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Genetic diversity based on osmotic stress tolerance-related morpho-physiological traits and molecular markers in traditional rice cultivars

Shamsun Nahar¹ · Lipika Lahkar¹ · Md Aminul Islam² · Debanjali Saikia¹ · Zina Moni Shandilya¹ · Lakshminarayana R. Vemireddy³ · Lingaraj Sahoo⁴ · Bhaben Tanti¹

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Abstract

Osmotic stress is one of the major global constraints for the rice cultivation, especially in the rainfed cropping system practiced in Assam, India. Hence, exploration of the discrepancy in functional along with germplasm structure among the traditional rice cultivars is needed. Morpho-physiological traits induced by osmotic and inter simple sequence repeat (ISSR) markers were considered to survey genetic array in growth chamber trials. Twenty one traditional rice cultivars from different agro-climatic regions of Assam, previously used in our laboratory were used in this study along with *Sahbhagi* and *IR64* as control that revealed a high differentiation in phenotypic performance under osmotic stress. On the basis of standardized osmotic tolerant related morpho-physiological parameters, all the rice cultivars could be assembled into five clusters. The cultivars included in a single group revealed almost identical osmotic tolerance levels. The rice cultivars from different agroclimatic zone viz., upper Brahmaputra valley, lower Brahmaputra valley and hill Zones revealed highest genetic diversity as judged by Jaccard coefficient matrix with polymorphism information content (PIC) 0.30. Furthermore, rice cultivars were observed to be constituted into five major clusters depending on ISSR markers that were relatively congruous to the agro climatic regions and specific traditional cultivation practices. The mantel test of the rice varieties between morpho-physiological and ISSR markers exhibited low correlation. The variation observed in morpho-physiological traits associated with genetic diversity has paved a potentiality to design osmotic stress resistant varieties in future breeding programme.

Keywords Genetic diversity · ISSR markers · Morpho-physiological traits · Osmotic tolerance · Traditional rice of Assam

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✉ Bhaben Tanti
btanti@gauhati.ac.in

- ¹ Department of Botany, Gauhati University, Guwahati, Assam 781014, India
- ² The Energy and Resources Institute, IHC Complex, Lodhi Road, New Delhi 110003, India
- ³ Department of Genetics and Plant Breeding, S.V. Agricultural College, Acharya NG Ranga Agricultural University (ANGRAU), Tirupati, AP 517502, India
- ⁴ Department of Bioscience and Biotechnology, Indian Institute of Technology Guwahati, Guwahati, Assam 781039, India

Introduction

Osmotic stress is a serious problem, especially for the rainfed rice cultivation. Rice is a major staple food for most of the South and Southeast Asian countries. Among the various abiotic stresses, osmotic stress is one of the major constraints that restrict the growth and productivity of rice. In some state of India, severe osmotic stress due to drought can cause as much as 40% yield loss. Water scarcity inhibits photosynthesis, constrain growth, and of course reduce productivity. Due to rapid climate change and increasing global temperature, study of various detrimental stresses could reveal significant impact in near future. For the alleviation of osmotic stress damage, identification of osmotic tolerant rice varieties would serve as a key way in future breeding programme. Availability of genetic diversity among the germplasm is highly relevant in the selection of osmotic tolerant variety. Both morpho-

physiological characteristics and molecular markers could be used for the evaluation of genetic variability (Lahkar and Tanti 2018; Lahkar et al. 2019). To study the diversity among the varieties, phenotypic characters are the convenient aids as a preliminary assessment. A few literatures available that wild rice germplasm undoubtedly provide immense genetic variations. However, genetic diversity may not always be mirrored by the phenotypic variation due to complex genetic nature and environmental influences, thereby, combination of more parameters would be useful for better prediction of genetic diversity in breeding programs (Sun et al. 2015; Shandilya and Tanti 2019).

Molecular markers were predominantly used to study the natural germplasm diversity of different species. It might be due to the markers which could directly characterize organism; but they are not affected by environment. A number of PCR-based markers are available to study genetic diversity, among these, inter simple sequence repeats (ISSR) are predominantly used to study genetic diversity due to its high polymorphisms, repeatability and simplicity, which does not require the sequence information. Along with this, molecular markers could detect specific targeted position of genome instead of a whole genome sequence. Thus phenotypic characters and molecular markers together serve as a significant tool for evaluation and utilization of genotypes, genetic diversity, genetic resources and breeding programs. In the present investigation, 21 collected traditional rice cultivars from different agro-climatic regions of Assam, India was surveyed to assess genetic diversity and a comparative analysis were carried out based on osmotic tolerance inducing morpho-physiological parameters and ISSR markers (Sun et al. 2015; Pradhan et al. 2019).

Material and methods

Plant materials, growth conditions and morpho-physiological measurements

Twenty one traditional rice varieties previously used in our laboratory were used for this experiment (Nahar et al. 2018a, b). Additionally, two hybrid varieties i.e., *Sahbhagi dhan*' (osmotic tolerant) and '*IR64*' (osmotic sensitive) were used as controls throughout the experiments (supplementary table S1).

For morpho-physiological analysis, rice kernels were grown for 2 weeks on ½ MS solid medium impregnated with 15% and 20% PEG-6000 to impose artificial osmotic stress. The cultures were allowed to grow under controlled conditions (light/dark regime of 16/8 h at 25/20 °C and relative humidity of 60–70%) with three replications.

Osmotic tolerant-related morpho-physiological traits were detected including germination index, root-shoot length, fresh

- dry weight, chlorophyll and relative water content (AOSA 1983; Kapoor and Pande 2015; Nahar et al. 2018a). Based on the morpho-physiological responses under osmotic stress, cluster analysis was done to distinguish all the experimental rice varieties and grouped into different clusters on the basis of osmotic tolerability (Gregorio et al. 1997).

Data analysis

Cluster analysis and correlation were analyzed for 23 experimental rice based on similarity indices of quantitative parameters using XLSTAT version 2017 packages. Similarity matrix was generated using Euclidean distance among all rice accessions using Ward's Minimum variance method of hierarchical clustering technique (Lahkar and Tanti 2017).

A total of 11 osmotic stress related morpho-physiological quantitative markers scored for the experimental rice under osmotic stress were analysed using analysis of variance (ANOVA) followed by Duncan's post hoc statistical tools for hierarchical cluster analysis and principal component analysis (PCA) using SPSS (version 22). A scatter plot was obtained from the mean values of the eleven traits across all the test rice varieties (Ray et al. 2013).

ISSR marker based diversity analysis

Fresh tender leaves of each cultivar were collected and genomic DNA was extracted using CTAB method (Doyle and Doyle 1987); quantified by a nanodrop and 0.8% agarose gel. PCR amplification was done initially with a panel of 12 ISSR primers for screening and based on the outcome; eight primers were finally screened to study the genetic diversity. PCR amplification was carried out for ISSR analysis using a panel of seven ISSR primers obtained from Operon Technologies Inc., CA, USA. ISSR-UBC 814 (5' CTCTCTCTCTCTCTCTCTA3'); ISSR-UBC 818 (5' CACACACACACACACAG 3'); ISSR-UBC 836 (5' AGAGAGAGAGAGAGAGYA 3'); ISSR-UBC 840 (5' GAGAGAGAGAGAGAGAYT3'), ISSR-UBC 843 (5' CTCTCTCTCTCTCTCTCTRA3'); ISSR-UBC 848 (5' CACACACACACACACARG 3'); ISSR-UBC 852 (5' TCTCTCTCTCTCTCTCRA 3') and ISSR-857 (5' ACACACACACACACACYG 3') primers were used in this experiment.

PCR amplification was performed in 25 mm³ containing 50 ng of DNA template, 2.5 mm³ 1X assay buffer, 1 U *Taq* DNA polymerase, 0.2 mM dNTPs, 1.5 mM MgCl₂ and 5 pmol of primers using a Thermal Cycler (Bio-Rad, USA). PCR was conducted with 94 °C for 5 min followed by 35 cycles of 94 °C (30 s) / 42 °C (45 s) / 72 °C (45 s), and a final extension at 72 °C for 10 min. Separation of amplification product was carried out in 1.5% agarose gel.

Genetic diversity analysis

The ISSR bands were scored manually and converted to binary data as ‘1’ (band present) or ‘0’ (band absent). Only distinct and unambiguous bands showing polymorphism were considered. Various band feature such as % polymorphism, and PIC (polymorphic information content) values for each marker were calculated (Roldan-Ruiz et al. 2000).

The pair-wise genetic similarities among cultivars were calculated from ISSR marker data using Jaccard’s coefficient (Jaccard 1908). The resulting similarity matrix data was used to construct the dendrogram using UPGMA method and the analysis was performed using NTSYSpc version 2.2 (Rohlf 2000). UPGMA based on Euclidean distance (osmotic stress tolerant morpho-physiological characters) and ISSR based genetic distances using neighbour-joining trees were prepared to study phylogenetic analysis. 1000 replications were carried out for evaluation of bootstrap percentage.

Finally, Mantel test was performed to study the osmotic stress coefficient of correlations between morpho-physiological traits (Euclidean distance matrix) and ISSR based genetic distance matrix (Nei’s genetic distances) (Mantel 1967; Nei 1987).

Results

The rice seeds were germinated and plants were grown on ½ MS medium supplied with 15% and 20% PEG-6000 for 2 weeks to induce osmotic stress in growth chamber and the effect was clearly visible. The growth of rice plants were gradually decreased with the increase of PEG-6000 concentration. A decreased growth rate was observed in 15% PEG-6000 while no growth was observed in 20% PEG-6000 treated plant as compared with control.

Analysis of morpho-physiological traits variation

Osmotic stress restricted the growth of almost all the traditional rice used in this experiment, yellowing and wilting were the main visually observed variation of leaves in the treated plants. The results of the osmotic stress of 23 rice varieties for eleven different morpho-physiological traits were recorded. The standard evaluation scores (SES) were used for screening the rice cultivars for clustering on the basis of osmotic stress related morpho-physiological parameters. This scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. SES analysis discriminated all the traditional rice cultivars as highly tolerant (8 cultivars), moderately tolerant (10 cultivars) and 3 cultivars as susceptible. The identified 8 osmotic tolerant rice varieties were SN03 (bora), SN04 (prosad bhog), SN05 (kola joha), SN06 (helash bora), SN08 (salihoi bao), SN12 (kola amona), SN20 (ronga

bora) and SN21 (sok-bonglong). The pattern of clustering was found to be identical with the previous report where drought stress was imposed in hydroponic conditions. Therefore, it was seemed that osmotic stress imposed in solid culture used in this investigation was in concordance with the hydroponic culture of the previous report (Nahar et al. 2018a). However, high variations in morpho-physiological parameters were revealed for most of the experimental rice cultivars in the analysis of variation. These variations indicate that there was a high degree of diversity among rice varieties under osmotic stress condition for phenotypic and physiological traits.

Cluster analysis

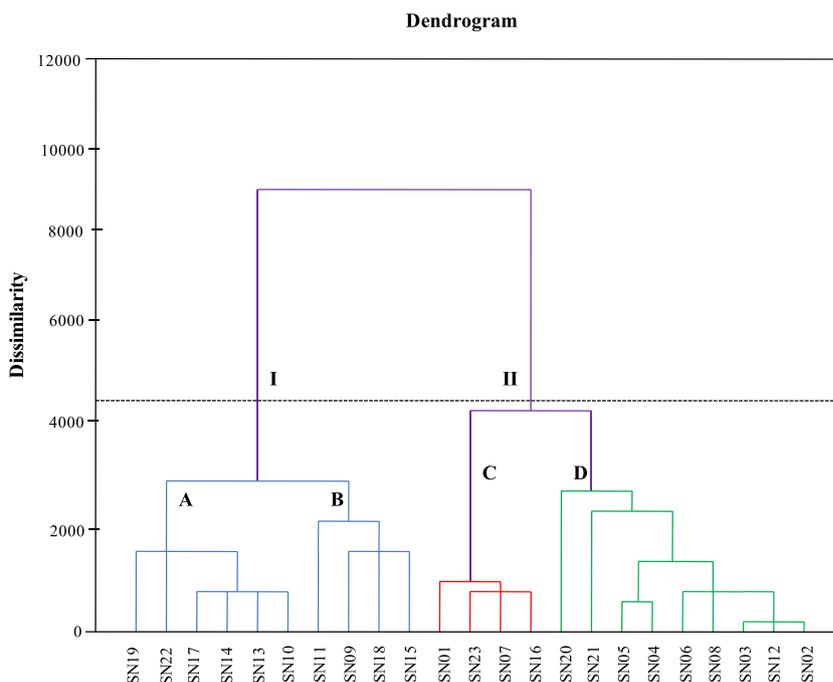
Cluster analysis was done based on distance matrices and dendrogram was constructed that revealed two major clusters; 10 rice cultivars in cluster - I and 13 cultivars included in cluster - II (Fig. 1). Cluster - I was further divided into group-A comprising 6 cultivars and group-B with 4 cultivars which showed moderately tolerant responses based on similarity in morpho-physiological characters. On the other hand, cluster-II further divided into two different groups; group-C included 4 sensitive rice cultivars while group-D included 9 tolerant rice varieties, respectively. The cluster analysis obtained from SES and distance matrix method divided all the rice varieties into three major groups viz., tolerant, moderately tolerant and sensitive (Nahar et al. 2018a).

Principle component analysis (PCA)

The principal component analysis (PCA) was carried out to investigate the various traits that played a key role in phenotypic diversity among the rice varieties (Supplementary Table S2). Four principal components (PCs) were observed; their Eigen values were found to be more than 1 (Fig. 2). While PC1, PC2, PC3 and PC4 contributed variations of 28.95%, 23.28%, 15.53% and 10.19%, respectively with cumulative contribution of 77.94% variations. It was revealed that the first six PCs jointly accounted for 91.12% (> 90%) of the total variations among all the studied varieties (Fig. 2; supplementary table S3). Out of all, eleven morpho-physiological traits revealed notably major contribution to PC1.

The initial number of factors was found to be identical as the number of variables used in the factor analysis. Initial Eigen values were the variances of the factors, since on the basis of correlation matrix the factor analysis was conducted, the variables were standardized that means each variable had a variance of 1, and the total variance was equal to the number of variables used in the analysis. The first factors showed the highest Eigen value of 3.184 while the next factor accounted for as much as the left over variance and so on. Percentage of total variance accounted for each factor which ranged from

Fig. 1 Dendrogram generated by Ward's Linkage method based on similarity in morpho-physiological traits for 23 traditional rice varieties growing in drought stress condition collected from Assam, India along with Sahbaghi and IR64



28.949–0.026, whereas the cumulative percentage of variance accounted for the current and all preceding factors starting from 28.949% among the entire variance. Extraction sums of squared loadings values in this panel of the table were calculated in the same way as the values in the left panel, except that here the values were based on the total variance, which was found to be identical with the Eigen value (supplementary Table S3; Fig. 2).

Two-dimensional principal component analysis

Two-dimensional PCA was carried out for 23 rice varieties with the 11 different morpho-physiological markers. The

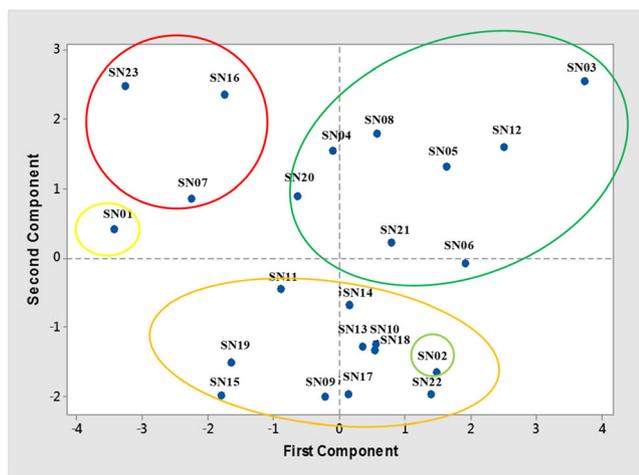
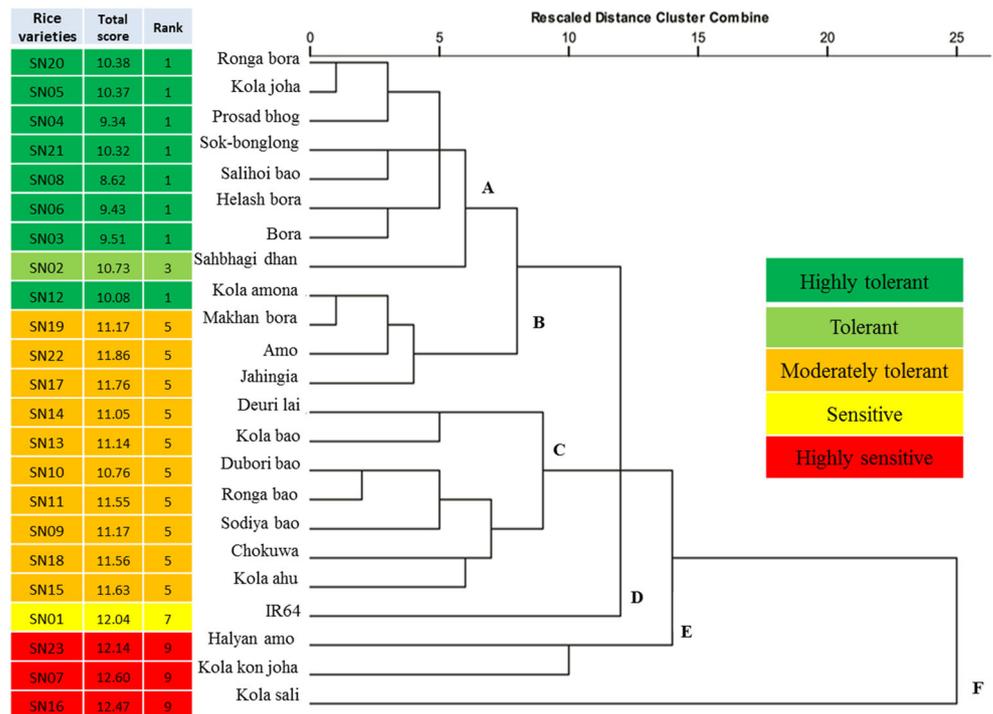


Fig. 2 Graphical representation of the total variance of morpho-physiological markers (extraction method: principal component analysis)

PCA and cluster analysis were found to be mostly correlated. Five different groups were revealed from two dimensional PCA analyses, of this 8 were highly tolerant cultivars in group I. Group II and III were merged where group II included 1 tolerant and group III included 10 moderately tolerant cultivars. Further, group IV consist of one sensitive cultivar. On the other hand, Group V encompassed 3 highly sensitive rice cultivars (Fig. 2). This result was identical to the cluster analysis generated by Ward's Linkage method.

The factor analysis is a useful tool for successful selection of rice in breeding program (Khorasani et al. 2011; Mostafavi et al. 2011). In the present study a UPGMA dendrogram was presented which separated the 23 rice cultivars into six major groups (Fig. 3). Cluster A contained 8 varieties which were identified as highly osmotic stress tolerant cultivars. Conversely, the cluster B and C altogether comprised of 11 varieties revealing moderate osmotic stress tolerance, except SN12 which was also scored as osmotic stress tolerant rice. Cluster D included only *IR64*, a well known as osmotic stress sensitive rice while, cluster E and F comprised of 3 highly osmotic stress sensitive varieties. Depending on morpho-physiological traits, a group of 8 osmotic stress tolerant rice varieties were recognised in cluster A that includes SN02 (*Sahbhagi dhan*), SN03 (*bora*), SN04 (*prosadhog*), SN05 (*kola joha*), SN06 (*helash bora*), SN08 (*salihoi bao*), SN12 (*kola amona*), SN20 (*ronga bora*) and SN21 (*sok-bonglong*). A remarkable similarity was found in between dendrogram constructed and the 2D scatter plot (Fig. 2). It was revealed that group A had genotype *Sahbhagi dhan* and other closely related tolerant

Fig. 3 Dendrogram showing clustering pattern based on euclidean distance from drought tolerant-related morpho-physiological data



varieties based on morpho-physiological traits. This result prevailed that the morpho-physiological traits would serve as a significant preliminary aid for improvement of grain yield under critical temperature and low irrigation conditions in future breeding program of osmotic stress resistant varieties. Moreover, these varieties can be used as a superior cultivar for better selection in future rice development program.

Principal coordinate analysis of morpho-physiological characters

The goal of PCO analysis is reducing the number of dimensions that describe a complex system from its complete set to a lower-dimensional representation which contains the most important data characterizing that system. In PCO analysis, the first principal coordinate axis accounted for the maximum variance of total variance, which separated the 11 tolerant rice varieties along with *Sahbaghi dhan* from the rest (Fig. 4). The second coordinate included other sensitive and moderately tolerant rice varieties.

Correlation coefficients among morpho-physiological parameters

The Karl Pearson’s correlation coefficients for different morphological and physiological parameters showed variations in correlation among the varieties (supplementary Table S4).

Osmotic stress tolerance indices related to parameters used in the experiment showed significant correlations. The shoot length had significantly positive correlations with shoot fresh weight ($r = 0.690, p < 0.05$) and RWC ($r = 0.516, p < 0.05$). Moreover, shoot fresh weight was positively correlated with relative water content ($r = 0.531, p < 0.05$). Moreover, Chl.-a and total chl. ($r = 0.842, p < 0.01$).

Genetic diversity by ISSR markers

Genetic diversity study of the 23 traditional rice cultivars were assessed using ISSR markers. DNA amplification results revealed the presence of a wide diversity of amplified bands. From all the rice accessions, polymorphisms was recorded in case of every primer indicating there may be presence of insertion/deletion mutation at the priming sites of amplified region. The presence of polymorphisms varied with the nature of primer used. (Reddy et al. 2002). Well-resolved and distinct bands for each ISSR primer were scored as present and absent using 1 and 0 numerical scale (Fig. 5a-b).

ISSR marker based genomic DNA amplification in all the 23 rice varieties yields a total of 62 scored amplified fragments, among this, 49 bands were polymorphic and rest were monomorphic (Supplementary table 4). Primer UBC-836 showed maximum numbers of bands whereas, UBC-852 and UBC-857 detects minimum numbers of bands. The numbers of amplified bands ranges from 5 to 11 with varied size of amplicon (150–1000 bp) in ISSR

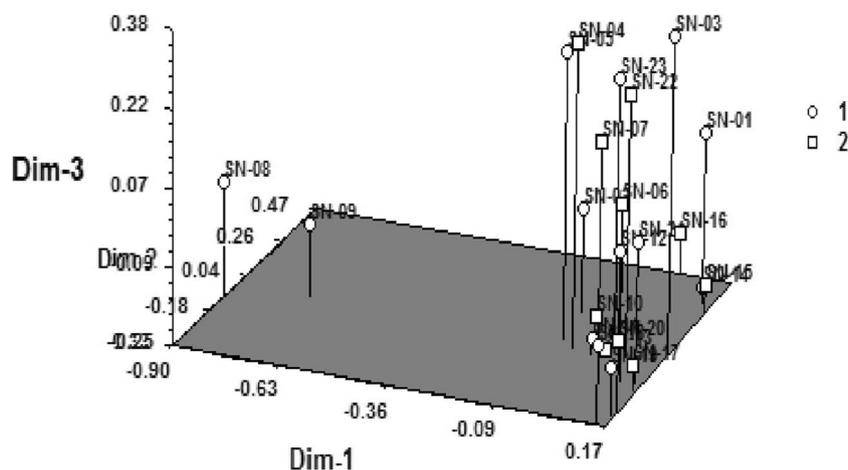


Fig. 4 Three dimensional PCO (principal coordinate analysis) scaling of 23 varieties of rice using morpho-physiological markers; 1 – *IR64*, 2 – *sahbhagi dhan*, 3 – *bora*, 4 – *prosad bhog*, 5 – *kola joha*, 6 – *helash bora*, 7 – *kola kon joha*, 8 – *salihoi bao*, 9 – *sodiya bao*, 10 – *dubori bao*,

11 – *ronga bao*, 12 – *kola amona*, 13 – *kola bao*, 14 – *deuri lai*, 15 – *kola ahu*, 16 – *kola sali*, 17 – *jahingia*, 18 – *chokuwa*, 19 – *makhan bora*, 20 – *ronga bora*, 21 – *sok-bonglong*, 22 – *amo*, 23 – *halyan amo*

analysis. Here, 49 (78.83%) polymorphic bands were recorded out of 62 amplified bands and each primer shared with an average of 9.87 polymorphic bands. The polymorphism percentage ranged between 50% (primer UBC 818) to 100% (primer UBC 836 and UBC 852). Primers having poly AG with YA at 3' produced highest number of amplified fragments (11 bands) while UBC-852 (poly TC with RA at 3') and UBC-857 (poly AC with YG at 3') generates minimum number of amplified fragments. Further, the average polymorphism information content (PIC) was 0.26 and the lowest and highest PIC value were 0.19 (UBC 848) and 0.36 (UBC 852), respectively. PIC value was related with the evaluation of marker ability to identify the germplasm. This suggested that all the primers used in this study were equally effective in determining polymorphisms and ISSR based PCR amplification produce reproducible amplification products.

A total of 49 polymorphic amplified fragments were used to evaluate the Jaccard's co-efficient and cluster analysis. Inter-variety genetic distance (D) ranged from 0.87 to 0.1 with an average value of 0.37. The highest similarity was between SN01 and SN08 and the lowest similarity was between SN04 and SN05. The details of similarity coefficient analysis have been given in supplementary Table S5.

Cluster analysis by molecular marker

The genetic distance tree made using cluster analysis by the UPGMA method in the NTSYS-pc showed three main divisions (A-C). In group A, four clusters were recorded. Cluster I comprised of 3 varieties (SN16, SN14, SN15); SN14 and SN16 showed more similarity value of 0.2 as compared to SN15 (0.16). Cluster II consisted of 2 varieties SN18 and SN01 with similarity matrix of 0.5. The cluster III consisted of three

varieties, namely SN10, SN11 and SN13. Cluster IV consisted of five varieties SN05, SN04, SN09, SN08, and SN07. Group B consisted of 7 rice varieties (SN20, SN19, SN02, SN17, SN06, SN03, SN12) with a range of 0.16–0.48 co-efficient values in the similarity matrix. Group C consisted of varieties SN21, SN22 and SN23. In group C, SN23, SN22 varieties showed similarity index of 0.24; SN21, SN23 varieties of 0.37 while 0.22 similarity index was found in case of variety SN21 and SN22 (Fig. 6a-b).

Using 8 ISSR primers, the genetic relationships among twenty one rice cultivars along with two control genotypes were tested. Neither all the selected 8 osmotic stress tolerant rice included in a single group. Interestingly, out of eight selected osmotic stress tolerant rice cultivars, four were found to be clustered in the same group (B) viz., SN03, SN06, SN12 and SN20 along with *sahbhagi dhan* (SN02), an osmotic stress resistant variety.

Mantel analysis among phenotypic and molecular markers

To establish the extent of correlations between morpho-physiological traits under osmotic stress and ISSR markers, mantel matrix test was carried out (Fig. 7). A low value of matrix correlation ($r = 0.13611$) was observed suggesting that no correlation was found between this two matrix's. Low correlation between morpho-physiological matrices and ISSR data revealed disagreement between phenotypic traits and molecular clustering. However, a positive significant correlation was observed among the replicas of morpho-physiological data sets.

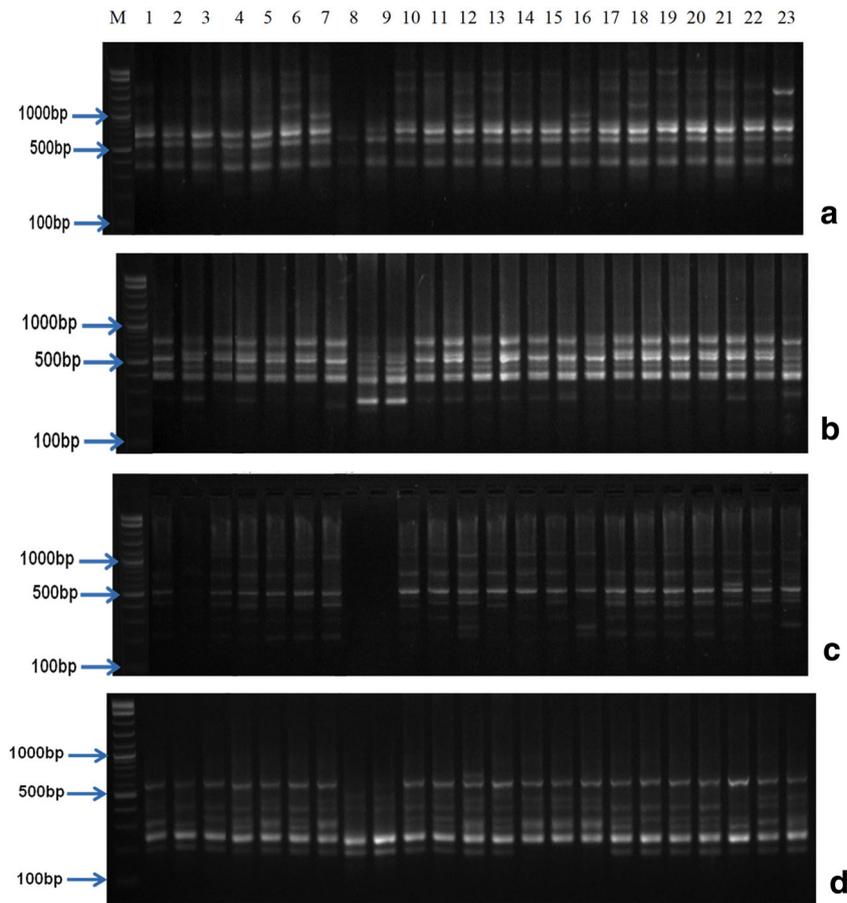


Fig. 5 **a** ISSR profiles generated from genomic DNA of 23 rice genotypes with primer **A.**UBC- 814; **B.** UBC-818; **C.** UBC-836; **D.** UBC-840. (**M** denotes the molecular weight marker; lane 1-*IR64*, lane 2-*sahbahgi dhan*; lane 3-*bora*, lane 4-*prosad bhog*, lane 5-*kola joha*, lane 6-*helash bora*, lane 7-*kola kon joha*, lane 8-*salihoi bao*, lane 9-*sodiya bao*, lane 10-*dubori bao*, lane 11-*ronga bao*, lane 12-*kola amona*, lane 13-*kola bao*, lane 14-*deurilai*, lane 15-*kola ahu*, lane 16-*kola sali*, lane 17-*jahingia*, lane 18-*chokuwa*, lane 19-*makhan bora*, lane 20-*ronga bora*, lane 21-*sok-bonglong*, lane 22-*amo*, lane 23-*halyan amo*). **b** ISSR

profiles generated from genomic DNA of 23 rice genotypes with primer **E.**UBC-843; **F.** UBC-848; **G.** UBC-852; **H.** UBC-857. (**M** denotes the molecular weight marker; lane 1-*IR64*, lane 2-*sahbahgi dhan*; lane 3-*bora*, lane 4-*prosad bhog*, lane 5-*kola joha*, lane 6-*helash bora*, lane 7-*kola kon joha*, lane 8-*salihoi bao*, lane 9-*sodiya bao*, lane 10-*dubori bao*, lane 11-*ronga bao*, lane 12-*kola amona*, lane 13-*kola bao*, lane 14-*deurilai*, lane 15-*kola ahu*, lane 16-*kola sali*, lane 17-*jahingia*, lane 18-*chokuwa*, lane 19-*makhan bora*, lane 20-*ronga bora*, lane 21-*sok-bonglong*, lane 22-*amo*, lane 23-*halyan amo*)

Discussion

Among the various abiotic stresses, osmotic stress is one of the major factors causing severe yield loss in most of the traditional rice cultivars, especially rainfed rice. To fight against damage induced by osmotic stress, identification of osmotic stress tolerant germplasm from natural gene pool would be a potential development. For successful development of well adapted abiotic stress tolerant plants, proper utilization of genetic resources, presence of genetic diversity in the germplasm is the prerequisite for breeding programme (Sharma et al. 2010). In this study, osmotic stress related morpho-physiological and ISSR markers characteristics were implemented among 23 rice varieties. This analysis was carried out to get a good perception of the genotypic diversity and recognised effective approach for breeding of osmotic stress tolerant traditional rice varieties.

Morpho-physiological characterization

Tolerance level of osmotic stress varies within and between the plant species that make it possible in the identification of stress tolerant accessions. Potential diversity had been estimated among 23 rice varieties that were collected from six different agro-climatic zones of Assam, India against eleven morpho-physiological traits. Three varieties were found to be susceptible at different degrees of osmotic stress induction that caused inhibition of growth, yellowing, wilting and even death of the plant (Sun et al. 2015; Kalita et al. 2018; Shandilya and Tanti 2020). Conversely, eight germplasm collected from various agro-climatic zones exhibited speculated level of tolerance, better adaption and resistance under osmotic stress treatment.

The dendrogram generated from the results of two different osmotic stress trials were concordant, further different

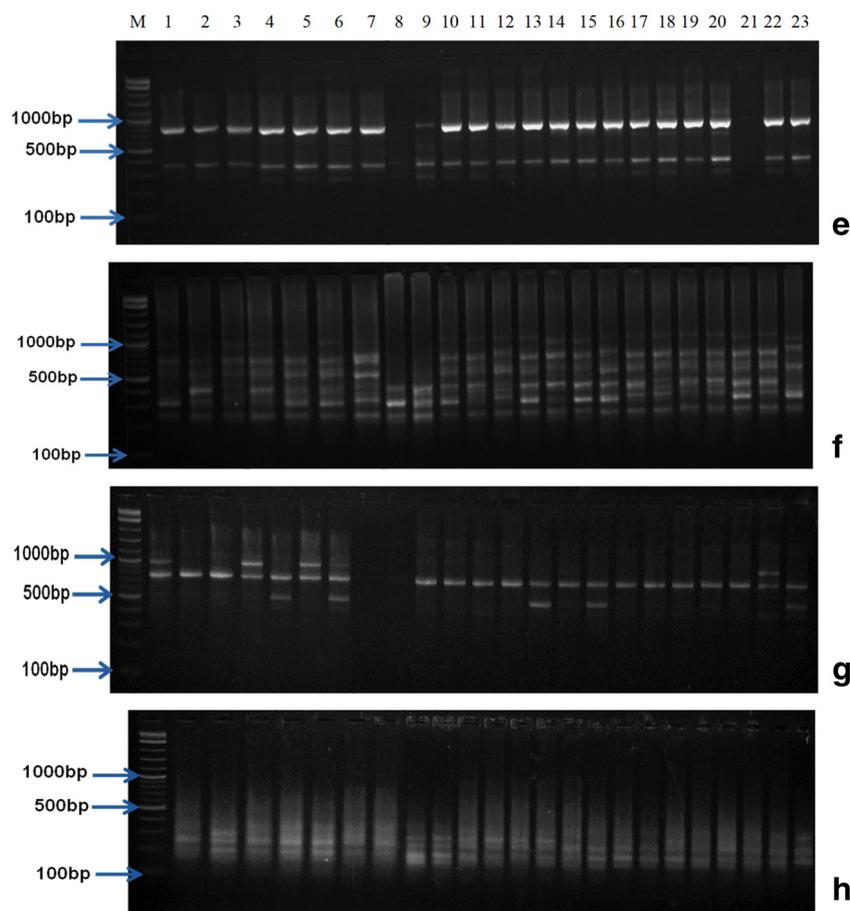


Fig. 5 continued.

varieties exhibited a diverse degree of osmotic stress tolerance against different characters indicating individual mechanisms of tolerance. In growth chamber conditions, osmotic stress caused evaporation, transpiration and wilting of leaves, root mortality rate drive and life span was gradually decreased in osmotic stress sensitive cultivars. Some of the osmotic stress sensitive germplasm root system showed withering. Various reports suggested that, the roots play a pivotal role in osmotic stress tolerance of plant and the growth of roots was more sensitive to stress than the growth of shoot.

Molecular characterization

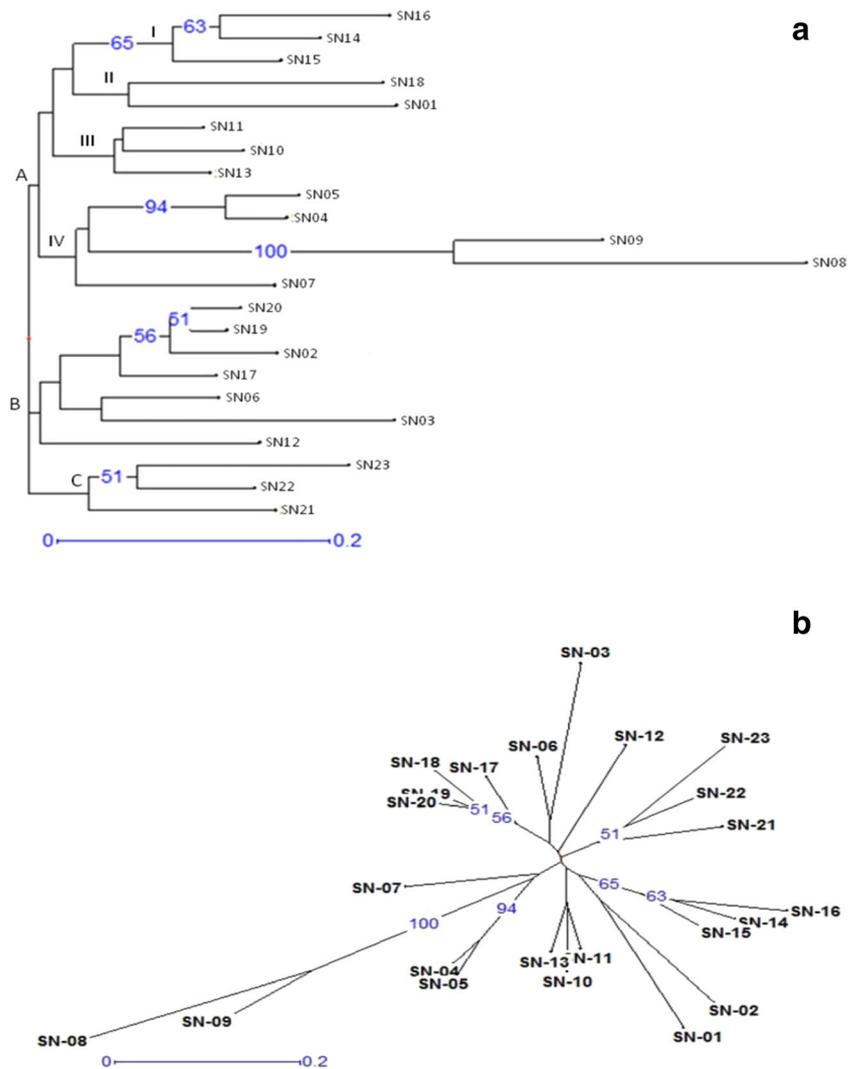
ISSR markers are widely used for molecular characterization of osmotic stress tolerance. In the present investigation, presence of a good number of ISSR alleles in each locus specified high potentiality of allelic abundance among the experimental germplasm, with an average of 7.75 alleles per locus (range between 5 to 11). In addition, ISSR markers exhibited high polymorphism levels (49 alleles, PIC = 0.26) since ISSR markers are presumed to be more conserved regions due to

its locations in gene (Crossa 1990; Khorasani et al. 2011; Mostafavi et al. 2011). PIC value is mainly used to study the potential of markers to identify the accessions. Here, in the maximum alleles per locus, PIC value obtained by ISSR markers was 0.36.

Analysis of comparative diversity between molecular and morpho-physiological data

Here in the mantel's test shows low but positive correlation between morpho-physiological traits under osmotic stress and molecular diversity. Various environmental and other factors may be attributed to the low level of correlation between the two matrices (Sun et al. 2015). For hierarchical classification, two independent patterns of clustering were used in the study of genetic diversity and clustering of identical group among a group of germplasm utilizing morpho-physiological parameter and molecular markers (Franco et al. 2001). Clustering of the experimental rice varieties were performed depending on osmotic stress related morpho-physiological traits using Euclidean distance matrix,

Fig. 6 a-b Phylogenetic tree of 23 rice varieties revealed by ISSR marker (bootstrap values per 1000 replicates)



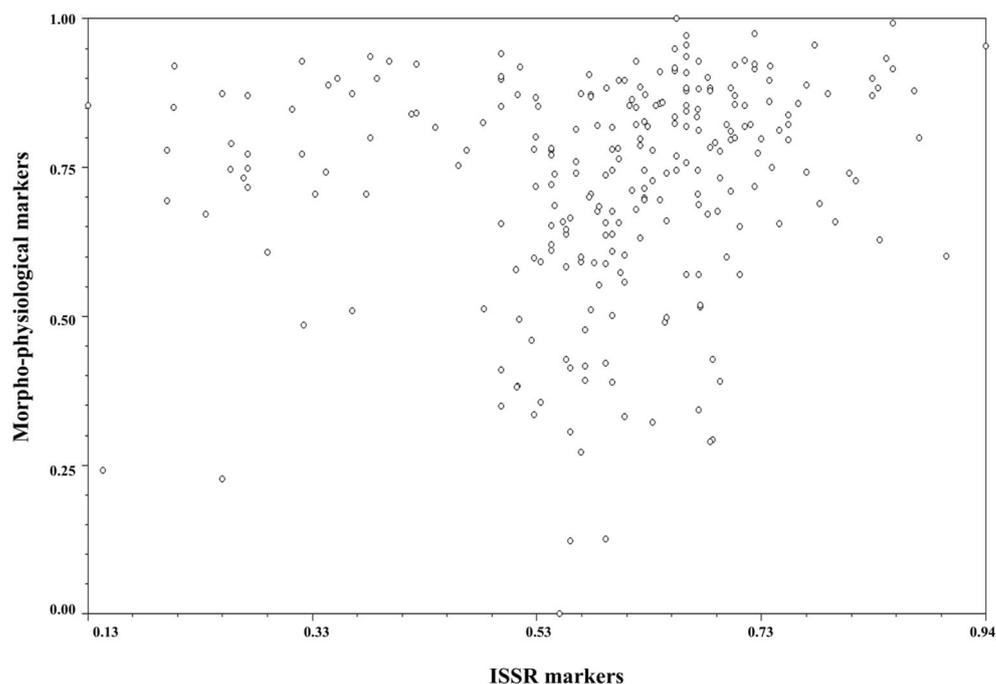
PCA and PCO analysis. Moreover, UPGMA and neighbor joining method were used to evaluate genetic similarities or dissimilarities of individuals and to construct ISSR based phylogenetic tree. The cluster from morpho-physiological and molecular data revealed that they have low consistency. The characterized rice germplasm were grouped according to the morpho-physiological adaptation made against osmotic stress; however, environmental factors influences their response towards the stress conditions since, a number of genes altogether regulate and promote the morpho-physiological characteristics. In contrast, ISSR markers are highly polymorphic that amplifies microsatellite core sequences as primers with a few selective nucleotides which could be efficiently used to closely related accessions. In this study, the morpho-physiological analysis of 23 rice varieties leads to formation of five clusters but ISSR based analysis grouped them into three clusters, which was not identical. A similar study on crop plants like rye grass,

durum wheat, sorghum, potato, etc. showed no correlation between morphological traits and molecular data (Sun et al. 2015). It can be suggested that the uneven marker distribution might lead to low correlation between the two matrices. Based on the results from morpho-physiological and molecular data of ISSR markers, it can be aided as a marker-trait association genetic study on rice cultivars in future plant improvement programmes.

Identification of osmotic stress tolerant and sensitive varieties

Ali (2008) proposed that segregating populations derived from the crosses between most diverse genotype pairs based on molecular markers and phenotypic traits would detect more informative polymorphic markers. This will be more beneficial to construct high density molecular linkage maps and QTLs compared to a

Fig. 7 Mantel correlogram comparing matrix of morphological descriptors with molecular markers



population derived from the most polymorphic potential parents based on phenotype only. In this experiment, from the osmotic stress tolerance induced morpho-physiological characters and F value integration, 8 efficient osmotic stress tolerant cultivars and 3 osmotic stress sensitive cultivars were identified among all the experimental cultivars that can be used for future breeding programs. These varieties can be used as osmotic stress specific variety since the results revealed similar outcome for all the data sets like cluster analysis generated by Ward's Linkage method, clustering pattern based on Euclidean distance matrices, PCA analysis and PCO analysis. By prevailing various obstacle, 8 excellent osmotic stress tolerant varieties with superior osmotic stress tolerant ability would have been recognised. This diverse gene pool of osmotic stress tolerant and sensitive varieties can be harnessed as a potent source for development of linkage mapping that could help in the detection of QTL related with osmotic stress tolerance.

Conclusions

In this investigation, significant information of various functional morpho-physiological traits and genetic variations, its relationship between and within genotypes were characterized among 23 rice cultivars that would be beneficial in maintaining diverse gene pool and in the improvement of potential

osmotic stress tolerant varieties for future rice breeding programs. In spite the fact, the morpho-physiological and genetic diversity study did not showed the similar description for the experimental rice varieties but there was a maximum proportion of identical results were obtained in osmotic stress condition. A few similarity between morpho-physiological and ISSR data was observed. The identified osmotic stress tolerant rice varieties in this experiment with varied genetic make-up could be used in gene mapping approach as potential parents for preparation of osmotic stress tolerant rice.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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