morphological parameters (independent variables) at different salinity level (salinity 6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) with submergence stress.

Parameters	Treatments	
	6 dSm <sup>-1</sup> salinity level with complete submergence	8 dSm <sup>-1</sup> salinity level with complete submergence
R-value	0.989**	0.979**
R <sup>2</sup> -value	0.959**	0.922**

\*\*\* correlation is significant at 1% level of probability.

**Table 8.** Regression coefficients for different morphological parameters (independent variables) at different salinity level salinity (6 dSm<sup>-1</sup> and 8 dSm<sup>-1</sup>) with submergence stress and constant (dependent variable is mean tolerance score).

Traits	Regression coefficients	
	6 dSm <sup>-1</sup> salinity level with complete submergence	8 dSm <sup>-1</sup> salinity level with complete submergence
Constant (MTS)	9.011**	9.064***
%LL	-.054**	-.042*
SL	-.011 <sup>NS</sup>	.118**
RL	-.091 <sup>NS</sup>	-.170*
SFW	.141 <sup>NS</sup>	.275**
RFW	.366 <sup>NS</sup>	-1.049*
SDW	-.782 <sup>NS</sup>	-2.768***
RDW	-.116 <sup>NS</sup>	-2.626 <sup>NS</sup>

MTS denote mean tolerance score. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg).

\*\*\* correlation is significant at 1% level of probability, and \*\* correlation is significant at 5% level of probability.

**Measurement of genetic variability, heritability, and genetic advance among the morphological parameters**

A wide range of genotypic and phenotypic variance was observed in the agronomic traits of rice seedlings (Table 9). In the present study, the phenotypic co-efficient of variation (PCV) was higher than the genotypic co-efficient of variation (GCV) for all the traits. Among the traits, shoot fresh weight (100% & 100.9%), shoot dry weight (88.8% & 88.9%), and root fresh weight (86.8% & 87.1%) exhibited high estimates of GCV and PCV respectively whereas the lowest phenotypic GCV and PCV were observed for leaf live (%) (57.7% & 58.3%), and shoot length (58.7% & 58.8%) (Table 9). All the studied traits in this experiment exhibited high heritability ranging from 97.9% to 99.7% and the highest value was recorded in shoot dry weight (99.7%), shoot length (99.6%), and root fresh weight (99.3%) (Table 9). The highest genetic advance (GA) was found in leaf live (%) (82.9) and shoot length (63.5) whereas the lowest value was found in root dry weight (0.78) (Table 9).

**Discussion**

Due to the adverse climatic change, a considerable number of agricultural lands in the world are simultaneously affected by multiple environmental stresses such as salinity and waterlogging combination is most common among them. Waterlogging and salinization both have several individual or simultaneous physical, chemical, and biological effects on plant and soil which can influence plant growth potential negatively and can decrease the usability of the fields (Boling et al. 2007; Singh 2014b). The influential mode of salinity stress on the plant growth and development are well documented (Munns and Tester 2008), but the interaction effects of salt stress with waterlogging stress on plant growth are not fully revealed in rice crops like most of the plants (Colmer and Flowers 2008; Gill et al. 2019). In the present study, therefore an attempt has been made to examine the response of 16 rice genotypes

**Table 9.** Estimation of genetic parameters for the morphological parameters in 16 rice genotypes.

Traits	σ <sup>2</sup> g	σ <sup>2</sup> p	GCV (%)	PCV (%)	h <sup>2</sup> <sub>b</sub> (%)	GA
LL (%)	1656.0	1691.0	57.7	58.3	97.9	82.9
SL	955.0	958.3	58.7	58.8	99.6	63.5
RL	114.6	115.6	70.0	70.3	99.1	21.9
SFW	104.3	105.4	100	100.9	98.9	20.9
RFW	2.103	2.116	86.8	87.1	99.3	2.97
SDW	3.581	3.590	88.8	88.9	99.7	3.89
RDW	0.146	0.148	78.9	79.2	99.1	0.78

σ<sup>2</sup>g genotypic variance, σ<sup>2</sup>p phenotypic variance, GCV genotypic coefficient of variation, PCV phenotypic coefficient of variation, h<sup>2</sup><sub>b</sub> narrow sense heritability, and GA genetic advance. The variables include LL (%) leaf live (%), RL root length (cm), SL shoot length (cm), RFW root fresh weight (mg), RDW root dry weight (mg), SFW shoot fresh weight (mg), and SDW shoot dry weight (mg).

grown under combined salinity and submergence stresses considering different morphological parameters.

The submerged plants are very sensitive to salinity, particularly at the early growth stages (Barrett-Lennard 2002). Waterlogging interrupts plant growth in two ways, directly by reducing the physiological activities and indirectly by decreasing the availability of essential nutrients with reduced aeration around the root zone whereas salt stress affects plant growth by enhancing the osmotic potential of the soil solution as well as by depleting the soil structure (Singh 2017). The results of our study revealed that the growth and development of rice seedlings in all genotypes are severely affected under combined stress of salinity and submergence (Table 2) however, some genotypes namely *ACM-18*, *ACM-35*, *RC-251*, *ACM-11*, *ACM-23*, and *ACM-4* displayed their ability to minimize combined stress-induced toxicity and maintain the higher value of growth parameters viz., higher live leaves (%), high root and shoot length, and high plant biomass production under stressed conditions in compared to other genotypes (Table 3). The ability of roots to uptake water with nutrients from the soil environment is depleted under salinity stress which may also disarrange many important physiological and biochemical processes in plants such as photosynthesis, redox homeostasis, and toxic ion assimilation in plant cells (Motos et al. 2017). Moreover, when salinized plants expose to waterlogging conditions, normal root function get damaged and the selectivity for  $K^+$  over  $Na^+$  is declined (Barrett-Lennard 2003), and thus the concentrations of  $Na^+$  and  $Cl^-$  considerably increased in the shoot of plants (Marcar et al. 2002; Araki 2006). When root parts become injured, shoot growth is impeded due to the less uptake of essential nutrients like  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , etc. through symplastic xylem loading in the root (Läuchli and Grattan 2007). In the present investigation, the root length, and shoot length was reduced in all genotypes with the increasing of salinity level under submergence condition (Table 5). In line with our results, several other researchers also manifested the negative effects of combined salinity and submergence stress with lower root and shoot growth in tomato (*Solanum lycopersicum* L.) (Horchani et al. 2010), barley (*Hordeum vulgare* L.) (Zeng et al. 2013) and rice (*Oryza sativa* L.) (Prusty et al. 2018). On the contrast, the genotypes viz., *ACM-18*, *ACM-35*, and *RC-251* showed the highest root and shoot length under salt stress coupled with waterlogging condition even at 10 days in the late vegetative stage whereas susceptible *ACM-23*, *ACM-5*, and *Binadhan-7* could not withstand combined salinity and submergence stress and thereby had lowest growth of root and shoot (Table 4). The higher root and shoot growth of the tolerant genotypes can be explained by the ability to maintain sufficient root aeration with the adaptation

of morpho-physiological mechanisms under salt-submergence stress (Colmer et al. 2014). Moreover, tolerant genotypes might consume excessive photo-assimilates and stored carbohydrates for rapid elongation of shoots by cell division and cell elongation as well as by photosynthetic fixation of  $CO_2$  when faced submergence (Anandan et al. 2015; Jackson and Ram 2003). Singh et al. (2001) also observed maximum root length in tolerant genotypes compared to sensitive one's along with less adverse effects on root volume and porosity in tolerant genotypes under submergence stress. On the other hand, the leaves of the susceptible genotypes usually senesced and depleted and recovered more slowly because of slower shoot elongation and lower leaf formation under submergence in saline water (Singh et al. 2014a). However, shoot growth usually demonstrated more susceptibility than root growth in both saline and submergence situations (Omisun et al. 2018; Fukao et al. 2019). The toxicity of salt stress occurs primarily in the aged leaves because  $Na^+$  and  $Cl^-$  accumulate in the transpiring leaves for long-time leading toxicity on leaves which ultimately reduces leaf live (%) of plants (Amirjani 2011). Besides, the salinized plant faced pervaded sunlight during submergence causing the reduction of the leaf chlorophyll content which sometimes causes the death of many leaves (Afrin et al. 2018). The results of this study revealed that the leaf live (%) was significantly declined in rice seedlings under combined stressed conditions compared to control condition plants but identified highly tolerant genotypes namely *ACM-3*, *ACM-35*, and *RC-251* minimized the salt-submergence induced toxicity and therefore, exhibited maximum live leaf (%) (Table 4), indicating that those genotypes could adopt faster recovery from stress-induced metabolic changes and might have the mechanism to maintain required photosynthesis for the synthesis of leaf chlorophyll content during combined submergence and salinity stress (Ella and Ismail 2006). On the other hand, sensitive genotypes viz., *ACM-5*, *ACM-6*, and *ACM-16* exhibited the higher reduction of leaf live (%) when faced salinity stress in waterlogging condition (Table 5). This is mainly because of continuous loss of oxygen in root atmosphere which exerts an adverse effect on respiration and photosynthesis of plants causes withering and death of active leaves (Yadav et al. 2018). Furthermore, the rapid degradation of leaf chlorophyll in sensitive genotypes under water reflected higher levels of leaf chlorosis as well as leaf senescence (Bui et al. 2019). Due to the reduction of the number of live leaves under stress conditions, the supply of carbohydrates and growth hormones to meristematic tissues badly impeded with the unusual photosynthetic process which ultimately decelerates the plant biomass production (Munns and Tester 2008). In the current study, the exposure of rice seedlings to com-



bined salt and submergence stress caused the significant reduction of shoot, and root biomasses which might be due to the inhibition of root and shoot growth (Table 5) as also previously observed in other plant species such as shrubby sea blight (*Suaeda salsa*), and birdsfoot trefoil (*Lotus corniculatus*) under combined salinity and waterlogging stress (Teakle et al. 2016; Song et al. 2011). The recorded plant biomass retrenchment in most of the genotypes probably because of conjunction of salt-induced osmotic stress and oxygen-deficient environment due to submergence in plant cells (Shani and Ben-Gal, 2005). Moreover, submergence stress considerably obstructs the generation of tiller number and other agronomic traits that collectively can reduce the fresh and dry weight of the plant (Afrin et al. 2018). Notably, the dry matter reduction was relatively higher in sensitive genotypes viz., *ACM-5*, *ACM-16*, *ACM-26*, and *ACM-29* under combined stressed conditions (Table 5), as also reported by Tatar et al. (2010), Kano-Nakata et al. (2011), and Talat et al. (2013) in rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) under salt stress or submergence stress. The maximum reduction of leaves and root dry weights of the sensitive genotypes might be due to the rapid death and decay of tissues with confined underwater photosynthesis in submergence stress situations (Winkel et al. 2013). On the contrast, tolerant genotypes namely *ACM-35*, *RC-251*, *ACM-4*, *ACM-23*, and *ACM-3* demonstrated the higher plant fresh and dry matter production even when exposed to combined salinity and submergence stress conditions (Table 4). The higher accumulation of dry matter in tolerant genotypes against combined stress might be due to the increase in culm dry matter content rather than leaf area or total leaf dry weight (Sarkar and Das 2003; Yin et al. 2010). Besides, the multiple stress-tolerant rice genotypes might adopt morpho-physiological mechanisms such as salt exclusion, salt reabsorption, root-shoot translocation, and leaf-to-leaf compartmentation through which they can exclude the accumulation of excessive salt on leaves in submerged conditions to maintain the normal cell metabolism and consequently plant achieve higher biomass production (Chen et al. 2018).

Several approaches exist for sorting out salt or submergence stress-tolerant genotypes such as standard evaluation score (SES) which is extensively used (Kranto et al. 2016). In the present study, the modified SES scoring system was employed to categorize the rice genotypes for salt-submergence tolerance at the late vegetative stage and there was a significant variation found in the mean tolerance score among the rice genotypes in terms of different growth parameters under combined stressed situation (Fig. 1; Table 3). This finding was in line with Ali et al. (2014) and Nishanth et al. (2017) who observed the substantial disparity in injury score among the rice

germplasm grown under salinity and submergence conditions in a separate experiment. In our study, rice seedlings exposed to 10 days of complete submergence in saline solution demonstrated several injury symptoms such as rolling and drying of leaves, brownish of leaf tip and stunted plant growth. However, tolerant genotypes *ACM-18*, *ACM-35* and *RC-251* considerably lessened the salt-submergence commenced visual toxicity symptoms and thereby exhibited a maximum value of growth attributes with lowest mean tolerance score (<2.5) (Fig. 1; Table 3) when combined stress is imposed. These genotypes might adopt a combination of anatomical, morpho-physiological, and metabolic strategies such as the formation of higher aerenchyma tissue in the nodal region, higher shoot elongation, restriction of chlorophyll degradation, less utilization of storage carbohydrates, more adventitious roots generation, the regulation of ion uptake, exclusion of excessive salt in plant parts and the increased activity of antioxidant defense system to withstand the hazardous effect of combined salinity and waterlogging stress conditions (Barrett-Lennard 2003; Carter et al. 2006; Yadav et al. 2018). On the other hand, some genotypes viz., *ACM-5*, *ACM-8*, *ACM-10*, and *Binadhan-7* displayed the maximum mean tolerance score with the greater reduction of morphological parameters in stressed conditions and therefore, these genotypes were depicted as stress-sensitive genotypes (Fig. 1; Table 3). Bharathkumar et al. (2015) and Afrin et al. (2018) also successfully separated the rice genotypes for salt-submergence tolerance based on the injury score under salinity and waterlogging stress in previous experiments. The findings of cluster analysis and PCA classification of rice genotypes produced similar results with the results obtained from the categorization of genotypes based on mean tolerance score in this investigation (Fig. 1 and 2). Ward's minimum-variance cluster analysis is the most frequently used exoteric scheme that can group genotypes from the diverse origin based on their similarities (Ravikiran et al. 2018). Besides, PCA can also classify the genotypes of similar categories together compared to dissimilar genotypes (Khatun et al. 2015; Kim et al. 2013). Furthermore, genotype versus variable biplot also can explore the strength and weaknesses of genotypes in a stress situation, which is fundamental for the selection of tolerant genotypes (Yan and Kang 2003). In the present study, both heatmap and PCA analysis clearly distinguished the tolerant rice genotypes from the sensitive genotypes considering the reduction (%) of growth attributes under salt-submergence stress (Fig. 1 and 2), similar with the findings of Sorkheh et al. (2012). In hierarchical cluster analysis, the highly tolerant genotypes viz., *ACM-4*, *ACM-35*, and *RC-251* were grouped in the same cluster (C) and similarly, these genotypes were also found in the right lower side together in the PCA biplot (Fig. 1

and 2). Those genotypes demonstrated normal growth and development when exposed to combined stress and had a higher value of growth traits in comparison with other genotypes (Fig. 3). The rice genotypes showing both salinity and waterlogging tolerance may be due to the adaptation of similar physiological mechanisms and co-expression of the related genes under stress conditions. On the other hand, sensitive genotypes (*ACM-5*, *ACM-8*, *ACM-10*, and *Binadhan-7*) also found in the same group both in PCA and cluster analysis which demonstrated the higher growth attributes reduction under stressed situation (Fig. 2 and 3; Table 5). The results of our investigation were also inconsistent with Rasel et al. (2020) and Chunthaburee et al. (2016) who successfully differentiated rice cultivars into distinct groups by PCA and cluster analysis in terms of salt tolerance considering agronomic traits. Moreover, several other researchers also previously used clustering and PCA analysis as useful tools for the classification of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) cultivars into different tolerance levels considering morpho-physiological traits under different stress conditions for instance salinity, submergence, drought etc. (Cha-um et al. 2012; Wijewardana et al. 2016; Siddiqui et al. 2017).

Correlation analysis is a novel method for exploring the relationships between the parameters and their principal components in phenotypic screening of genotypes under stress conditions (Dehbalaei et al. 2013; Sun et al. 2013). In the present study, morphological attributes such as LL (%), RL, RFW, RDW, and SFW showed the highest negative correlation and association with mean tolerance score under combined stress situation (Table 6) reflecting that tolerant genotype (having lower mean tolerance score) exhibited a higher value of those growth attributes in stressed condition, similar with the findings of Rasel et al. (2018). Thus, these traits might be used as the best descriptors to evaluate the tolerance level of rice genotypes as well as to find out the tolerant genotypes at the seedling stage under combined salinity and waterlogging stress in comparison to other growth parameters. Ali et al. (2014) also found the strongest and significant correlations between shoot length, and plant biomass with SES at the seedling stage of rice plants and used these traits as important selection criteria to find out salt-tolerant rice genotypes. Similarly, the genetic analysis also showed that the growth parameters viz., LL, RL, RFW, RDW, and SFW had the highest value for PCV (%), GCV (%), heritability (%), and genetic advance (Table 9) indicating that the phenotypic expression of these traits are less influenced by environment and therefore, selection can be done by using these traits for the identification of rice genotypes that can tolerate combined salinity and waterlogging stress condition simultaneously and the higher

value of these traits would facilitate better scope for the improvement of rice genotypes against multiple stress.

## Conclusion

A large variability was observed among the rice genotypes for all growth traits under combined salinity and waterlogging stress. Several parameters such as leaf live (%), root length, root fresh weight, shoot fresh weight, and root dry weight exhibited a strong and negative correlation with mean tolerance score reflecting their importance as useful indicators for the selection of tolerant rice genotypes in combined stress salinity and submergence. Rice seedlings showed the reduction of morphological parameters due to the interactive effects of salinity and waterlogging. However, some genotypes namely *ACM-18*, and *ACM-35* significantly minimized the stress-induced reduction of growth attributes likewise tolerant check *RC-251* and therefore, those genotypes were depicted as highly tolerant. The results of clustering and PCA also revealed those genotypes in the same group. Besides, *ACM-11*, *ACM-23*, and *ACM-14* also performed very well and demonstrated the higher value in agro-morphological assays under stress situations. Therefore, the genotypes viz., *ACM-18*, *ACM-35*, *ACM-11*, *ACM-23*, and *ACM-14* could be used as a potential donor of *Saltol* and *Sub1* gene as well as in marker-assisted backcrossing for the development of salt-submergence tolerant high-yielding rice variety.

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