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Study of Genetic Diversity and Drought-Tolerance Characteristics of Few Rice Varieties Using Morphological and Molecular Markers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important cereal crops. Increasing rice production is constrained by various stresses and drought is one of the major limiting factors. For future food security, assessments of genetic resources are necessary. Indigenous varieties contain a high level

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of genetic diversity and can serve as potential genetic resources for improving yield, and resistance to various biotic and abiotic stress conditions. The present investigation was conducted in the Biotech Hub, Biswanath College of Agriculture, AAU, Biswanath Chariali during 2022-23. In this study, the genetic diversity of 27 indigenous rice germplasm using SSR markers was assessed. Out of the total 28 SSR markers screened, 17 were found polymorphic across twenty-seven genotypes with PIC values ranging from 0.076 to 0.499. The genetic diversity was estimated by the Jaccard dissimilarity coefficient. The phylogenetic tree, using unweighted neighbor-joining (UPGMA) drawn from the analysis divides 27 genotypes into 3 clusters. Nine genotypes were further characterized by exposure to drought stress compared to the control condition. Plants were grown in PVC pipes and subjected to drought by withdrawing water at 45 days after showing (DAS) for 25 days. A comparative study was done for a few morphophysiological parameters i.e. root length, root biomass, root-shoot ratio, root length density, chlorophyll content etc. It was observed that genotype Dehangi followed by N22 and Shahabhagi showed the best performance for all parameters under drought stress. This information will help in the selection of varieties with better root characteristics for drought tolerance in future breeding programs.

Keywords: Genetic diversity; markers; drought tolerance; root architecture.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food crop in India and many other countries that feeds half of the world's population. India is one of the centers for rice diversity, where both inter- and intra-specific levels of diversity have been identified [1]. Northeast India is the home to large indigenous rice varieties. A significant level of genetic diversity has been found in indigenous crop varieties that have been traditionally grown and maintained by farmers. These varieties can be used as potential genetic resources to increase production and tolerance to a variety of biotic and abiotic stress conditions.

Estimates indicate that more than 50% of the world's total rice is impacted by drought, significantly limiting rice production (Fukao et al. 2011; Bouman et al. 2005). A total of 34 million ha of rainfed lowland rice and 8 million ha of upland rice are affected by drought stress each year in Asia [2] In India, around 6.3 million ha of highland and 7.3 million ha of lowland are considered drought-prone zones [3]. Drought stress can impede floret initiation during the flowering, booting, and terminal periods. Additionally, spikelet sterility lowers grain weight and ultimately results in lower grain output. The crop growth stage, duration, and degree of water stress all affect the amount of grain production. [4], Kumar and Dwivedi, 2014). Drought phenotype is very complex and is linked to a number of morpho-physiological and biochemical characteristics. Numerous data exist suggesting a sizable variability in rice genotypes for different adaptation-related parameters during drought. In response to drought, root system architecture (RSA) is crucial to increase water intake [5]. The nodal roots which make up the rice root system differ in the lateral and vertical patterns of roots between rice cultivars. The capacity of a deep root system helps the plant to absorb or take out water from the deeper soil layer. According to Yoshida and Hasegawa [6] and O'Toole [7], plants with few and early tillers typically have deep root systems. Root depth and diameter were found positively correlated with plant vigor under drought stress. The thickness and penetrating power of rice roots vary genetically.

In this study, the diversity of some indigenous and improved rice varieties at both nuclear and organellar genome levels are evaluated with the help of the simple sequence repeat (SSR) marker. Since SSR markers have a high amount of polymorphism and can establish relationships between individuals even with a small number of markers, they are frequently utilized in investigations of genetic variation in rice. In addition, morphophysiological characteristics of a few indigenous rice emphasizing root and shoot architecture were evaluated in drought and control conditions. Current rice development programs have placed a strong emphasis on increasing the effectiveness of the rice root system in terms of moisture absorption and water use efficiency. In the upcoming years, root system research will likely become the most crucial component of rice breeding.

2. MATERIALS AND METHODS

2.1 Plant Materials

The investigation was conducted in the Biotech Hub, Biswanath College of Agriculture, AAU,

Biswanath Chariali during 2022-23. The materials for the present investigation consisted of a set of 27 rice cultivars which were used to study genetic diversity (Table 1).

2.2 SSR Analysis

DNA was extracted from leaves by following the CTAB method (CSHL protocols). In short 1-1.5 gm of leaves were grounded in 1.5 ml of CTAB buffer (Tris buffer, EDTA, 20% CTAB, Nacl, 1% polyvinyl pyrrolidone and Marcaptoethanol). After centrifugation, the aliquot was mixed with chloroform isoamyl alcohol. The aqueous phase was taken out and DNA was precipitated using isopropanol and dissolved in 50 ul of molecular-grade water. The DNA concentration and purity were estimated using spectral reading (A260/A280). Agarose gel electrophoresis was performed to judge the integrity of the isolated DNA of each rice genotype.

A sum of total 28 markers were designed for the experiment, out of which 17 were nuclear SSR and 11 were organelle SSR. The markers' list and sequences are given in the Supplementary Table 1. The PCR was performed using Emerald Amp® GT PCR Master Mix, forward and reverse primer (10 pM each), template DNA (100 ng) and adjust the total volume to 20 µl with water. The PCR temperature cycling conditions were, initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 45 sec. Denaturation, annealing and elongation were repeated 35 times. The final cycle was followed by 5 min extension at 72°C. The PCR product after the amplification was run on 1.8 % agarose gel in TAE (1X) buffer along with a 100bp DNA ladder. The gel was visualized in the Gel Doc EZ System (Bio-Rad) and the gel image was captured using the image lab (Bio-Rad). The amplified fragments were scored as band present (1) and absent (0).

The PIC value of each marker was calculated using the formula PIC= 1- [f2 + (1-f)2], where f is the marker frequency in the data set. The binary matrix based on the amplification pattern was used to gauge the similarity and diversity of sample sets based on the Jaccard dissimilarity coefficient.

$$d_{ij}=\frac{b+c}{a+(b+c)}$$

d_{ij}=dissimilarity between units *i* and *j*.
a=number of variables where x_i=presence and x_j=presence
b=number of variables where x_i=presence and x_j=absence,
c=number of variables where x_i=absence and x_j=presence

The genetic diversity was estimated by the Jaccard dissimilarity coefficient and phylogenetic tree was drawn by DARwin (https://darwin.cirad.fr/) using unweighted neighbor-joining (UPGMA).

2.3 Drought Stress

Out of 27 varieties that were listed in Table 1, Nine varieties were selected for the comparative study of root and shoot characteristics under both drought and control conditions. Those 9 varieties were N22, Shahabhagi, Dehangi, Maizubiron, Ranjit sub1, IR64, Basantabahar, Vandana and Luit. The investigation was conducted in a playhouse by using PVC (Poly Vinyl Chloride) tubes of 1 m in length and 20 cm in diameter (Fig. 1). The experiment was carried out in two sets containing 27 numbers of PVC pipes in each set up as each genotype is replicated thrice. A 4:1 mixture of soil and compost was created for the soil medium that

Table 1. List of 27 rice cultivars taken for the investigation

Sl No.	Variety Name	Sl No.	Variety name	Sl No.	Variety name
1	N22	11	Mashuri	21	Haccha
2	Shahabhagi	12	KarbiDhan	22	Sok jongthi
3	Dehangi	13	AborSali	23	Sok votung
4	Maizubiron	14	Baismuthi	24	Maguri
5	Ranjit Sub-1	15	Kola joha	25	Mala
6	IR64	16	Betguti	26	Manipuri joha
7	Basantabahar	17	RongaSali	27	Basantasali
8	Vandana	18	Moinagiri		
9	Luit	19	Sok Soi Soi		
10	Inglonkiri	20	Maibee		

was used to fill the PVC pipes. Five to six seeds were placed in each PVC pipe which was treated as one experimental unit. After germination and seedling establishment, three well-spaced (equally on all experimental units) seedlings were retained and others were discarded. Each entry was replicated thrice and plotted as per the "factorial RBD" experimental design. After 45 days of sowing one set up containing 27 pipes/seedlings of 9 genotypes was exposed to complete drought for 25 days by withdrawing water and the other set was watered regularly to maintain the moisture in the soil. After 25 days when the moisture percentage reached up to 2.5% in the stress plot, plants were uprooted and observations were recorded. Maximum root length(cm), Minimum root length(cm), Maximum Shoot length(cm), Root fresh weight(gm), Root dry weight(gm), Shoot fresh weight(gm), Shoot dry weight(gm), Root shoot ratio, Root length density, Total chlorophyll content, and Chlorophyll stability Index (CSI) were recorded in sample plants of each genotype. Maximum root length and minimum root length were calculated by measuring the distance (cm) from the collar region to the tip of the longest root and shortest root respectively. As with maximum root length, Maximum shoot length was calculated by measuring the distance between the plant's highest point and the stem's base (at the soil's surface) in centimeters. Root fresh weights and Root dry weight were taken by measuring the weight(gm) of the entire below-ground part of the plant just after harvesting and after oven drying respectively. The same protocol was followed for the Shoot fresh weight and shoot dry weight but here weight of the entire above-ground portion of the plant was taken. Root Shoot ratio was calculated from the ratio of maximum root length to the maximum shoot length and root length density is the length of roots per unit volume of soil. Total chlorophyll was measured as per the standard protocol given by Shoaf and Lium (1976). Formulae for chlorophyll estimation and Chlorophyll Stability Index are given below,

Chlorophyll a={12.7(A_{663})+2.69(A_{645})}x(V/1000W) mgg-1fw.

Chlorophyll b={22.9(A_{645})+4.68(A_{663})}x(V/1000W)mgg-1fw.

Total chlorophyll content={20.2(A_{645})+8.02(A_{663})}x(V/1000W) mgg-1fw

Where, V=Final volume of extract (ml), W=weight of leaf sample taken, fw= Fresh weight of leaf sample(g), A_{645} & A_{663} are the optical density(OD) values at 645nm & 663nm wavelength of light respectively.

CSI= (Chlorophyll content of the treated leaf / Chlorophyll content of normal leaf) X 100

All the experimental data were collected from three plants of each genotype in every experimental condition. Means and standard deviation were calculated and presented in graphs.

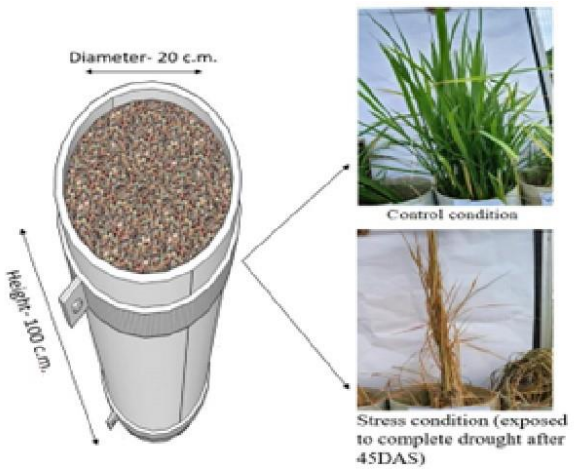

Fig. 1. Experimental design for drought stress showing a representative plant picture after

3. RESULTS

3.1 Diversity Analysis by SSR Marker

The amplified products of SSR markers were visualized by gel electrophoresis and scored. A representative picture of RM8213 and RM10864 markers amplification across all 27 genotypes is shown in Fig. 2. The PIC value of SSR markers is shown in Table 2, which ranges from 0.0767 (RM314) to 0.3746 (RM3472).

The genetic distance between the varieties was measured using Jaccard's dissimilarity coefficient matrix (Supplementary Table 2). The dissimilarity coefficient ranged from 0.150 to 0.818. The maximum dissimilarity was observed between the varieties 'Maibee' and 'Kola Joha' and the minimum dissimilarity was observed between the varieties 'Sok votung' and 'Manipuri Joha'. The phylogenetic tree based on Jaccard's dissimilarity coefficient matrix was constructed following Unweighted Neighbor-Joining (UPGMA) (Fig. 3). The tree grouped the 27 genotypes into mainly 3 clusters and each cluster consists of 9 varieties. The cluster I consisted of "N22', 'Shahabhagi', 'Dehangi', 'Ranjit Sub-1', 'Maizubiron', 'IR64', 'Basantabahar', 'Vandana' and 'Luit'. The cluster II includes- 'Sok soi soi', 'Haccha', 'Sok jongthi', 'Maguri', 'Mala', 'Basantasali', 'Manipuri joha', 'Sok votung', 'Maibee'. Cluster III included 9 varieties, namely-

stress

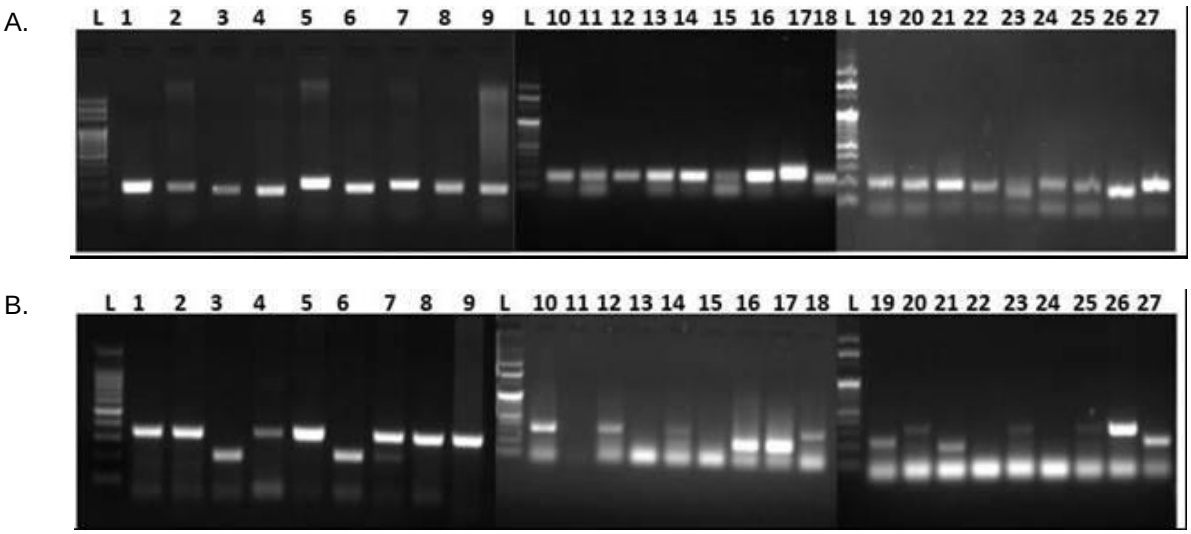

Fig. 2. Amplification pattern of A. RM8213 and B. RM10864 markers across all the genotypes

Table 2. List of SSR markers with PIC value

Sl NO	NAME	PIC VALUE	Sl NO	NAME	PIC VALUE
1	RM10864	0.359	16	RM190	0.246
2	RM207	0.363	17	RM314	0.077
3	RM6378	0.373	18	RMT01	0.000
4	RM3866	0.373	19	RMT02	0.000
5	RM186	0.290	20	RMT06	0.113
6	RM480	0.372	21	RMT07	0.000
7	RM8213	0.370	22	RMT12	0.000
8	RM2615	0.372	23	RMT13	0.000
9	RM336	0.370	24	RMT14	0.000
10	RM8020	0.124	25	RMT23	0.000
11	RM590	0.000	26	RCL03	0.000
12	RM1375	0.263	27	RCL04	0.000
13	RM4862	0.168	28	RCL14	0.000
14	RM2935	0.173			
15	RM3472	0.375			

'Inglonkiri', 'Mahsuri', 'KarbiDhan', 'AborSali', 'Baismuthi', 'Kola joha', 'Betguti', 'RongaSali', and 'Moinagiri. Genotypes which are present in the different clusters are genetically diverse from each other and the genotypes which are present in the same cluster are less genetically diverse. Under cluster I, N22 and Shahabhagi showed minimum dissimilarity percentage (15.8%), which means they have least genetic diversity. Under cluster II 'Sok votung' and 'Manipuri joha' have least genetic diversity with 15% dissimilarity. In cluster III. 'Abor Sali' and 'Baismuthi' showed the least dissimilarity percentage (22.2%) and the highest genetic distance is recorded between 'Rongasali' and 'Moinagiri'.

3.2 The Morphophysiological Trait of Few Rice Genotype under Drought Stress

Maximum root length(cm), Minimum root length(cm), Maximum Shoot length(cm), Root fresh weight(gm), Root dry weight(gm), Shoot fresh weight(gm), Shoot dry weight(gm), Root shoot ratio, Root length density, Total chlorophyll content(mg/g fw), and Chlorophyll stability(%) Index were recorded in control and drought stress (Table 3).

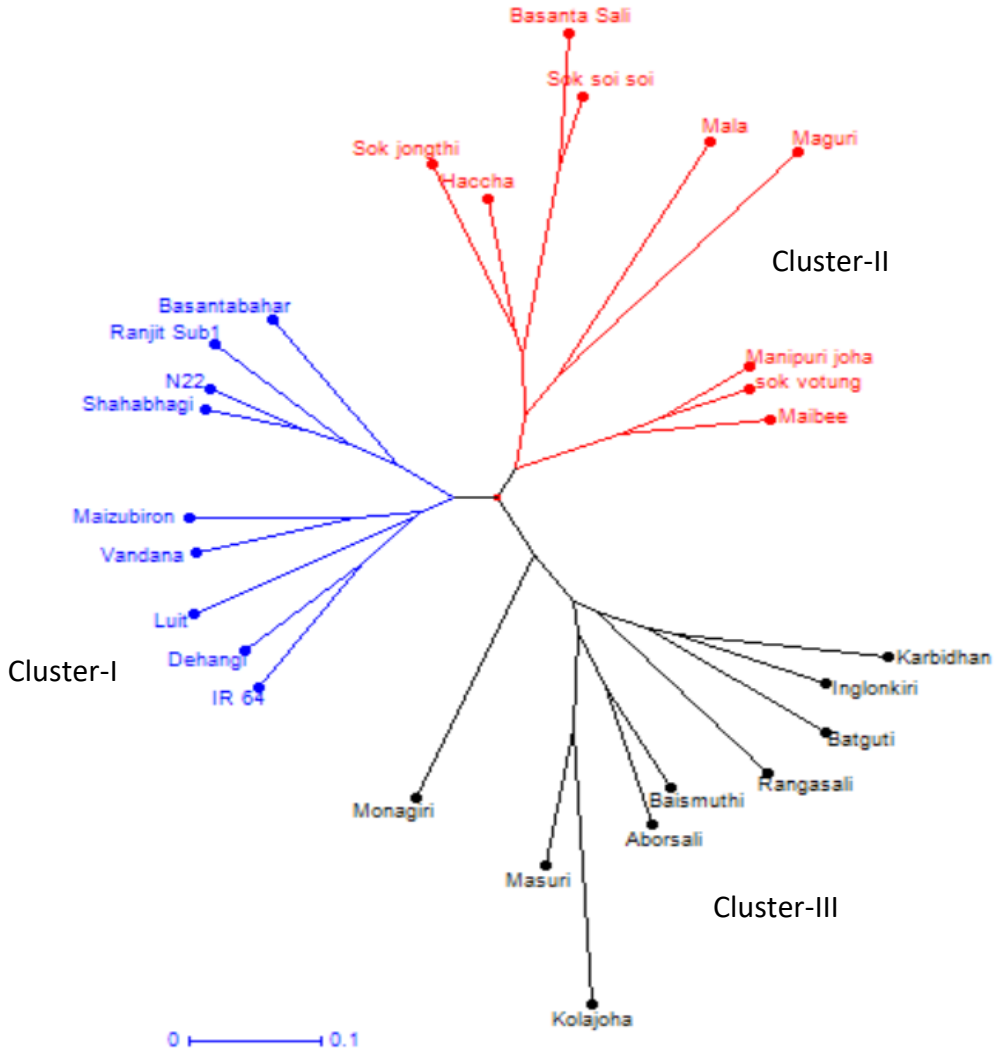

Fig. 3. Phylogenetic tree of 27 genotypes based on SSR markers

Table 3. The comparative mean performance of all the nine varieties along with their difference

Variety/ Trait	CD	Growing condition	N22	Shahabhagi	Dehangi	Maizubiron	Ranjit Sub1	IR64	Basanta bahar	Vandana	Luit	Mean
Max root length	11.91	Normal	71.00	70.00	51.33	83.33	65.67	75.67	81.00	86.33	93.33	75.30
		Stress	86.00	86.33	88.67	62.33	53.67	36.33	80.33	88.33	85.00	74.11
		Mean	78.50	78.17	70.00	72.83	59.67	56.00	80.67	87.33	89.17	
		Difference	15.00	16.33	37.33	21.00	12.00	39.33	0.67	2.00	8.33	16.89
		Significance	S	S	S	S	S	S	NS	NS	NS	
Fresh Root Weight	5.97	Normal	35.11	33.14	36.65	13.38	12.23	13.8	35.25	27.54	20.31	25.27
		Stress	25.20	17.31	20.71	12.72	6.70	7.04	23.91	22.24	10.47	16.26
		Mean	30.16	25.23	28.68	13.05	9.47	10.42	29.58	24.89	15.39	
		Difference	9.91	15.83	15.94	0.66	5.53	6.76	11.34	5.30	9.84	9.01
		Significance	S	S	S	NS	NS	S	S	NS	S	
Dry root weight	5.26	Normal	27.88	26.95	33.34	9.76	7.67	12.50	29.98	24.57	17.47	20.01
		Stress	13.83	15.92	11.83	7.15	4.47	4.17	20.91	19.50	7.20	11.66
		Mean	20.86	21.44	22.59	8.46	6.07	8.34	20.45	22.04	12.34	
		Difference	14.05	11.03	21.51	2.61	3.20	8.33	0.93	5.07	10.27	8.56
		Significance	S	S	S	NS	NS	S	NS	NS	S	
Fresh Shoot weight	14.55	Normal	50.81	67.85	118.78	66.22	90.83	59.36	77.94	81.43	108.34	80.17
		Stress	29.02	36.3	69.94	31.57	43.18	26.59	53.5	42.93	72.83	45.10
		Mean	39.91	52.08	94.36	48.90	67.01	42.98	65.72	62.18	90.59	
		Difference	21.79	31.55	48.84	34.65	47.65	42.77	24.44	38.50	35.51	36.19
		Significance	S	S	S	S	S	S	S	S	S	
Dry shootweight	15.6	Normal	40.85	61.87	108.98	56.11	80.83	49.54	67.5	71.77	98.17	70.62
		Stress	19.77	26.43	69.80	21.47	32.89	17.26	43.5	36.11	70.17	37.49
		Mean	30.31	44.15	89.39	38.79	56.86	33.4	55.5	53.94	84.17	
		Difference	21.08	35.44	39.18	34.64	47.94	32.28	24.00	35.65	28.00	33.13
		Significance	S	S	S	S	S	S	S	S	S	
Maximum Shoot length	10.42	Normal	91.33	88.67	92.67	87.67	82.00	66.33	101.67	99.00	91.00	88.93
		Stress	85.67	78.33	77.33	64.67	59.33	48.33	92.33	85.00	70.33	73.48
		Mean	88.50	83.50	85.00	76.17	70.67	57.33	97.00	92.00	80.67	
		Difference	5.67	10.33	15.33	23.00	22.67	18.00	9.33	14.00	20.67	15.44
		Significance	NS	NS	S	S	S	S	NS	S	S	
Root:shoot ratio	0.20	Normal	0.64	0.61	0.43	0.75	0.63	0.95	0.63	0.64	0.76	0.67
		Stress	0.82	0.90	0.92	0.79	0.72	0.58	0.65	0.77	0.90	0.78
		Mean	0.73	0.76	0.67	0.77	0.68	0.77	0.64	0.71	0.83	
		Difference	0.18	0.29	0.49	0.04	0.09	0.37	0.02	0.133	0.14	0.19

		Significance	NS	S	S	NS	NS	S	NS	NS	NS	
Root length density	0.174	Normal	0.76	0.73	0.94	0.27	0.21	0.34	0.82	0.67	0.48	0.579
		Stress	0.85	0.81	1.32	0.24	0.15	0.14	0.84	0.67	0.24	0.584
		Mean	0.61	0.64	0.65	0.26	0.18	0.24	0.76	0.66	0.36	
		Difference	0.29	0.19	0.51	0.03	0.06	0.20	0.12	0.01	0.23	0.18
		Significance	S	S	S	NS	NS	S	NS	NS	S	
Minimum root length	1.67	Normal	8.90	9.40	7.96	4.57	3.10	2.00	11.00	9.6	9.07	7.29
		Stress	4.50	6.67	9.267	6.4	5.27	4.43	8.03	7.167	13.10	7.20
		Mean	6.7	8.04	8.62	5.49	4.19	3.22	9.52	8.38	11.09	
		Difference	4.40	2.73	1.30	1.83	2.17	2.43	2.97	2.43	4.03	2.70
		Significance	S	S	NS	S	S	S	S	S	S	
Total chlorophyll	0.69	Normal	2.98	3.13	3.87	3.47	3.65	2.83	2.63	3.40	3.82	3.31
		Stress	2.89	2.78	3.78	3.21	2.57	1.97	2.12	2.78	3.00	2.79
		Mean	2.94	2.96	3.83	3.34	3.11	2.40	2.37	3.09	3.41	
		Difference	0.09	0.35	0.09	0.26	1.08	0.86	0.51	0.62	0.82	0.52
		Significance	NS	NS	NS	NS	S	S	NS	NS	S	
Chlorophyll stability index (%)			75.98	73.82	76.67	58.51	54.41	51.61	70.00	71.76	63.53	

The maximum root length of all the nine varieties under control and drought environment is shown in Fig. 4 along with the representative photograph. It was observed that N22, Shahabhagi and Dehangi shows significant increases of their root length in stress condition compared to control. The highest root length increases was observed in Dehangi (72.72%) followed by Shahabhagi (23.32%) and N22 (21.12%). N22 and Shahabhagi had longer roots in control than Dehangi.

The fresh root weight for all the varieties was decreased (average 35%) under stress conditions. However, the decrease in Maizubiron, Ranjit sub-1 and Vandana was non-significant. Similarly, the dry root weight for all the varieties was also decreased under stress conditions (average 43.30%). However, Maizubiron, Ranjit sub-1, Basantabaha and Vandana shows non-significant decreases. The root length density for N22, Shahabhagi and Dehangi shows significant increases in drought conditions. Out of these three varieties, Dehangi (59%) shows the highest increases of root length density followed by N22(39%) and Shahabhagi (26%).

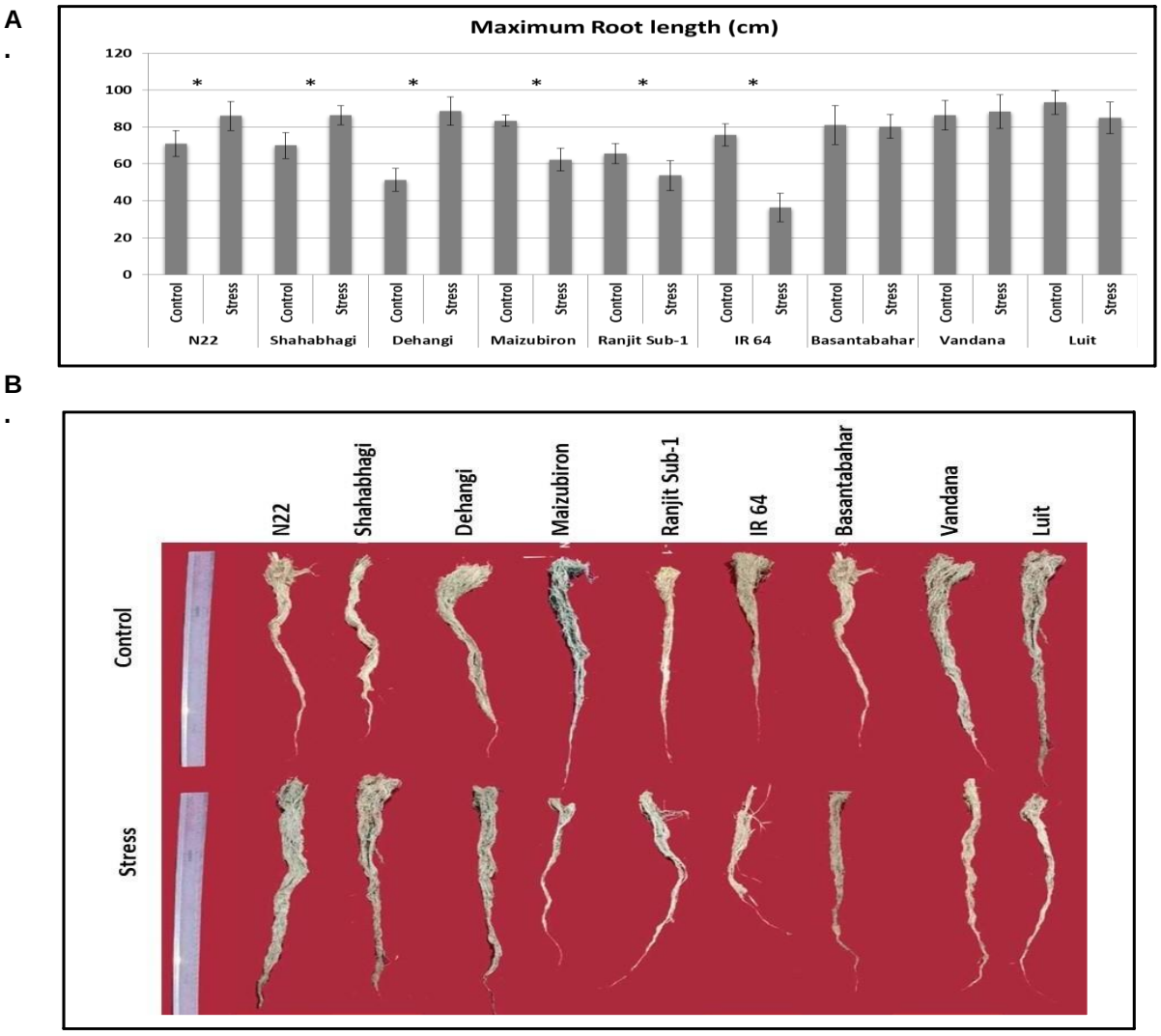

Fig. 4. (A) Graphical representation of maximum root length shown by all the nine varieties under control and drought environment and (B) Representative pictures of maximum root length

** means significance, $p < 0.05$*

The maximum shoot length was decreased for all the varieties under drought conditions compared to the control. Fresh shoot weight for all the varieties was decreased significantly in drought conditions. In stress conditions, the dry shoot weight ranged from 17.26 gm to 70.17gm with a mean value of 37.49gm under drought stress. The highest value was recorded by 'Luit' and 'IR64' showed the lowest value. The root: shoot ratio for 'Shahabhagi' and 'Dehangi' showed significant increases in drought compared to control whereas IR 64 showed significant decreases (Fig. 4). Dehangi shows the highest increase of root- shoot ratio followed by Shahabhagi.

The total chlorophyll content was measured under drought stress that ranged from 1.97 mg/g fw to 3.78 mg/g fw. The highest value was recorded by Dehangi and IR64 showed the lowest value. The total chlorophyll content has decreased for all the varieties under drought conditions as compared to control conditions. However, N22, Shahabhagi, Dehangi, Maizubiron, Basantabahar and Vandana shows non-significant decreases of total chlorophyll content in drought condition. Chlorophyll stability index (CSI) indicates how well chlorophyll performs under stress conditions. The range for chlorophyll stability index was recorded from 61.61% to 76.67%. The highest value for chlorophyll stability index was recorded from the Dehangi and the lowest value was recorded in IR64.

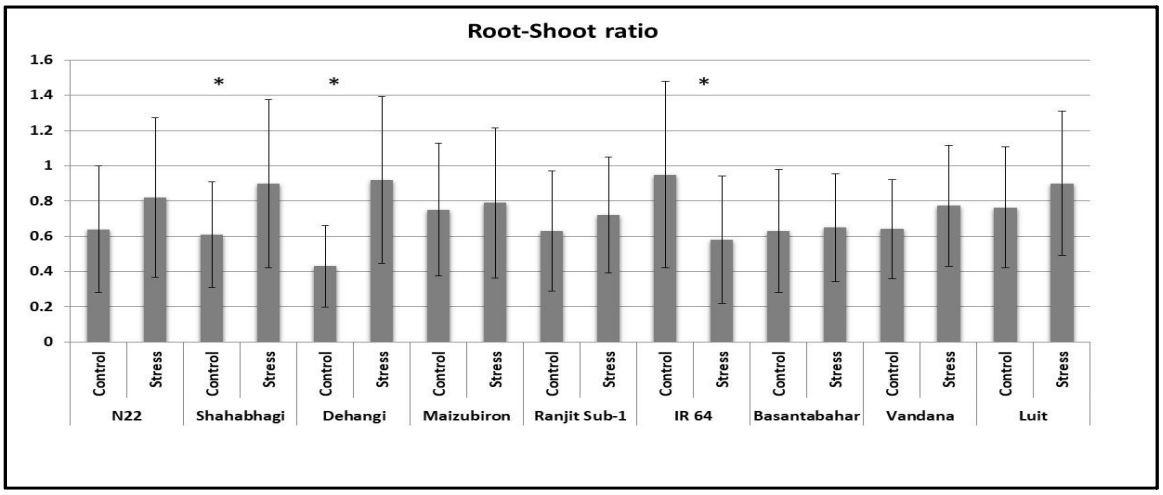

Fig. 5. Root-shoot ratio shown by all the 9 genotypes under control and drought stress environment

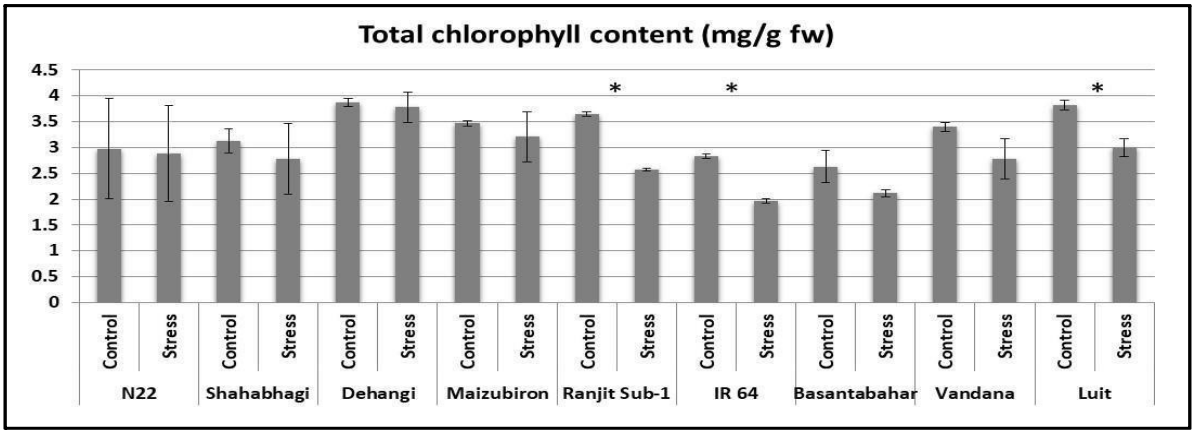

Fig. 6. Amount of total chlorophyll content shown by all the 9 genotypes under control and drought stress environment

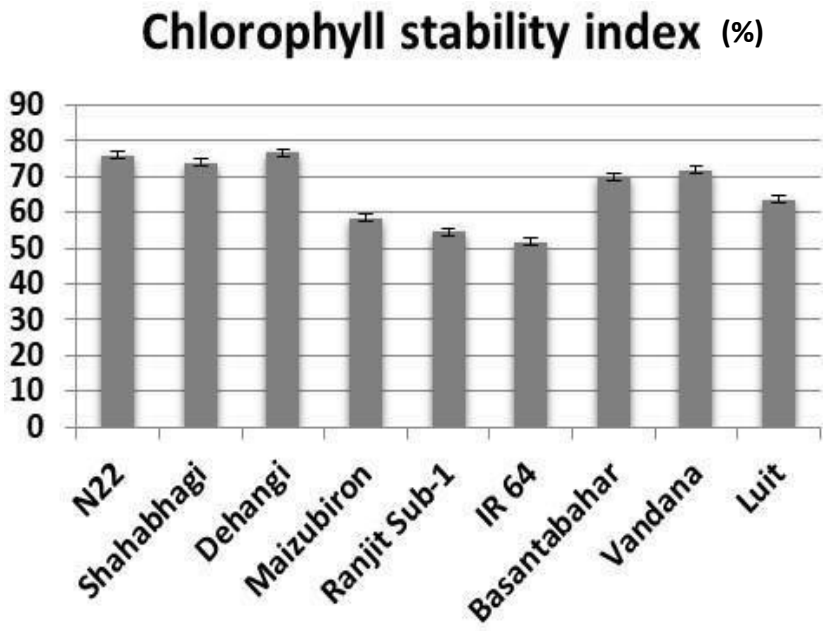

Fig. 7. Chlorophyll stability index (%) shown by all the 9 genotypes after subjecting to drought stress

4. DISCUSSION

To effectively plan a breeding program for higher yields in a rainfed ecosystems, information on the nature and magnitude of nuclear and organelle genomic variability present in the existing cultivars, association between the various traits of importance, as well as roots architecture and behavior during the drought period, would be essential. In this study, both nuclear and organellar SSR were used to study genetic diversity as the nuclear genome inherits biparentally and the organelle genome in rice inherits maternally and both the genomes are necessary to maintain cellular homeostasis. Nuclear SSR markers showed greater polymorphism whereas only one mitochondrial SSR marker showed polymorphism in our study. It was reported earlier that mitochondria and chloroplast SSRs in rice accession exhibited polymorphism [8,9]. The lack of polymorphism in our study may be explained by the same maternal origin of the genotypes under study or there may be technical difficulty to resolve small differences of PCR amplified products in agarose gel that we have used in gel electrophoresis. Unlike the nuclear genome, copy number of organellar genome may also influence phenotypes which needs to be investigated in the future.

Genetic diversity of rice genotypes of North East India using SSR markers and phenotypic data showed considerable variation across genotypes for root, shoot and drought tolerance traits [10]. In this study phylogenetic tree using SSR marker showed three clusters of rice genotypes under investigation. Root morphology of nine varieties under cluster I was investigated under control and drought. The verities under cluster one include N22, Shahabhagi, Dehangi which were earlier reported to show drought tolerance characteristics [11,12,13,14]. N22 also reported for maintaining its grain yield even after it was subjected to drought at the reproductive stage [15] In the control experiment Maizubiron, IR64, Basantabahar, Vandana and Luit show similar root length as compared to N22. As these varieties have a longer root system, which may be helpful to the plant to withstand drought. It was also observed that N22, Shahabhagi, Dehangi and Vandana shows inducible root character under drought stress. Dehangi shows the highest inducible root trait (72.72%) followed by Shahabhagi (23.32%) and N22 (21.12%). It was previously reported that varieties that show inducible root traits have better capability to relocate their resources for root development [6,16,17]. This increasing root length character will be the most desirable character for the plants to withstand drought stress as longer roots will help the plant absorb water from deeper soil layers under drought stress conditions which ultimately increases the yield [18,19,20]. In Root-

Shoot ratio measurement Maizubiron, IR64, Vandana and Luit show a similar root-shoot ratio as compared to N22. Out of these four varieties, Luit shows the highest value of the root-shoot ratio which may be helpful to the plant to withstand drought. The increase in Root Shoot ratio is due to the alteration of carbohydrate partitioning and enzymatic activity in rice seedlings during drought [21,22,23. It was observed that 'Shahabhagi' and 'Dehangi' show a significant increase in root-shoot ratio in drought stress. This increasing root-shoot ratio character will also be one of the most desirable characteristics for the plants to withstand drought stress as longer roots will help the plant to absorb water from deeper soil layers under drought stress conditions. Also, a lesser shoot will help to reduce transpiration which ultimately helps the plant to conserve water and use that water during the stress condition. Plant roots are also adapted to increase root length density (RLD) during drought stress conditions [7]. Root length densities from 0.5 to 1 cm^{-3} are usually capable to meet moisture demand in plants [24]. In the present investigation, it was observed that 'N22', 'Shahabhagi', 'Dehangi', 'Basantabahar' and 'Vandana' shows significant increased in length density in drought condition than the control condition. Out of these five varieties 'Dehangi' with RLD 0.906 cm^{-3} was the highest in drought. That means 'Dehangi' will show better results to withstand drought conditions as higher root length density means more water will be absorbed. Measurements of fresh and dry root weight show that in control condition 'N22', 'Dehangi' and 'Basantabahar' shows higher fresh and dry root weight compared to other varieties. 'Maizubiron', 'Ranjit sub-1' and 'Vandana' shows non-significant decreases in the fresh and dry root weight in stress condition which means there are fewer changes in the root weight of these varieties under water stress. This may also be a desirable characteristic for drought stress because lesser changes lead to better function of the roots.

The total chlorophyll content is one of the significant drought-tolerant indicators [25]. At control condition, we saw that Shahabhagi, Dehangi, Maizubiron, Ranjit sub-1, Vandana and Luit showed higher total chlorophyll content which is a desirable trait as the higher chlorophyll means a better rate of photosynthesis. When we compared total chlorophyll content in the stress condition with the control condition, there was a reduction in chlorophyll content in all the genotypes when they were exposed to drought stress. The reduction may be due to oxidative stress or chlorophyll degradation [26]. But out of those 'N22', 'Shahabhagi', 'Dehangi', 'Maizubiron', 'Basantabahar' and 'Vandana shows non-significant decreases in total chlorophyll content in stress condition. That means these six varieties are showing stability during drought environment. In the case of the chlorophyll stability index, out of nine genotypes, Dehangi shows the highest CSI followed by 'N22' and 'Shahabhagi'. Plants with higher CSI have a better potential to survive under drought conditions [27]. Considering the mean performance of all the parameters in drought stress conditions, 3 promising varieties were named 'Dehangi' followed by 'N22' and 'Shahabhagi' [28].

5. CONCLUSION

In India, rice is cultivated over 44 million ha, which gives a total production of 117.47 million tones and a productivity of 2659 kg/ha. However, because of a limited amount of available water, a large portion of this agricultural yield is destroyed by disease and drought. The assessment of genetic variability present in indigenous rice genotypes helps in strategic breeding program that have the potential to produce new cultivars with a wider genetic base and wider adaptability towards various abiotic as well as biotic stresses. Therefore, using native rice genotypes as the main source of variation and introducing desired traits from contemporary cultivars may be a successful method for creating drought resistance. A hybridization program between diverse genotypes with different morpho-physiological characters would provide a mapping population that could then be used to pinpoint the genes underlying traits related to moisture stress tolerance. Additionally, this will open the door for the creation of molecular and marker-assisted selection for trait-based breeding for drought stress tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Supplementary Table 1. List of markers that were used in the investigation

Sl No	Sequence name	Chromosomeno.	Repeat motif	Sequence (5'-3')	Length	Expected size of the product
1	RM1360	1	$(AG)_{25}$	TTACCTCAGGCTCTTCAGGC	20	154
				AGAAGTGAGCAATCATGGCC	20	
2	RM10864	1	$(GT)_{27}$	GAGGTGAGTGAGACTTGACAGTGC	24	239
				GCTCATCATCCAACCACAGTCC	22	
3	RM207	2	$(CT)_{25}$	CCATTCGTGAGAAGATCTGA	20	118
				CACCTCATCCTCGTAACGCC	20	
4	RM6378	2	$(GAA)_{19}$	ATAGGGTGGGTGTGCTGAAC	20	167
				TGCACAAAACTGCAGGTCTC	20	
5	RM422	3	$(AG)_{30}$	TTCAACCTGCATCCGCTC	18	385
				CCATCCAAATCAGCAACAGC	20	
6	RM186	3	$(CGG)_{5}$	TCCTCCATCTCCTCCGCTCCCG	22	124
				GGGCGTGGTGGCCTTCTTCGTC	22	
7	RM3866	4	$(GA)_{29}$	AGTTGGTCATCTACCAGAGC	20	161
				GATCTTCTTGCCTCAGAAAG	20	
8	RM8213	4	$(TC)_{10}$	AGCCCAGTGATACAAAGATG	20	177
				GCGAGGAGATACCAAGAAG	20	
9	RM480	5	$(AC)_{30}$	GCTCAAGCATTCTGCAGTTG	20	225
				GCGCTTCTGCTTATTGGAAG	20	
10	RM2615	6	$(AT)_{30}$	CAGAGTGCTTTAGACAATCA	20	164
				AAATTGGTAAGAGATTCTGC	20	
11	RM2381	7	$(AT)_{26}$	AACCTCAAATATTTAAACTC	20	142
				GCTAGAGAAAATAGAGAAAC	20	
12	RM336	7	$(CTT)_{18}$	CTTACAGAGAAACGGCATCG	20	154
				GCTGGTTTGTTTCAGGTTCG	20	
13	RM80	8	$(TCT)_{25}$	TTGAAGGCGCTGAAGGAG	18	142
				CATCAACCTCGTCTTCACCG	20	
14	RM8020	8	$(TA)_{20}(GA)_{19}$	ATCCTCGATGAATTGTATAT	20	167
				GAAGAGGTGTACATGAATAA	20	
15	RM6839	9	$(TCT)_{17}$	CTACTGTTGCAGGCTTGCAG	20	104
				CAGAGGAGGAGATCGAGAGG	20	
16	RM590	10	$(TCT)_{10}$	CATCTCCGCTCTCCATGC	18	137
				GGAGTTGGGGTCTTGTTCG	19	
17	RM1375	10	$(AG)_{31}$	CTACACGCGCAAACTCTGTC	20	180
				ATGAAGGTCTAGGCTGCACC	20	
18	RM206	11	$(CT)_{21}$	CCCATGCGTTTAACTATTCT	20	147
				CGTTCCATCGATCCGTATGG	20	
19	RM4862	11	$(TA)_{28}$	CAACTTTCTGGCATAAACTA	20	164
				TGGTGAAAGATATTTCAGAC	20	
20	RM2935	12	$(AT)_{39}$	CAGCAAATTTGTTACTTATG	20	165
				TGCTATGTTTTTTTATAACG	20	
21	RM347 2	12	$(CT)_{21}$	ATCGCAAGAACTCCGTGAAG	20	215
				CGCTTTTGAGCTCGCCTC	18	
22	RM190	6	$(CT)_{11}$	TTTGTCTATCTCAAGACAC	19	124
				TTGCAGATGTTCTTCCTGATG	21	
23	RM314	6	$(GT)_{8}(CG)_{3}(GT)_{5}$	CTAGCAGGAACTCCTTTCAGG	21	118
				AACATTCCACACACACACGC	20	
24	RCL03	_	$(TCT)_{4}(T)_{11}$	GTTTCCTTAGCCCACTC	17	306

				GCATTCTACCCGCAATC	17	
25	RCL04	_	$(TAAA)_4$	GTTAAAAGTGGCACCAATC	19	288-292
				GATTTATGTCGTGCCAATC	19	
26	RCL14	_	$(T)_{12}$	ACGGAATTGGAACTTCTTTGG	21	
				AAAAGGAGCCTTGGAATGGT	20	
27	RMT01	_	$(GTAG)_4$	TTCATACGGCGGGAGTC	17	208-216
				AGCTCTCAGACGAGCTG	17	
28	RMT02	_	$(ACA)_4$	GGAACTCAGACCCGATC	17	241-247
				ATTTATTGCCCGTCGAG	17	
29	RMT06	_	$(AT)_6$	GGGTTTAGAGTCGCCAC	17	198-200
				GATGGTTTGGAAGGCTG	17	
30	RMT07	_	$(AG)_6$ $(TC)_6$	GAGGATTTCGAGTCCTC	17	211-233
				GAATTCTTCGAGGCCTG	17	
31	RMT12	_	$(AT)_6$	TCATTACTTTGGCCACCTAAGC	22	187-197
				GGCTTTCGTGAAAGCACC	18	
32	RMT13	_	$(AG)_6(TC)_6$	GCTCTAACCAGCCAGAACC	19	198-206
				CCATAGAATTCTTCGAGGCCTG	22	
33	RMT14	_	$(ACA)_4$	TCTAGCCGAACGGATGC	17	132-138
				GGTACTCAACGTTGAAGCCAC	21	
34	RMT23	_	$(TAAT)_3$	GCCATGTTACCACGTTCG	18	229-234
				CCTGAGTTGTACTGGGTCG	19	

Supplementary Table 2. Matrix for Jaccard co-efficient of dissimilarity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2	0.158																									
3	0.455	0.542																								
4	0.381	0.409	0.300																							
5	0.273	0.227	0.480	0.348																						
6	0.478	0.500	0.250	0.250	0.440																					
7	0.391	0.273	0.458	0.391	0.292	0.417																				
8	0.409	0.435	0.478	0.250	0.375	0.435	0.348																			
9	0.429	0.381	0.350	0.263	0.391	0.381	0.364	0.455																		
10	0.583	0.542	0.522	0.522	0.538	0.654	0.577	0.478	0.500																	
11	0.652	0.609	0.524	0.591	0.600	0.667	0.522	0.400	0.571	0.368																
12	0.667	0.630	0.500	0.560	0.621	0.630	0.556	0.577	0.478	0.286	0.429															
13	0.591	0.667	0.368	0.450	0.600	0.545	0.583	0.316	0.571	0.450	0.353	0.500														
14	0.542	0.615	0.333	0.409	0.556	0.500	0.538	0.364	0.522	0.333	0.476	0.391	0.222													
15	0.762	0.773	0.632	0.700	0.800	0.773	0.739	0.579	0.750	0.556	0.357	0.526	0.357	0.500												
16	0.750	0.714	0.478	0.600	0.700	0.500	0.593	0.615	0.583	0.333	0.476	0.318	0.476	0.364	0.579											
17	0.538	0.556	0.538	0.643	0.600	0.655	0.586	0.556	0.679	0.348	0.478	0.462	0.478	0.375	0.571	0.375										
18	0.625	0.583	0.500	0.565	0.520	0.522	0.500	0.522	0.476	0.500	0.500	0.478	0.421	0.455	0.611	0.455	0.630									
19	0.545	0.500	0.609	0.720	0.615	0.625	0.542	0.680	0.708	0.667	0.619	0.786	0.682	0.680	0.800	0.625	0.500	0.591								
20	0.300	0.250	0.522	0.455	0.417	0.409	0.391	0.600	0.350	0.640	0.708	0.615	0.708	0.654	0.818	0.600	0.643	0.500	0.476							
21	0.381	0.478	0.455	0.583	0.538	0.478	0.577	0.542	0.680	0.583	0.591	0.714	0.524	0.478	0.700	0.542	0.417	0.625	0.316	0.455						
22	0.450	0.400	0.652	0.652	0.542	0.609	0.583	0.609	0.636	0.591	0.600	0.731	0.667	0.667	0.722	0.609	0.542	0.636	0.389	0.368	0.278					
23	0.333	0.435	0.409	0.333	0.500	0.286	0.538	0.435	0.381	0.600	0.667	0.577	0.545	0.500	0.714	0.560	0.607	0.522	0.625	0.250	0.409	0.476				
24	0.667	0.619	0.727	0.727	0.720	0.682	0.652	0.739	0.714	0.667	0.684	0.696	0.684	0.739	0.750	0.550	0.609	0.650	0.556	0.444	0.526	0.438	0.550			
25	0.421	0.524	0.750	0.636	0.583	0.652	0.625	0.524	0.739	0.636	0.650	0.720	0.650	0.652	0.706	0.708	0.583	0.682	0.600	0.500	0.421	0.500	0.450	0.500		
26	0.435	0.391	0.500	0.435	0.462	0.391	0.500	0.520	0.333	0.560	0.625	0.538	0.625	0.577	0.727	0.520	0.621	0.409	0.583	0.200	0.500	0.429	0.150	0.500	0.545	
27	0.625	0.583	0.680	0.778	0.630	0.692	0.560	0.692	0.769	0.731	0.636	0.793	0.696	0.692	0.810	0.640	0.520	0.609	0.176	0.565	0.429	0.500	0.692	0.650	0.682	0.654

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