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Genetic variation of 12 rice cultivars grown in Brunei Darussalam and assessment of their tolerance to saline environment

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Genetic variations of 12 different rice cultivars in Brunei Darussalam were studied using 15 different SSR markers and their salinity tolerance mechanism was also assessed. Eight SSR markers, RM 151, 187, 206, 226, 276, 310, 320 and 334, showed polymorphic alleles while the other seven were monomorphic. A total of 158 alleles were amplified for all these rice cultivars using 15 SSR markers, with an average of 10.53. The allele frequencies per locus or marker range from 0 in RM 307 to five alleles in RM 226. PIC values varied from 0.00 to 0.7521. Similarity distance varied from 0.00 to 1.00. Dendrogram showed three distinct clusters, where both *Kuaci* and *Sp1* significantly diverted from the other ten rice cultivars. *Bandul berminya*k was the most tolerant to salinity. Quantum yield for *B. berminyak* were unaffected and it showed the least reduction in growth parameters studied when expose to salinity stress. From both salinity tolerance and genetic variation investigations for these 12 cultivars, it may probably be better to intercross between *Arat* (moderately tolerant) and *Sp1* (susceptible) as both are from different clusters, showed low genetic similarity with 0.33 and different salinity tolerance level.

Key words: Genetic variability, rice cultivars, SSR markers, salinity

INTRODUCTION

Extensive efforts have been carried out in Brunei to increase rice production which includes cultivation of fast-growing inbred paddy *Laila*, increase paddy cultivation area and improvements in facilities. However,

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Abbreviations: RFLP, Restriction fragment length polymorphism; **RAPD,** random amplified polymorphic **DNA; AFLP,** amplified fragment length polymorphism; **ISSR,** inter-simple sequence repeat**; SSR,** single sequence repeats; PIC, polymorphism information content; **RM,** rice marker.

the country has yet been able to achieve the target 20% self-sufficiency. Soil salinity is one of the major constraints that cause this situation. Low rice productivity is also caused by narrow range of genetic variability and lack of sufficient genetic information about traits inherited (Mahajan et al., 2012). Standard conventional breeding techniques which involved selection of desired parent plants using morphology and physiology study (Allard, 1999; Collard and Mackill, 2008) has been replaced by molecular breeding. The conventional breeding techniques may take five to 10 years to be completed and more work on morphology and physiology are needed for studying genetic diversity among parents (Zeng et al, 2004; Collard and Mackill, 2008). Zeng et al. (2004) have reported that the morphology measurement might not reveal the actual genetic relationships among genotypes studied. Thus, both measurements might not be discriminative enough to differentiate all the genotypes studied (Behera et al., 2012).

The constraints encountered by conventional breeders on selection of parents can be solved by using molecular breeding techniques. Some molecular markers that can be used in molecular breeding include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and single sequence repeats (SSR). These techniques are different in terms of speed, cost and degree of polymorphism. According to Garcia et al. (2004), RAPD is a much simpler technique when compared to RFLP, as the latter requires radioactive materials. However, RAPD lack in reproducibility cause by mismatch annealing. SSR solve these limitations as its sequence present in most eukaryote genomes, informative and reproducible. According to Saini et al. (2004), SSR is more efficient for differentiating rice genotypes when compared to both AFLP and ISSR and they also stated that both AFLP and SSR are more reliable and reproducible than ISSR.

According to Shams et al. (2012), SSR markers are usually utilized to study genetic variation in different rice germplasm as they are inexpensive, simple, rapid, easily detected by PCR and importantly only require small amount of tissue samples. These markers helped to increase efficiency and precision when selecting the parents before intercrossing and this would lead to the production of new cultivar with improved characteristics (Zeng et al., 2004; Collard and Mackill, 2008). Assessing salinity tolerance level of rice cultivars is also necessary to select the parental line for breeding program as it helps to understand salinity tolerance mechanism in the cultivars. Growth and photosynthetic efficiency measurements were taken for determining salinity tolerance of the studied rice cultivar (Cha-um et al., 2007).

Cultivar that possess high tolerance and show less reduction in these parameters would be the best parent. Rice varieties adapt to saline environments differently due to the diverse genetic background and different salinity tolerance mechanisms. Improving salt tolerance can be achieved by selecting suitable parents before intercrossing based on information provided from microsatellite markers.

Different salt tolerance components can be combined into a cultivar by intercrossing parents from different microsatellite clusters with wide salt tolerance mechanisms (Zeng et al., 2004). Investigation on genetic similarity, cluster analysis and also salt tolerance ability provide useful information for plant breeders to select best parents with diverse genetic background prior to intercrossing (Kanawapee et al., 2011).

In this study, the genetic diversity of 12 different rice cultivars grown in Brunei, with different adaption to salinity stress, was assessed using 15 SSR markers. Fifteen (15) markers with high polymorphism information content (PIC) values from Temnykh et al. (2000) were selected for the study. The result obtained from this study may help breeder to increase efficiency of breeding as it provides detailed information about genetic diversity of the 12 cultivars studied, thus allowing one to select the suitable parents for crossing to produce germplasm with better traits. Besides, no extensive investigations have yet been carried out for genetic study of the rice cultivars grown in the country using microsatellites.

MATERIALS AND METHODS

Plant material

Twelve (12) different rice cultivars: *Adan, Arat, Bandul berminyak, Jongkok, Kuaci, Laila, Pusu, Pulut Keladi, Pusu Merah*, *Raden Pinang*, *Salleh*, and an unknown rice cultivar which was named *Sp1* were used in the present study.

Genomic DNA extraction

Genomic DNA was extracted from frozen leaves (0.2 g) of the rice cultivars using GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich Co. LLC, St. Louis, Missouri) according to the manufacturer's instructions.

PCR amplification

A total of 15 microsatellites with high PIC value were chosen from Temnykh et al. (2000) and used for the study (Table 1). The polymerase chain reaction (PCR) amplification mixture was prepared in 0.5 ml Eppendorf tubes. Each reaction mixture contained 2.5 µl of 10X PCR buffer, 1.5 µl 50 mM $MgCl_2$, 0.5 µl 10 mM dNTP, 5 µl of 1 µM for each primer (forward and reverse), 100 ng of DNA, 0.3 µl Taq DNA polymerase and TE buffer (pH 8.0) was added to adjust the final volume to 25 µl. The amplification programme consisted of the following cycles: 94°C for 4 min, 30 cycles of 94°C for 45 s, 39.5 to 57.2°C (standardized for each primer) for 1 min and 72°C for 1 min, and a final extension at 72°C for 10 min, following method of Nadia et al. (2014).

Table 1. List of SSR markers used in this investigation.

Primer	Size range (bp)	PIC	Tm (°C)	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
RM 72	152-198	0.85	43.8	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
RM 151	205-317	0.87	54.2	GGCTGCTCATCAGCTGCATGCG	TCGGCAGTGGTAGAGTTTGATCTGC
RM 159	238-252	0.87	57.2	GGGGCACTGGCAAGGGTGAAGG	GCTTGTGCTTCTCTCTCTCTCTCTCTCTC
RM 187	136-164	0.84	45.3	CCAAGGGAAAGATGCGACAATTG	GTGGACGCTTTATATTATGGG
RM 206	121-137	0.87	39.5	TAGTTTAACCAAGACTCTC	GGTTGAACCCAAATCTGCA
RM 226	264-342	0.82	49.2	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC
RM 264	148-178	0.83	46.7	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
RM 276	85-153	0.84	46.7	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
RM 287	98-118	0.83	42.6	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
RM 307	124-176	0.83	46.7	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM 310	85-120	0.83	38.5	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC
RM 320	153-254	0.85	46.7	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC
RM 333	164-215	0.83	47.5	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACTCGC
RM 334	146-197	0.83	47.3	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG
RM 335	104-155	0.84	49.2	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG

Electrophoresis of PCR products

Amplified products were separated on 2.5% agarose gel containing 0.5 µg/ml ethidium bromide using 1xTBE buffer as explained by Behera et al. (2012) at 100 V for 1 h. A volume of 10 µl amplified product was mixed with 2 µl loading dye. The gel was visualized under gel documentation (Compact CCD Image System, Major Science, USA).

Salinity treatments for rice cultivars in soil

Salinity tolerance levels for these 12 cultivars were studied by treating the plants with 0 mM salinity (control) and at 100 mM salinity level. Once every two days, 1 L of 100 mM saline solution was supplied to salinity treatment pot and 1 L of distilled water to control pot. Experiments were carried out for three (for *Laila* cultivar only) to seven months (the other 11 cultivars). Three days after the first salinity treatment, the plantlets were subjected to physiological investigations which include photosynthesis efficiency and growth measurements.

Quantum yield measurement

Quantum yield (Fv/Fm) was taken using Fluorpen FP100 (Photon Systems Instruments, Brno, Czech Republic) once a week. Measurement was taken from the same leaf at three different locations (at the leaf base, middle and near the leaf tips). Prior to the quantum yield measurement, the leaf was dark-adapted with aluminium foil for 30 min (Wankhade et al., 2013).

Growth measurements

Towards the end of the project (after 3 months for paddy *Laila* and seven months for the other 11 cultivars), fresh and dry root weight were measured. Root fresh weights were measured immediately after harvesting. Topsoil of the extracted roots were flushed away using tap water and dry weights were taken after plants were dried at 70°C until a constant weight was attained (Zeng et al., 2004). Plant height was measured from the stem base to the tip of the top most leaf (Zeng et al., 2004) at the commencement, mid project time (one and half month or four month) and final project time (three or seven months).

Salinity tolerance scores

The salinity symptoms observed in the cultivars studied were scored according to standard evaluation systems described by Lee et al. (2007) for salinity tolerance.

Data analysis

The amplified band or allele was scored manually as present (1) or absent (0) for each genotype and primer combination. Data was entered in binary matrix using Excel and was used to calculate simple matching similarity coefficient using DARWin 5.0 with 500 bootstraps (Perrier and Jacquemoud-Collet, 2006). Dendrogram was constructed using unweighted pair group with arithmetic mean (UPGMA) following method done by Tabkhkar et al. (2012) to separate the rice cultivars into clusters and from here genetic relatedness among cultivars studied was deduced. The procedure adopted by Nei and Li (1979) was used with FreeTree software (Pavlicek et al., 1999) to calculate the distance matrix. To measure alleleic diversity of SSR markers, PIC was calculated using this formula:

$$PIC=1-\Sigma(Pi\ j)^2$$

Where, Pi j is the frequency of the ith allele in the jth population for each SSR locus (Botstein et al., 1980; Mahajan et al., 2012).

For physiology experiment, Non parametric Kruskal Wallis (if data was not normally distributed) or Two-Way Anova Tukey Test (if data was normally distributed) was used to test the following hypothesis:

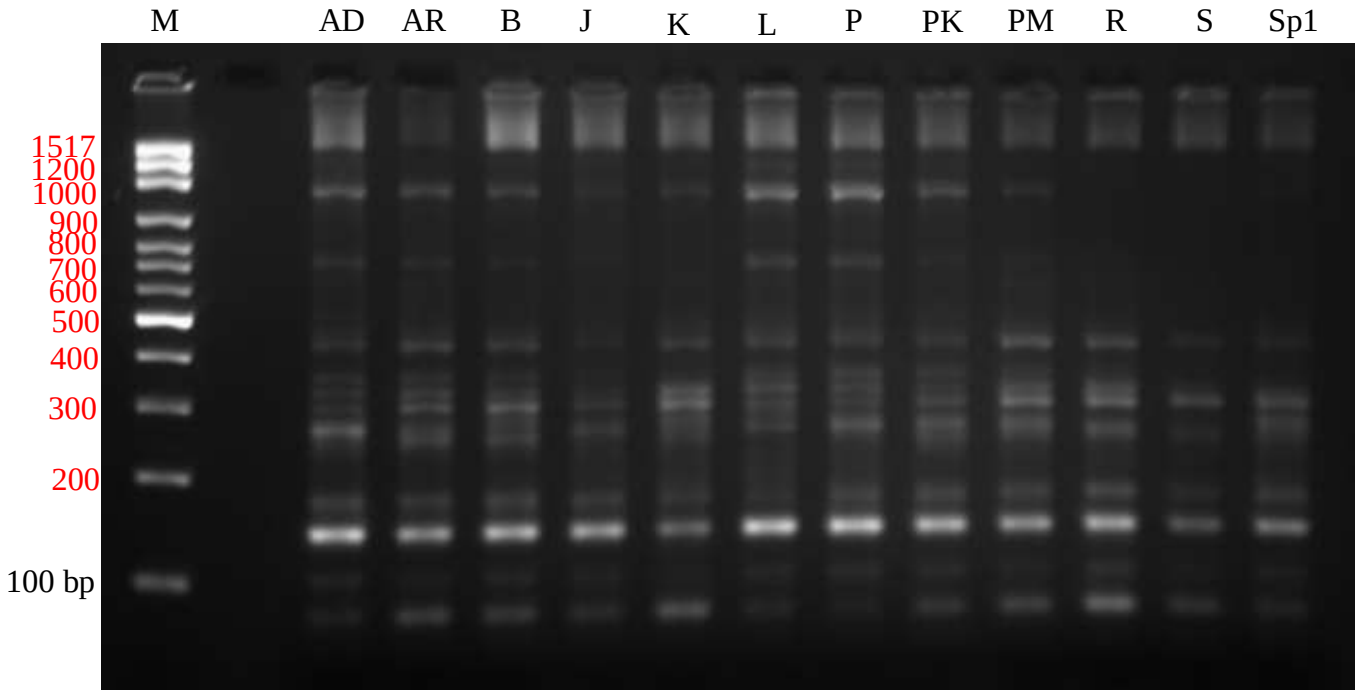

Figure 1. PCR amplification for 12 different rice cultivars (AD=*Adan*, AR=*Arat*, B=*Bandul berminya*k, J=*Jongkok*, K=*Kuaci*, L=*Laila*, P=*Pusu*, PK=*Pulut Keladi*, PM=*Pusu Merah*, R=*Raden Pinang*, S=*Salih* and *Sp1*=unnamed rice cultivar) using SSR marker RM 226 (M=marker (100 bp)).

yield for instance) of the rice cultivar; H_1: there is significant effect of salinity to parameter (quantum yield for instance) of the rice cultivar.

RESULTS

Polymorphism in SSR markers

A total of 158 alleles were amplified for all these rice cultivars using 15 SSR markers with an average of 10.53. Out of 15 markers, 8 were polymorphic while the others were monomorphic. Rice marker (RM) 151, 187, 206, 226, 276, 310, 320 and 334 resulted in polymorphic alleles while the primers, RM 72, 159, 264, 287, 333 and 335, gave monomorphic alleles. The allele frequencies per locus or marker range from 0 in RM 307 to 5 alleles in RM 226. Figure 1 shows amplified bands or alleles for 12 different rice cultivars using SSR markers RM 226. PIC values varied from 0.00 to 0.7521 (RM 226). SSR markers that produce high PIC value with 0.5 and above were obtained from RM 151, 206, 226, 310 and 320.

Genetic diversity and relationship among 12 rice cultivars studied

Both similarity distance and dendrogram were analysed using polymorphic marker RM 226. Similarity distance varied from 0.00 to 1.00 (Table 2). Genotype *Laila* showed highest similarity with *Adan* (1.00). Genotype *Pusu* and *Adan*, *Pulut Keladi* and *Bandul berminyak* and *Pusu Merah* with *Jongkok* also showed the highest similarity. Genotype *Salleh* or *Sp1* and *Kuaci* showed the least similarity (0.00). The dendrogram (Figure 2) showed 3 distinct clusters. Group 1 consists of *Sp1* while Group 2 consists of *Kuaci* and the other ten cultivars are in Group 3. Group 3 was further sub-divided into 5 groups with *Salleh* diverted from the other nine cultivars. Data shown in Figures 2 and 3 show that *Kuaci* and *Sp1* were most genetically different from the other ten rice cultivars.

Salinity tolerance

Figure 4 shows the paddy of *Arat*, *Adan* and *Bandul berminyak* grew upright and showed healthier leaves compared to the rest of the cultivars after being exposed to salinity stress for 3 months. Leaves for some of the cultivars *(Laila* and *Pusu*) died due to the high salinity stress. The other cultivars (*Jongkok, Kuaci, Pulut Keladi, Pusu Merah*, *Raden Pinang*, *Salleh* and *Sp1*) were slightly affected due to salinity stress as some of their leaves showed symptoms of dying. According to results

summarized in Table 3, *Adan* and *Arat* can be classified as moderately tolerant to salinity stress and *Bandul berminyak* was tolerant. Quantum yield of *Bandul*

Table 2. Similarity distance among 12 different rice cultivars cultivated in Brunei Darussalam investigated using RM 226 markers.

	Adan	*Arat*	*Bandul berminyak*	*Jongkok*	*Kuaci*	*Laila*	*Pusu*	*Pulut Keladi*	*Pusu Merah*	*Raden Pinang*	*Salleh*	*Sp1*
Adan												
Arat	0.75											
Bandul berminyak	0.89	0.89										
Jongkok	0.57	0.86	0.75									
Kuaci	0.67	0.67	0.57	0.40								
Laila	1.00	0.75	0.89	0.57	0.67							
Pusu	0.75	1.00	0.89	0.86	0.67	0.75						
Pulut Keladi	0.89	0.89	1.00	0.75	0.57	0.89	0.89					
Pusu Merah	0.57	0.86	0.75	1.00	0.40	0.57	0.86	0.75				
Salleh	0.33	0.67	0.57	0.80	0.00	0.33	0.67	0.57	0.8	0.8		
Sp1	0.67	0.33	0.57	0.40	0.00	0.67	0.33	0.57	0.40	0.40	0.50	

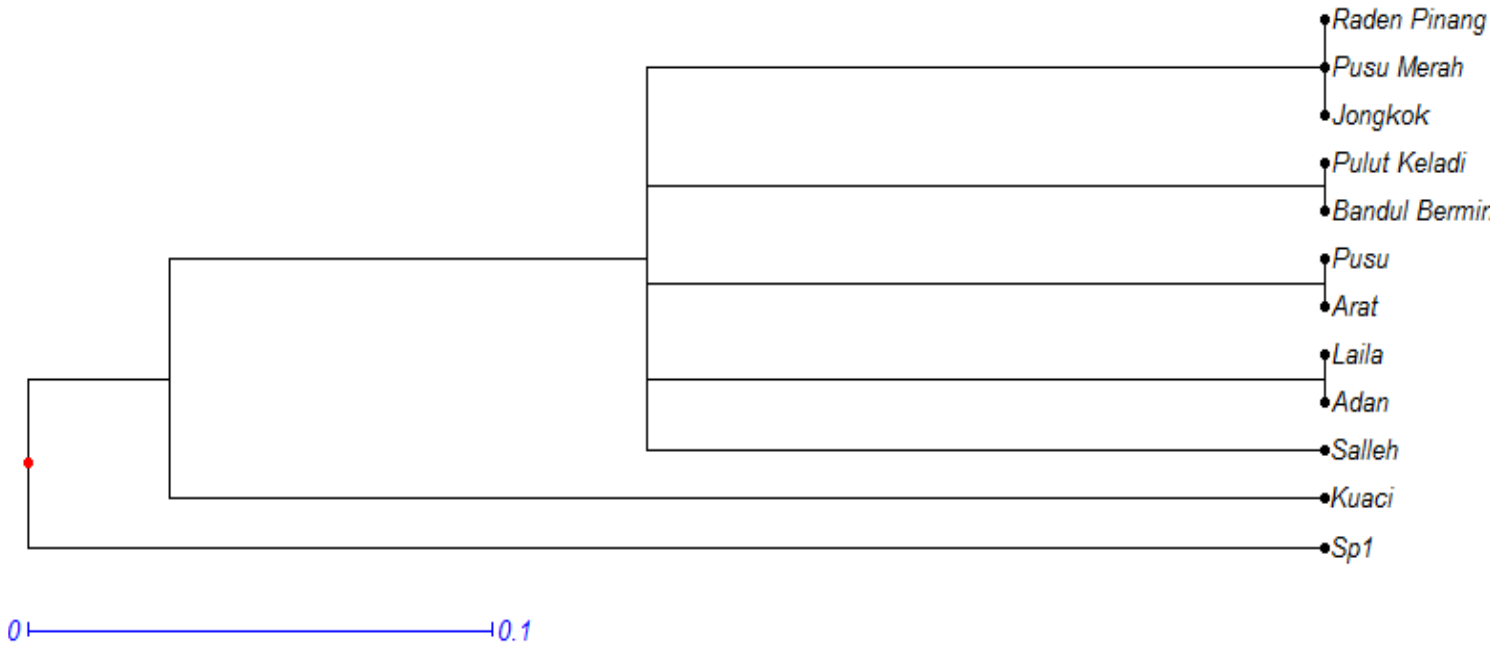

Figure 2. UPGMA based dendrogram for all rice cultivars.

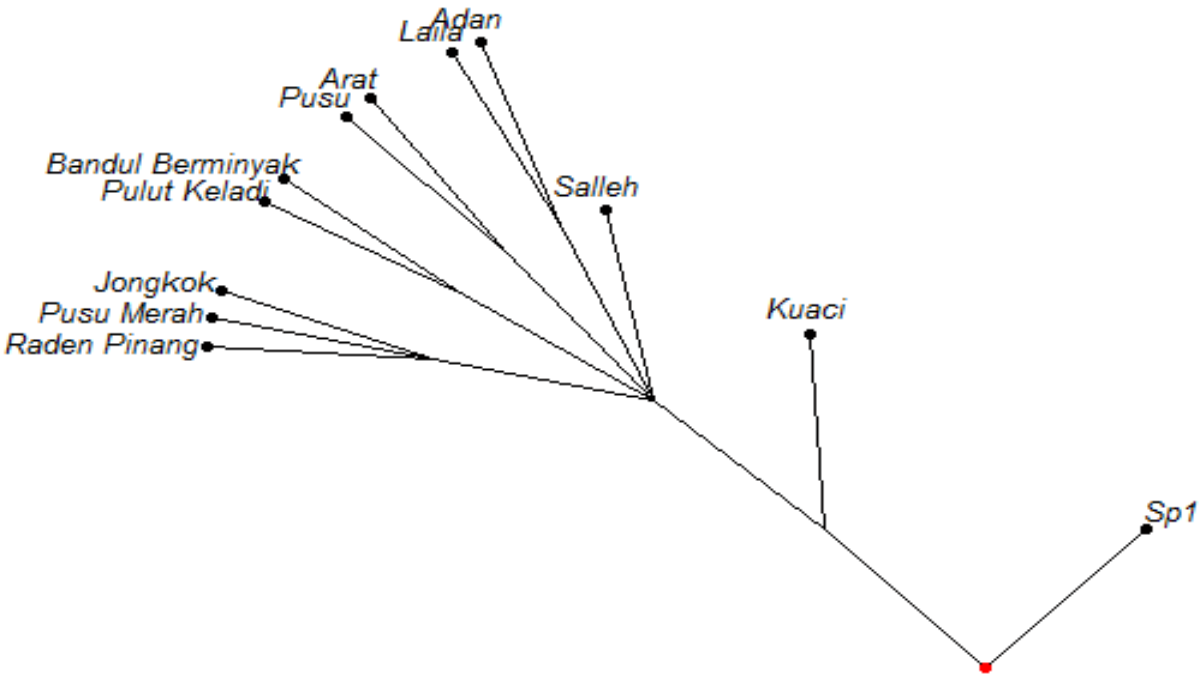

Figure 3. Tree based on neighborhood joining method showing genetic dissimilarity between all 10 rice cultivars investigated.

berminyak in control and exposed to 100 mM salinity level showed no significant different ($p>0.05$). All rice varieties showed reduction in all growth performance when exposed to salt stress, regardless whether they are tolerant or susceptible to salinity stress (Tables 4 and 5). High reduction in plant height occurred in susceptible and highly susceptible varieties except *Pusu Merah* and *Raden Pinang* while *Bandul berminyak* showed the lowest with only 2.98% (Table 4). Only *Bandul berminyak* showed less than 50% reduction in both fresh and dry root weight (Table 5). Moderately tolerant rice cultivars, *Adan* and *Arat*, showed less than 80% reduction when exposed to salinity stress. With the exception of *Raden Pinang*, all susceptible varieties showed more than 80% reduction while highly susceptible varieties showed more than 90% reduction in both fresh and dry root weights.

DISCUSSION

Polymorphism in SSR markers

Our study shows an average number of 10.53 alleles per locus, which was higher than Sarawak bario rice cultivars reported by Wong et al. (2009) and for Iranian and Malaysian rice cultivars reported by Etemad et al. (2012). These reports showed an average of 2.6 and 3.57 alleles per locus, respectively. However, our finding was lower when compared to Bangladesh rice cultivars reported by Rahman et al. (2008) with 15.6 alleles per locus. The mean allele in our study was almost comparable to Rahman et al. (2010) where they detected 11.7 alleles per SSR locus from 28 Bangladesh rice cultivars using seven markers. Etemad et al. (2012) and Shah et al. (2013) reported that the different values of average number of alleles per SSR locus among all these reports could be because of the different genotypes used and the selection of microsatellite markers. SSR markers that produce high PIC value with 0.6 and above were obtained from all loci studied except RM 334, RM 287, RM 307 and RM 151. All these markers gave monomorphic alleles, thus would not help in discriminating the genotypes studied. Wong et al. (2009) and Shah et al. (2013) explained that high PIC value of markers indicated that the number of alleles detected were also high. Their findings are in agreement with this study as the highest PIC value of RM 226 detected 5 numbers of alleles while the lowest PIC value in RM 307 gave only one allele which was monomorphic. High average PIC value of 0.59 obtained in this study might be due to high genetic diversity in all rice cultivars investigated and the fact that the markers used were chosen due to their high PIC value as reported earlier by Temnykh et al. (2000) and Behera et al. (2012). According to Sajib et al. (2012), markers with high PIC value (>0.50) could be used for genetic studies as they are greatly informative and highly polymorphic. These primers would help to differentiate the genotypes studied.

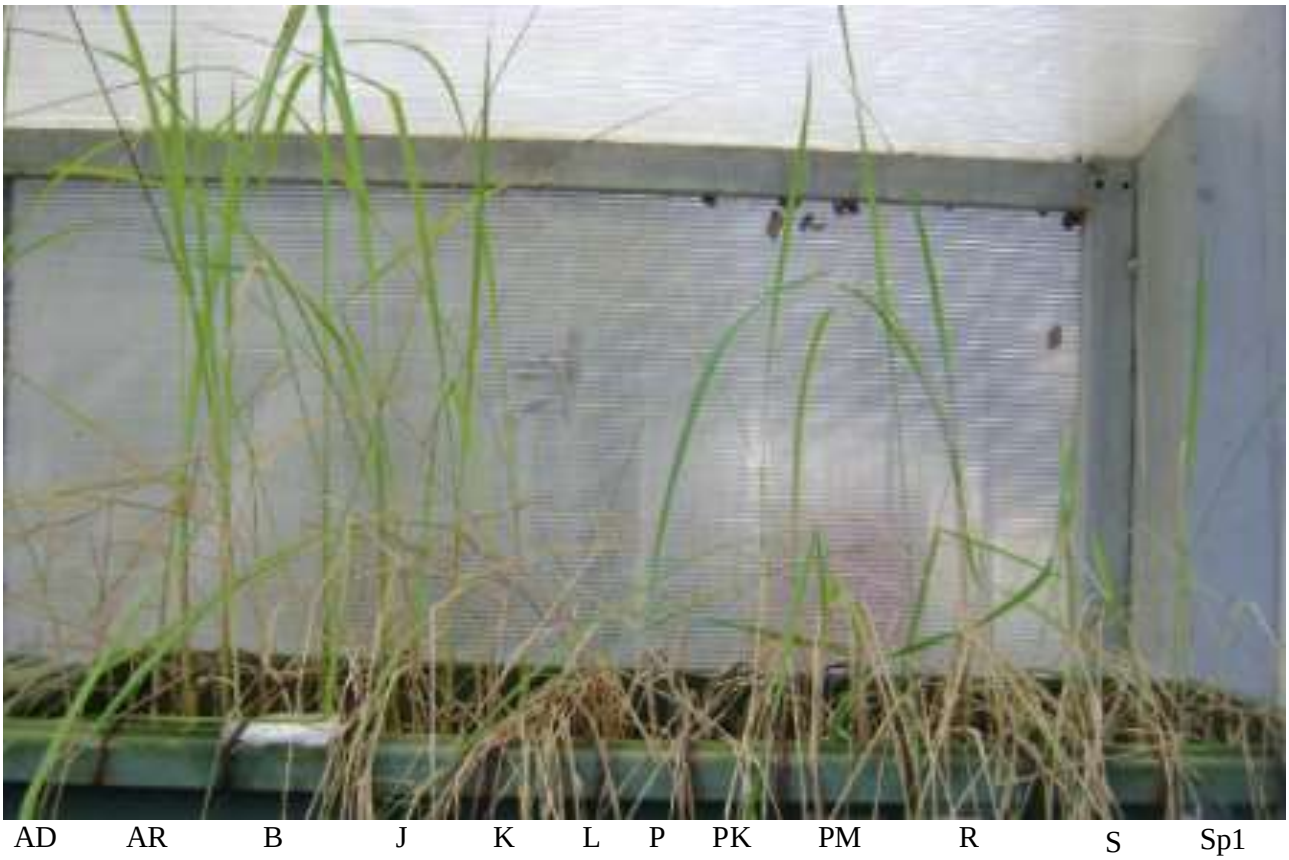

Figure 4. Salinity stress effect at 100 mM on growth of 12 rice cultivars studied.

Table 3. Salinity Tolerance status for all 12 rice cultivars studied.

Cultivar name	Score	Tolerance
Adan	5	Moderately tolerant
Arat	5	Moderately tolerant
Bandul berminyak	3	Tolerant
Jongkok	7	Susceptible
Kuaci	9	Highly susceptible
Laila	9	Highly susceptible
Pusu	9	Highly susceptible
Pulut Keladi	7	Susceptible
Pusu Merah	7	Susceptible
Raden Pinang	7	Susceptible
Salleh	7	Susceptible
Sp1	7	Susceptible

Genetic diversity and relationship among 12 rice cultivars studied

The lowest similarity distance was obtained between *Kuaci* and *Salleh* or *Sp1* while the highest was between *Laila* and *Adan, Pusu* and *Arat, Pulut Keladi* and *Bandul berminyak, Jongkok* and *Pusu Merah*. Cultivars with low genetic similarity as that for *Kuaci* and *Salleh or Sp1* can be chosen and used in breeding program to obtain higher grain quality by intercrossing as suggested by Etemad et al. (2012). Some rice cultivars showed high similarity distance with 1.00, for example, between *Pulut Keladi* and *Bandul berminyak*. According to Wong et al. (2009) such cultivars may probably originate from same source but were given different local names.

Salinity tolerance

High quantum yield for tolerant *Bandul berminyak* compared to other susceptible cultivars was in agreement to result obtained by Cha-um et al. (2007) for two cultivars exposed to 342 mM salinity stress, whereby quantum yield in salt sensitive GS No. 7032 was lower than the salt-tolerant GS No. 4371 when both were exposed to 342 mM salinity stress. This implied that the maximal quantum yield of Photosystem II of this cultivar was not affected by this stress and that this cultivar could be tolerant to salinity. Reduction in growth parameters for all 12 cultivars caused by salinity was in agreement to the

Table 4. Percentage reduction in plant height for 12 cultivars grown in salinity stress.

Rice variety		Reduction in plant height (%)
Tolerant	*Bandul berminyak*	2.98
Moderately Tolerant	*Adan*	9.05
	Arat	8.76
Susceptible	*Jongkok*	14.66
	Pulut Keladi	10.28
	Pusu Merah	8.54
	Raden Pinang	9.04
	Salleh	10.55
	Sp1	19.72
Highly susceptible	*Kuaci*	16.47
	Laila	25.28
	Pusu	22.86

Table 5. Fresh and dry root weight for 12 cultivars grown in 0 and 100 mM salinity levels with percentage reductions.

Rice variety		Reduction in root fresh weight (%)	Reduction in root dry weight (%)
Tolerant	*Bandul berminyak*	25.25	51.13
Moderately tolerant	*Adan*	72.11	74.54
	Arat	73.48	78.83
Susceptible	*Jongkok*	87.65	91.20
	Pulut Keladi	92.43	93.84
	Pusu Merah	90.04	91.04
	Raden Pinang	77.89	67.72
	Salleh	76.74	81.91
	Sp1	90.23	95.41
Highly susceptible	*Kuaci*	95.02	93.35
	Laila	97.71	97.86
	Pusu	93.69	94.77

result reported by Cha-um et al. (2007, 2009). The growth reduction is caused by decreased in photosynthesis due to salt toxicity (Cha-um et al., 2009). Photosynthetic efficiency of salt-sensitive varieties affected more than tolerant varieties. Tolerant *Bandul berminyak* shows similar pattern to salt tolerant rice variety Homjan studied by Cha-um et al. (2009), whereby both showed lower reduction in growth parameters compare to salt-sensitive varieties such as KDML105.

Plant height of all 12 cultivars showed no significant variation due to exposure to salinity stress. However, *Bandul berminyak* showed the lowest reduction in plant height, thus confirming that it is tolerant to salinity stress. Susceptible cultivars in this study showed high reduction in plant height which was similar to susceptible *japonica* variety *Daegudo* and *Guweoldo* reported by Lee et al. (2003). Reduction in plant height of susceptible varieties may have been caused by inhibition of cell expansion in leaf growth zone caused by salinity (Setter et al., 1983; Fraga et al., 2010).

In our study, highly susceptible cultivars showed the highest reduction in both fresh and dry root weights. Our results are in agreement with those reported for *Daegudo* and *Guweoldo* cultivars (Lee et al., 2003). Reduction in root weight may have been caused by the suppression in root growth cause by the production of cytokinins as a result of high saline level (Bottger et al., 1978; Hosseini et al., 2012). From this result, it can be deduced that root weight was the most affected trait by salinity stress. As stated by Lee et al. (2003) and Hosseini et al. (2012), root dry weight is a good parameter to determine salinity tolerance in these 12 cultivars. This is the most obvious

parameter affected by salinity stress, where all cultivars showed more than 50% reduction when exposed to salinity. The grouping shown in cluster and tree diagram analysis (Figure 2 and 3, respectively) does not explicitly reflect salinity tolerance levels. For example, *Pusu* and *Arat* were grouped together when the *Pusu* was regarded as highly susceptible and *Arat* was considered as moderately tolerant. This result was similar to result obtained by Kanawapee et al. (2011) whereby the moderately tolerant cultivar IR64 was grouped together with highly susceptible rice cultivar Khao Kaset and IR34. Zeng et al. (2004) had stated that it was not surprising to observe some sensitive cultivars mixing with tolerance ones in a same cluster. Cluster analysis shown by Kanawapee et al. (2011) produced from RAPD markers did show groupings based on the cultivars salinity tolerance ability. Thus, suggesting RAPD markers are more accurate in addressing cultivars affected by salinity stress. The cluster analysis produced for the 12 rice cultivars studied may have been grouped according to their location and genetic origin (Kanawapee et al., 2011).

Kanawapee et al. (2011) have recommended intercrossing cultivars from different clusters as these cultivars possess different genetic background. Out of 30 different rice cultivars investigated by Kanawapee et al. (2011), they have suggested to intercross KDML 105 with salinity tolerant SPR90 because both possess different genetic background and different physiological tolerance levels to salinity and also have different characteristics and physiology. They reported that the KDML 105-derived progeny would possess improved characteristics than both parents. Thus, SSR markers are the promising marker for rice breeder as they provide faster and precise genetic information of potential parents than the conventional breeding and also helps to produce improved rice cultivar that possess better characteristics than both parents.

Results obtained for physiological responses to salinity tolerance and genetic variation studies conducted for 12 cultivars here implies that it is desirable to intercross *Arat* (moderately tolerant) and *Sp1* (Susceptible) as both of them are from different clusters, show low genetic similarity with 0.33 and possess contrasting salinity tolerance levels. Even though *Kuaci* and *Sp1* or *Salleh* showed the lowest genetic similarity, intercrossing these cultivars may not produce an improved cultivar when compared to *Arat* and *Sp1*.

Further investigations should be carried out to assess the success of interbreeding *Arat* and by comparing the growth performance and yield between their progeny and both parents to confirm that the progeny would show better quality.

Conflict of Interests

The authors have not declared any conflict of interests.

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