Genetic diversity of Vietnamese lowland rice germplasms as revealed by SSR markers in relation to seedling vigour under submergence

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ABSTRACT

In the direct-seeding rice cultivation system, seedling vigour is one of the most important traits for stable stand establishment during early seedling stages, particularly under submergence that is caused by temporal flash flood. We studied the genetic diversity in a set of 40 Vietnamese lowland rice varieties using 30 simple sequence repeat (SSR) markers covering all rice chromosomes. A total of 111 alleles were detected, with a mean of 3.7 alleles per locus. The number of polymorphic alleles detected by each SSR marker ranged from 2 to 6. The fragment size of a given SSR locus varied between 85 and 650 bp and the frequency of a major allele at each locus ranged from 32.5% to 76.9%. Polymorphism information content value varied from 0.355 to 0.774 with an average of 0.594. The genetic similarity calculated between pairs of rice varieties ranged from 0.03 to 0.97 with an average of 0.27. According to a constructed dendrogram of unweighted pair group method with arithmetic mean based on the SSR marker analysis, the tested rice varieties were clustered into two major groups consisting of five subgroups. Significant correlations existed between the mean genetic similarity and the mean seedling vigour estimated by shoot length under submergence among the tested varieties. Our results suggested usefulness of the SSR marker system to assess genetic diversity in Vietnamese rice germplasms in relation to their seedling vigour under submergence.

KEYWORDS

Genetic diversity; simple sequence repeat (SSR) markers; allele frequency; seedling vigour; submergence stress; rice (Oryza sativa L.)

Introduction

Rice (Oryza sativa L.) is a key staple crop providing food for half of the world’s population and a valuable cash crop particularly in rice-exporting countries including Vietnam. Rice cultivation has been impacted by various environmental stresses. Flooding is one such stress and is thought to affect about 20 million hectares of rice growing areas in Asia (excluding China) as well as significant areas of lowland rice production in Africa.[1] In Vietnam, rice planting area occupies ca. 7.89 million hectares, of which 6.97 million hectares (88.3%) are lowland. Recently, annual flooding has been a big problem for rice production in these regions, causing significant economic losses. Seedling vigour, in general, is a trait that expresses itself as the ability of seedlings to rapidly elongate after germination and emerge above the water surface for escaping submergence stress.[2–5] This trait is particularly important for optimum stand establishment during early seedling stages in the direct-seeding rice cultivation system in paddy field.[6,7]

Seedling vigour is a complex trait influenced by the interaction between many genes and environmental conditions.[8] Genetic diversity is a prerequisite and a vital resource for plant breeders to improve the effective breeding programmes. Many rice genotypes have evolved to adapt to various environments, including irrigated, rain-fed lowland and upland ecosystems between 55 N° and 36 S° latitude.[9,10] However, it has been estimated that not even 15% of the potential diversity has been utilized. This implies that a vast majority of valuable allelic variations in agronomic traits of economic significance remain unutilized.[11] Vietnam is a reservoir of diverse rice germplasms and is a home land to many traditional rice varieties or landraces with unique and important traits. Since 1996, more than 3000 Vietnamese traditional rice accessions have been collected from different ecosystems by the national project conducted by National Plant Resource Centre, Vietnam Academy of Agricultural Sciences. These rice germplasms have a great potential for the future rice breeding programmes in Vietnam. However, the evaluation of germplasms has
so far only been conducted on morpho-physiological traits. In addition, the lowland rice fields of deltaic regions have often been inundated during the rainy season. Many rice direct-seeding fields have been almost completely destroyed by surging floodwaters. So far, molecular tools have not been effectively utilized in studying the mechanisms of controlling rice seedling vigour under flooding conditions. The floods often cause severe reduction of rice yield at regions vulnerable to submergence in Vietnam. Thus, genetic evaluation and improvement of rice germplasms for submergence tolerance is one of the most urgent goals toward breeding rice cultivars with enhanced seedling vigour under submerged conditions.

Compared to the conventional morpho-physiological traits, the molecular markers are more powerful tools for the assessment of genetic variation and elucidation of genetic relationships within and among species.[12] Many different types of molecular markers are now available for evaluating the genetic diversity in rice, such as restriction fragment length polymorphism markers, random amplified polymorphic DNA markers, simple sequence repeats (SSRs) markers and single nucleotide polymorphisms markers, the last of which is the newest type. We selected the SSR markers, which have been widely used in genetic diversity analysis and mapping works because of their merits, such as quickness, simplicity, richness in polymorphism and stability.[13,14] Furthermore, approximately 20,000 SSR markers have been mapped to specific chromosomal sites throughout the whole rice genome [15] and they have been extensively used to assess the genetic diversity in a variety of rice germplasms. Such study includes diversity analysis of high yielding cultivars,[16] Indian aromatic and quality rice [17] and traditional lowland rice.[18] Recently, SSR markers have been applied for the molecular characterization and genetic diversity analysis of salinity tolerant rice,[19] aromatic landraces [20] and Vietnamese upland rice cultivars.[21] These studies have indicated that SSR markers are efficient in detecting genetic polymorphisms and in reliably discriminating genotypes among diverse rice germplasms.

Vietnam is located in the sub-climatic regions and has been suffering from the negative impacts of climate changes on the agricultural production. Rice cultivation in lowland paddy fields in Vietnam can be divided into three major regions including the Red River Delta (Northern Delta), Mekong River Delta (Southern Delta) and Central Delta. In these regions, poor crop establishment poses a major problem for rice production, particularly in lowland fields prone to early-season floods. Therefore, deployment of research on the genetic system for abiotic stress tolerance, such as seedling vigour under submergence, plays an important role in achieving high productivity in rice in these flood prone regions. Keeping the foregoing threat in mind, we evaluated the usefulness of SSR markers in determining the genetic diversity among the selected local rice germplasms that have been collected from a wide range of lowland regions in Vietnam. Possible correlation between the genetic relatedness and the level of seedling vigour among local cultivars from the three major rice-growing regions was also studied.

Materials and methods

Plant materials and bioassay conditions for seedling vigour

Rice germplasms used in this study comprised a set of 40 accessions including 36 local lowland rice varieties with different origins collected from 20 provinces of Vietnamese Deltas plus 4 check commercial cultivars (Table 1). Seedling vigour was evaluated based on shoot (coleoptile) lengths of germinating seedlings after five days of dark submergence, according to the test tube method. [22] Briefly, imbibed seeds were sterilized in 1% (w/v) sodium hypochlorite (NaClO) solution for 10 min and rinsed with distilled water. They were then allowed to germinate on wet glass petri dishes (70-mm diameter) in a dark incubator at 28 °C for three days. The seeds were washed every day before germination. Ten germinating seeds per accession at the pigeon breast stage were transferred to a glass test tube filled with 10 cm deep distilled water. Uncovered test tubes were placed in dark without changing the water for five days. Shoot lengths were measured from the base to the tip of the shoots at the end of the five days’ stress period. These accessions were selected from the rice germplasm collection maintained by the National Gene Bank of Plant Resource Center of Vietnam for studying the genetic diversity in Vietnamese rice, because they represent different unique lowland varieties from various provinces of the three Vietnamese Delta regions. A japonica cultivar Nipponbare and an indica cultivar Kasalath were used as high and low seedling vigour control cultivar, respectively, according to Manangkil et al. [22]

Genomic DNA extraction and simple sequence repeat assay

Total genomic DNA was extracted from rice seedlings according to the modified hexadecyltrimethylammonium bromide method.[23] DNA quality was ensured by electrophoresis in an agarose gel and quantification was accomplished using a spectrophotometer (NanoDrop 2000). After extraction, DNA was stored at −20 °C until
further use. A total of 30 SSR markers covering all 12 rice chromosomes (Table 2) were selected from the Gramene Markers Database (http://www.gramene.org/markers/) for genetic diversity analysis. Polymerase chain reaction (PCR) was performed in 96-well plates using the Eppendorf thermocycler (Eppendorf AG, Germany). A reaction mixture (15 μL) contained 2 μL DNA template, 1.5 μL PCR buffer (10X DreamTaq buffer containing 20 mM MgCl2), 1 μL 2mM of dNTPs, 0.5 μL of each of the forward and reverse primers (Invitrogen, USA), 8.5 μL MiniQ water (Merck Millipore, Germany) and 1 μL (5 U/μL) DreamTag DNA Polymerase (Thermo Scientific, USA). The PCR profile started with 94 °C for 5 min, followed by 35 cycles, each comprising denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and a final extension step at 72 °C for 5 min. The amplified PCR products were mixed with bromophenol blue dye and separated in non-denaturing 8% polyacrylamide gel electrophoresis. A molecular weight DNA marker (100 bp, Nacalai Tesque, Japan) was loaded on either side of the gel. After electrophoresis, the gel was stained with ethidium bromide in dark for 15 min. The gels were scanned on the high performance UV transilluminator (UVP, USA) and photo images were saved on a

### Table 1. List and mean shoot length of Vietnamese lowland rice varieties under submerged conditions collected from different regions.

| No. | Accession no. | Designation | Name of variety | Mean shoot length (cm ± SE) | Location |
|-----|---------------|-------------|----------------|----------------------------|----------|
| 1   | 124           | V1          | Du Thom Hai Duong | 3.28正 ± 0.12 | Red River Delta |
| 2   | 215           | V2          | Tam den Hai Phong | 2.95正 ± 0.34 | Red River Delta |
| 3   | 303           | V3          | Tam thom Hai Duong | 2.98正 ± 0.17 | Red River Delta |
| 4   | 467           | V4          | Nep hoa vang Bac Ninh | 2.74正 ± 0.09 | Red River Delta |
| 5   | 542           | V6          | Du den Thai Binh | 3.43正 ± 0.09 | Red River Delta |
| 6   | 543           | V7          | Du Ninh Binh | 3.18正 ± 0.22 | Red River Delta |
| 7   | 799           | V8          | Du trang Nam Dinh | 3.14正 ± 0.20 | Red River Delta |
| 8   | 868           | V9          | Lua di Son Tay | 3.00正 ± 0.14 | Red River Delta |
| 9   | 888           | V11          | Te loc Hoa Binh | 3.20正 ± 0.18 | Red River Delta |
| 10  | 892           | V12          | Hom rau Nam Dinh | 3.16正 ± 0.19 | Red River Delta |
| 11  | 1048          | V13         | Tam xoan Hai Hau | 4.55正 ± 0.19 | Red River Delta |
| 12  | 1126          | V15         | Canh nong Bac Giang | 3.62正 ± 0.14 | Red River Delta |
| 13  | 1290          | V19         | Bau Hai Duong | 3.05正 ± 0.20 | Red River Delta |
| 14  | 2442          | V21         | Hom Binh Luc | 2.73正 ± 0.22 | Red River Delta |
| 15  | 2463          | V23         | Tep Hai Phong | 4.47正 ± 0.23 | Red River Delta |
| 16  | 3550          | V25         | Chanh trui | 3.57正 ± 0.18 | Red River Delta |
| 17  | 5127          | V26         | Lua cham | 3.77正 ± 0.07 | Red River Delta |
| 18  | 6188          | V27         | Cuom dang 1 | 2.95正 ± 0.16 | Red River Delta |
| 19  | 6189          | V28         | Cuom dang 2 | 3.67正 ± 0.16 | Red River Delta |
| 20  | 6192          | V29         | Nep oc | 3.29正 ± 0.08 | Red River Delta |
| 21  | 6196          | V30         | Nep non tre | 3.19正 ± 0.16 | Red River Delta |
| 22  | 6206          | V31         | Re nuoc | 4.64正 ± 0.06 | Red River Delta |
| 23  | 12749         | V36         | Doc do | 3.32正 ± 0.18 | Red River Delta |
| Mean |               |             |               | 3.39正 ± 0.16 |          |
| 24  | 473           | V5          | Nep thom Nghe An | 2.94正 ± 0.26 | Central Delta |
| 25  | 882           | V10         | Bang muon Nghe An | 3.02正 ± 0.16 | Central Delta |
| 26  | 1108          | V14         | Ba la Nghe An | 3.05正 ± 0.09 | Central Delta |
| 27  | 1159          | V16         | Chiem so 1 Thanh Hoa | 2.70正 ± 0.22 | Central Delta |
| 28  | 1274          | V17         | Ven lua Nghe An | 2.88正 ± 0.09 | Central Delta |
| 29  | 1289          | V18         | Nep dau Thua Thieng Hue | 2.66正 ± 0.18 | Central Delta |
| 30  | 2456          | V22         | Ba thang nuoc Nghe An | 3.59正 ± 0.07 | Central Delta |
| 31  | 3362          | V24         | Lua heo Quang Nam | 3.18正 ± 0.32 | Central Delta |
| 32  | 6337          | V32         | Chiem do | 3.82正 ± 0.24 | Central Delta |
| Mean |               |             |               | 3.10正 ± 0.18 |          |
| 33  | 1613          | V20         | Nang thom Cho Dao | 4.48正 ± 0.07 | Mekong River Delta |
| 34  | 9218          | V33         | Tep hanh | 4.45正 ± 0.13 | Mekong River Delta |
| 35  | 12099         | V34         | OM35056 I (OMCS21) | 4.60正 ± 0.18 | Mekong River Delta |
| 36  | 12748         | V35         | Nanth chon | 4.57正 ± 0.19 | Mekong River Delta |
| Mean |               |             |               | 4.53正 ± 0.14 |          |
| 37  | N/A           | V37         | TL6          | 4.05正 ± 0.20 | Northern cultivated |
| 38  | N/A           | V38         | DS3          | 4.38正 ± 0.11 | Temperate country |
| 39  | N/A           | V39         | JO1          | 5.41正 ± 0.08 | Temperate country |
| 40  | N/A           | V40         | G184-Pelanh | 4.97正 ± 0.11 | Northern cultivated |

Note: Significantly lower (**/) and higher (#/##) than the high seedling vigour check variety Nipponbare at 1% and 5% levels, respectively. Not significantly different from Nipponbare (ns); standard error (SE). Do not have an accession number (N/A). Least significant difference (LSD) (5%) = 0.32; LSD (1%) = 0.41.
computer. The reproducibility of the obtained amplification products was confirmed by two independent experiments for each primer.

**Scoring SSR alleles and analysis of SSR data**

The size of the amplified band for each SSR marker was determined based on its migration in comparison with the size standard (100 bp DNA ladder) using AlphaEase FC 5.0 software. All alleles were scored manually and double-checked by two independent experimenters. The number of alleles per locus, major allele frequency, genetic diversity/similarity and polymorphism information content (PIC) value was calculated using PowerMarker Version 3.25. Genetic similarity indices (Dice coefficient) based on the 30 SSR markers were calculated. To detect unique alleles, the number of accessions having a specific allele was counted for each locus. Alleles that were observed in only one or two of the 40 accessions were considered unique for the specific locus. For each marker, allelic bands were scored at each locus using the binary coding system, ‘1’ for the presence of band, ‘0’ for the absence of band and ‘9’ for missing data. The data matrices were made and analysed to produce a dendrogram by using an unweighted pair group method with arithmetic mean (UPGMA) with the module of sequential agglomerative hierarchical nested (SAHN) in the NTSYS-pc version 2.1.

**Statistical analyses**

For bioassay, 10 seedlings were used per genotype (germplasm accession). All experiments were laid out following a complete randomized block design with three replications. Analysis of variance was performed using Microsoft Excel Programme. Least significant differences were calculated using a software programme Analyze-it General 1.68 version. The levels of seedling vigour under submergence measured based on the mean shoot length data were compared by the Tukey’s honest significance difference (HSD) test and further compared among the SSR marker-based UPGMA subgroups, as well as among accessions representative of different locations.

The matrix of similarity was created from all pairs of rice genotypes. Dice’s genetic distance matrix was based on the SSR markers and the seedling vigour matrix was based on the mean shoot length. The correspondence of

### Table 2. Summary results of 30 SSR markers across 40 genotypes.

| No. | Marker | Chr. no. | Position (Mb) | Size range (bp) | Number of alleles | Major allele frequency (%) | PIC value | Genetic diversity |
|-----|--------|----------|---------------|----------------|-------------------|----------------------------|-----------|-------------------|
| 1   | RM6    | 2        | 29.57         | 163–400        | 4                 | 47.5                       | 0.644     | 0.710             |
| 2   | RM16   | 3        | 23.13         | 150–200        | 4                 | 50.0                       | 0.646     | 0.704             |
| 3   | RM80   | 8        | 24.48         | 142–200        | 6                 | 32.5                       | 0.774     | 0.807             |
| 4   | RM85   | 3        | 23.1         | 85–107         | 3                 | 53.7                       | 0.581     | 0.656             |
| 5   | RM152  | 8        | 0.68          | 151–180        | 3                 | 45.0                       | 0.626     | 0.704             |
| 6   | RM159  | 5        | 0.49          | 210–248        | 4                 | 55.0                       | 0.596     | 0.660             |
| 7   | RM162  | 6        | 24.04         | 200–229        | 3                 | 65.0                       | 0.485     | 0.564             |
| 8   | RM163  | 5        | 19.19         | 124–180        | 3                 | 52.5                       | 0.604     | 0.676             |
| 9   | RM224  | 11       | 120.1        | 110–157        | 3                 | 47.5                       | 0.636     | 0.708             |
| 10  | RM227  | 3        | 34.93         | 106–120        | 2                 | 62.5                       | 0.469     | 0.579             |
| 11  | RM229  | 11       | 18.41         | 116–130        | 3                 | 65.0                       | 0.515     | 0.570             |
| 12  | RM235  | 12       | 26.11         | 100–124        | 2                 | 76.9                       | 0.355     | 0.413             |
| 13  | RM257  | 9        | 17.72         | 110–180        | 4                 | 45.0                       | 0.625     | 0.699             |
| 14  | RM307  | 4        | 0            | 100–300        | 6                 | 46.7                       | 0.683     | 0.724             |
| 15  | RM316  | 9        | 1.8          | 192–250        | 3                 | 72.6                       | 0.399     | 0.447             |
| 16  | RM341  | 2        | 82.7         | 100–172        | 4                 | 43.9                       | 0.642     | 0.708             |
| 17  | RM485  | 2        | 0.93          | 291–650        | 6                 | 57.1                       | 0.601     | 0.649             |
| 18  | RM493  | 1        | 12.28         | 211–250        | 5                 | 45.0                       | 0.708     | 0.750             |
| 19  | RM518  | 4        | 2.03          | 171–190        | 3                 | 50.0                       | 0.620     | 0.693             |
| 20  | RM528  | 6        | 26.55         | 232–250        | 4                 | 55.0                       | 0.570     | 0.650             |
| 21  | RM562  | 1        | 14.63         | 200–243        | 3                 | 60.0                       | 0.549     | 0.617             |
| 22  | RM1243 | 7        | 3.55          | 157–200        | 5                 | 37.5                       | 0.746     | 0.786             |
| 23  | RM7075 | 1        | 15.12         | 110–155        | 4                 | 34.1                       | 0.694     | 0.745             |
| 24  | RM7338 | 7        | 15.38         | 130–164        | 4                 | 62.5                       | 0.554     | 0.599             |
| 25  | RM10793| 1        | 12.56         | 123–190        | 4                 | 37.2                       | 0.706     | 0.759             |
| 26  | RM16589| 4        | 11.23         | 195–210        | 2                 | 73.8                       | 0.566     | 0.637             |
| 27  | RM25022| 10       | 3.58          | 150–300        | 5                 | 52.5                       | 0.618     | 0.660             |
| 28  | RM25181| 8        | 8.84          | 162–180        | 3                 | 47.5                       | 0.611     | 0.691             |
| 29  | RM27877| 12       | 9.18          | 185–200        | 3                 | 75.0                       | 0.395     | 0.443             |
| 30  | RM28746| 12       | 26.39         | 157–190        | 3                 | 52.5                       | 0.596     | 0.670             |

| Total | 111 |
| Mean  | 3.70|

Note: Chromosome number (Chr. No.); *marker position is expressed in centimorgan (cM); position in Mb is not known; polymorphism information content (PIC).
the two matrices of Dice similarity index and the mean shoot length was performed by means of MXCOMP procedure of NTSYS-pc.[27] Also, the significance of the correlation between the two matrices was analysed with the Mantel test.[28]

**Results and discussion**

**Seedling vigour under submergence**

Seedling vigour in germinating seeds plays a key role in maintaining uniform and optimum stand establishment and in avoiding or escaping flooding stress in the direct-seeded rice cultivation system. In our study, 36 local varieties plus 4 commercial cultivars were selected after a survey of Vietnamese rice germplasms based on the evaluation of seedling vigour under submergence by the test tube bioassay method.[22] This method, based on the ability of shoot elongation, is reliable and effective for the evaluation of the significant and consistent cultivar differences in seedling vigour under submerged conditions. The results showed that the levels of seedling vigour of nine varieties, including seven local ones, were significantly higher than that of high seedling vigour check Nippobare (Table 1). Four among these seven were from Mekong River Delta. Among the four commercial cultivars, a japonica type JO1 and glutinous G184-Pelanh showed very high levels of seedling vigour.

**Overall allelic diversity of SSR markers**

Unlike the morphological and biochemical markers, molecular markers are affected neither by environmental factors nor by growth practices.[29] SSR markers have played an important role in studying germplasm diversity in rice.[30] In the present study, the genetic diversity was estimated by the total number of alleles, mean number of alleles, mean genetic diversity/similarity and PIC values, all of which are shown in Table 2.

All 30 SSR markers were found to be polymorphic across the 40 tested varieties and thus generated informative allelic profiles. The overall size of amplified products ranged from 85 bp (for RM85) to 650 bp (RM485). A total of 111 different reproducible alleles were detected, ranging from 2 (RM227, RM235 and RM16589) to 6 (RM80, RM307 and RM485) with an average of 3.7 alleles per locus. An example of SSR marker with multiple alleles (four alleles) as resolved by PCR assay for RM10793 locus is shown in Figure 1. The average number of alleles per locus obtained in our study was comparable to those reported in two earlier studies with an average of 3.8 alleles per SSR locus,[20,31] but quite lower than an average of 4.53 and 4.5 alleles per locus reported by Hossain et al.[11] and Pervaiz et al.,[32] respectively. On the other hand, our mean value was higher than those reported in studies performed by using smaller numbers of germplasms, in which an average of 2.6 [33] or 3.33 [34] alleles per locus were reported. Such variability in the number of detectable alleles per locus might primarily be due to the degree of diversity existing in the tested germplasms and differences of SSR markers with scorable alleles used in different studies. Our result suggested that the local varieties and check cultivars selected in our study harboured enough genetic divergence. As few as 30 SSR markers covering all rice chromosomes could provide effective means to assess the genetic diversity of rice germplasms of various sources in Vietnam.

The frequency of a major allele at each locus ranged from 32.5% (RM80) to 76.9% (RM235). On the average, 53.4% of the 40 rice accessions shared a common major allele at any given locus (Table 2). The level of genetic diversity existing among 30 SSR loci studied across the 40 accessions ranged from 0.413 (RM235) to 0.807 (RM80) with an average of 0.656. Our results indicated that these local lowland rice cultivars collected from three different geographical regions in Vietnam contain a high genetic diversity.

**PIC value and unique alleles**

PIC value that reflects the allele frequency and diversity among the accessions varied from one locus to another. The PIC values for the 30 SSR markers varied from the lowest value of 0.355 (RM235) to the highest value of 0.774 (RM80), with a mean of 0.594 (Table 2). Markers showing PIC value higher than 0.5 are considered informative and useful for the detection of more alleles in germplasm accessions and distinguishing the polymorphic rate of a marker at a specific locus.[35] Because the majority (83%) of the SSR markers used in this study showed PIC values higher than 0.5, they were all judged to be highly informative. The mean PIC value that was obtained in the present study agreed very well with the mean PIC value (0.60) reported by using 35 Asian rice
These mean PIC values were higher than 0.508, recorded by using aromatic rice germplasm,[20] although they were lower than those obtained in some earlier reports, with average PIC values ranging from 0.66 to 0.78.[12,21,36]

Many unique alleles can be found in traditional varieties including local landraces. This is because they are widely adapted to stressed local environments and often possess some unique genes that have enabled them to survive. SSR markers could generate unique SSR profiles in rice by using a few primers that covered all rice chromosomes.[37] In our study, alleles that were observed in only one or two of the 40 cultivars were considered unique for the specific locus. A total of eight unique alleles (7.2% of 111) that were associated with seven rice cultivars were detected at six SSR loci (Table 3). The number of unique alleles per locus was either one or two. Most of the markers that amplified such unique alleles showed PIC values higher than 0.60, except for RM316 that showed a low PIC value of 0.399. All of the unique alleles were detected in the gene pools of traditional varieties (V10 and V22 from Central Delta and V6, V8, V15, V25 and V26 from Red River Delta in Tables 1 and 3). A possible relationship of these unique alleles with quantitative trait locus (QTL) regions associated with seedling vigour under submergence in rice [7] must be studied.

Table 3. SSR loci that amplified unique alleles in different rice genotypes.

| No. | Markers | Number of unique alleles | Allele size (bp) | Genotype |
|-----|---------|--------------------------|-----------------|----------|
| 1   | RM6     | 1                        | 400             | Ba thang nuoc Nghe An (V22) |
| 2   | RM357   | 1                        | 180             | Bang muon Nghe An (V10) |
| 3   | RM307   | 2                        | 250/300         | Chanh trui (V25); Du trang Nam Dinh (V8) |
| 4   | RM316   | 1                        | 250             | Canh nong Bac Giang (V15) |
| 5   | RM485   | 1                        | 550             | Du trang Nam Dinh (V8) |
| 6   | RM25022 | 2                        | 250/300         | Du den Thai Binh (V6); Lua cham (V26) |
| Total | 8      |                          |                 | Seven cultivars |

Genetic diversity and relationship among Vietnamese lowland rice germplasms

Genetic diversity and relationship among the accessions including 36 lowland rice varieties was further studied by UPGMA cluster analysis, as shown in Figure 2. Genetic similarity indices based on the 30 SSR markers were calculated by using Dice coefficient [25,26] and varied from 0.97 to 0.03 with an average of 0.27 (Table 1S in the Online Supplementary Appendix), indicating a wide range of genetic variations among the rice germplasms in Vietnam. Other studies using SSR markers revealed similar results by obtaining different levels of variation in lowland [38] and upland [21] rice. Our highest genetic similarity value (0.97) corresponded to a pair of ‘Cuom dang 1 (V27)’ and ‘Nep oc (V29)’. The least similarity value (0.03) was found between ‘Nang thom Cho Dao (V20)’ and ‘Nep dau Thua Thien Hue (V18)’ as well as ‘Re nuoc (V31)’ (Table 1S in the Online Supplementary Appendix). An UPGMA dendrogram revealed two distinct groups (Groups I and II) at a level of genetic similarity of 0.14 (Figure 2). Group I consisted of 31 accessions, which were sorted into three subgroups: Subgroup 1 contained most of the lowland rice accessions collected from Red River Delta, Subgroup 2 consisted of three accessions from Mekong River Delta and one from Red River Delta, and Subgroup 3 with 13 accessions consisted mostly of ones from Red River Delta and Central Delta. Group II comprised nine remaining accessions and was further divided into two subgroups. Subgroup 4 contained of two accessions from Red River Delta. Three out of the four commercial check cultivars were clustered into subgroup 5 together with three Central Delta and one Red River Delta varieties (Figure 2).

Next, we compared the level of seedling vigour under submergence based on the mean shoot length data among the SSR marker-based UPGMA subgroups and also among accessions representative of different locations. Tukey’s HSD test revealed significant ($\alpha = 0.05$)

Figure 2. An UPGMA cluster dendrogram showing the genetic relationship between 40 Vietnamese lowland rice varieties (V1–V40 shown in Table 1) based on 30 SSR markers. Note: Group (I, II); subgroups (1)–(5).
differences in the mean seedling vigour values among the three locations, Mekong River Delta (Southern delta) group being the highest level of seedling vigour (Table 4). There was a significant difference (α = 0.05) between Groups I and II classified by the 30 SSR markers. Significant differences among the five subgroups were also observed: Subgroup 2, consisting of most accessions from Mekong River Delta, showed the highest seedling vigour when compared to the other subgroups. Subgroups 1 and 3, consisting of almost all cultivars originated from the Red River Delta and Central Delta, which are at a close geographic distance, showed the lowest seedling vigour. Further, we studied a possible relationship between the genetic similarity indices based on SSR markers and the level of seedling vigour by comparisons between the two different matrices. The study showed a low but significant correlation between the genetic similarity and seedling vigour (r = 0.233; P = 0.001) among all the accessions, indicating that varieties with higher genetic similarity indices tended to show more similar levels of seedling vigour under submergence (Figure 3). Our result suggests that UPGMA clustering based on SSR markers can give good information on the level of seedling vigour under submergence among Vietnamese rice germplasms with diverse genetic backgrounds.

Our result showed that the rice varieties collected from the same region were grouped together according to two criteria. This suggested the existence of a certain level of genetic similarity in gene pools corresponding with its geographic distribution. Success of crop improvement programmes depend on the magnitude of genetic diversity and the extent to which the desirable characters are heritable.[37,39] The genetic diversity assessment with a high level of marker polymorphism in this study provided useful information for future breeding. This may improve seedling vigour under submergence that supports the direct-seeded rice ecosystem in Vietnam. A QTL mapping study of seedling vigour under submergence is underway using a suitable mapping population and a larger number of SSR markers by taking into consideration the information obtained in our previous study.[7]

**Conclusions**

This is the first study on characterization of the genetic diversity at a molecular level in lowland rice germplasms originated from various regions of Vietnamese Deltas. The local varieties showed a high degree of genetic diversity, according to SSR marker polymorphism, which was correlated with seedling vigour under submergence. The allelic diversity, revealed in this study, provides us with tools for the future QTL mapping study and marker assisted selection of this important agronomic trait.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Supplemental data**

Supplemental data for this article can be accessed at [http://dx.doi.org/10.1080/13102818.2015.1085330](http://dx.doi.org/10.1080/13102818.2015.1085330).
References

[1] Bailey-Serres J, Fukao F, Ronald P, et al. Submergence tolerant rice: SUB1’s journey from landrace to modern cultivar. Rice. 2010;3:138–147.

[2] Cisse ND, Ejeta G. Genetic variation and relationships among seedling vigor traits in sorghum. Crop Sci. 2003;43:824–828.

[3] Cui KH, Peng SB, Xing YZ, et al. Molecular dissection of seedling-vigor and associated physiological traits in rice. Theor Appl Genet. 2002;105:745–753.

[4] Ranawake AL, Amarasinghe U, Senanayake SGJN. Submergence tolerance of some modern rice cultivars at seedling and vegetative stages. J Crop Weed. 2014;10:240–247.

[5] Vu HTT, Manangkil O, Mori N, et al. Post-germination seedling vigor under submergence and submergence-induced SUB1A gene expression in indica and japonica rice (Oryza sativa L.). Aust J Crop Sci. 2010;4:264–272.

[6] Magneschi L, Perata P. Rice germination and seedling growth in the absence of oxygen. Ann Bot. 2009;103:181–196.

[7] Manangkil OE, Vu HTT, Mori N, et al. Mapping of quantitative trait loci controlling submergence in rice (Oryza sativa L.) under submergence. Euphytica. 2013;192:63–75.

[8] Jackson MB, Ram PC. Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. Ann Bot. 2003;91:227–241.

[9] Khush GS. Origin, dispersal, cultivation and variation of rice. Plant Mol Biol. 1997;35:25–34.

[10] Lin HY, Wu YP, Hour AL, et al. Genetic diversity of rice cultivars differing in salinity tolerance based on RAPD and SSR markers. Electron J Biotechnol. 2011;14:2–2.

[11] Ovesna J, Polakova K, Leisova L. DNA analyses and their applications in plant breeding. Czech J Genet Plant Breed. 2007;20:1–10.

[12] Thomson MJ, Septiningsih EM, Suwardjo F, et al. Genetic diversity analysis of traditional and improved Indonesian rice (Oryza sativa L.) germplasm using microsatellite markers. Bangladesh J Genet Plant Breed. 2007;20:1–10.

[13] Ma H, Yin Y, Guo ZF, et al. Establishment of DNA fingerprinting of Liaojing series of japonica rice. Middle-East J Sci Res. 2011;8:384–392.

[14] Zhang SB, Zhu Z, Zhao L, et al. Identification of SSR markers closely linked to eui gene in rice. Hereditas (Beijing). 2007;29:365–370.

[15] International Rice Genome Sequencing Project. The map-based sequence of the rice genome. Nature. 2005;436:793–800.

[16] Ni J, Colowit PM, Mackill DJ. Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci. 2002;42:601–607.

[17] Jain S, Jain RK, McCouch SR. Genetic analysis of Indian aromatic and quality rice (Oryza sativa L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theor Appl Genet. 2004;109:965–977.

[18] Bhuyan N, Borah BK, Sarma RN. Genetic diversity analysis in traditional lowland rice (Oryza sativa L.) of Assam using RAPD and ISSR markers. Curr Sci. 2007;93:967–972.

[19] Kanawatee N, Sanitchon J, Srihaban P, et al. Genetic diversity analysis of rice cultivars (Oryza sativa L.) differing in salinity tolerance based on RAPD and SSR markers. Electron J Biotechnol. 2011;14:2–2.

[20] Hossain MM, Islam MM, Hossain H, et al. Genetic diversity analysis of aromatic landraces of rice (Oryza sativa L.) by microsatellite. Genes Genomes Genomics. 2012;6:42–47.

[21] Nguyen TT, Nguyen TMN, Hoang HL, et al. Genetic diversity in Vietnamese upland rice germplasm revealed by SSR Markers. J Fac Agr Kyushu Univ. 2012;57:383–391.

[22] Manangkil OE, Vu HTT, Yoshiida S, et al. A simple, rapid and reliable bioassay for evaluating seedling vigor under submergence in indica and japonica rice (Oryza sativa L.). Euphytica. 2008;163:267–274.

[23] Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987;19:11–15.

[24] Liu K, Muse SV. Power Marker: an integrated analysis environment for genetic marker analysis. Bioinformatics. 2005;21:2128–2129.

[25] Dice LR. Measures of the amount of ecologic association between species. Ecology. 1945;26:297–302.

[26] Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA. 1979;76:5269–5273.

[27] Rohlf FJ. NTSYS-pc: numerical taxonomy and multivariate analysis system. Version 2.1. New York, NY: Exeter Publications; 2000.

[28] Mantel NA. The detection of disease clustering and a generalized regression approach. Cancer Res. 1967;27:209–220.

[29] Ovesna J, Polakova K, Leisova L. DNA analyses and their applications in plant breeding. Czech J Genet Plant Breed. 2002;38:29–40.

[30] Yu GQ, Bao Y, Shi CH, et al. Genetic diversity and population differentiation of Liaoning weedy rice detected by RAPD and SSR markers. Biochem Genet. 2005;43:261–270.

[31] Nagaraju J, Kathirvel M, Ramesh KR, et al. Genetic analysis of aromatic landraces of rice (Oryza sativa L.) of Assam using fluorescently-labeled microsatellite markers. Hereditas (Beijing). 2007;114:559–568.

[32] Najaraju J, Kathirvel M, Ramesh KR, et al. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence based ISSR–PCR and SSR markers. Proc Natl Acad Sci USA. 2002;99:5836–5841.

[33] Sajib AM, Hossain MM, Mosnaz ATMJ, et al. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (Oryza sativa L.). J BioSci Biotech. 2012;1:107–116.

[34] Dewoody JA, Honneycutt RL, Skow LC. Microsatellite markers in white-tailed deer. J Heredity. 1995;86:317–319.

[35] Upadhyay P, Singh VK, Neeraja CN. Identification of genotype specific alleles and molecular diversity assessment of popular rice (Oryza sativa L.) varieties of India. Int J Plant Breed Genet. 2011;5:130–140.
[37] Ravi M, Geethanjali S, Sameeyafarheen F, et al. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica. 2003;133:243–252.

[38] Joshi RK, Subudhi E, Kar B, et al. Comparative genetic analysis of lowland rice cultivars of India using microsatellite markers. Biores Bull. 2010;4:213–223.

[39] Singh A, Sengar RS. DNA fingerprinting based decoding of *indica* rice (*Oryza sativa* L) via molecular marker (SSR, ISSR, & RAPD) in aerobic condition. Adv Crop Sci Tech. 2015;3:167. doi:10.4172/2329-8863.1000167