Genetic Diversity Studies in Ragi (Eleusine coracana (L.) Gaertn.) With SSR and ISSR Markers

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Abstract

Investigation was carried out on a collection of forty ragi genotypes to study the genetic diversity using ten SSR and 30 ISSR markers. There were successful amplification SSR and ISSR markers in ragi. Of the ten SSR primers used, six were found to be polymorphic. The ten primer pairs generated a total of 23 alleles. Of the thirty ISSR primers used for molecular analysis, twenty primers showed polymorphism among forty ragi genotypes. The twenty polymorphic primers generated a total of 101 alleles. Forty genotypes grouped into thirteen clusters (SSR) and five clusters (ISSR), respectively in UPGMA analysis.

Keywords

Ragi, SSR marker, ISSR marker, Genetic diversity, UPGMA analysis

Background

Finger millet or Ragi (Eleusine coracana (L.) Gaertn.) 2n=4x=36, is a poor man’s crop, originated in Ethiopia (Vavilov, 1951). It belongs to the tribe Chloridae of the family Poaceae. Finger millet is the primary food for millions in dry lands of east and central Africa and southern India. Finger millet is third in importance among millets in the country in area and production after sorghum and pearl millet. Genetic diversity is normally assessed by common morphological traits. However, such traits are affected by effects of environment, development stage of the plant and the type of plant material and also it require several replications to establish the genotypic contributions. Hence, there is a need to go in for a highly reliable and precise method for assessment of genetic variability with no environmental effects. Assessment of genetic diversity with molecular markers overcomes this problem. The use of molecular markers allows the direct assessment of genotypic variation at the DNA level. Marker analysis helps to understand the genetic makeup of the accessions and also make it possible to analyze the global organization of genetic diversity within a species. Several statistical techniques are available for the analysis of genetic diversity using DNA fingerprinting data. The present study, a set of ten SSR and 30 ISSR markers were used to assess the genetic diversity in ragi.

Result and Discussion

Ten SSR and thirteen ISSR primers were tested for their ability to amplify in the forty ragi genotypes. Of the ten SSR primers used, six were found to be polymorphic. The ten primer pairs generated a total of 23 alleles. SSR Marker profile of forty ragi genotypes generated by RM 2 (Figure 1). The number of alleles produced by different primers ranged between two and five with an average of 2.3 alleles per primer. The percentage of amplification ranged from 58 (RM 570) to 92 (RM 3351). Of the thirty ISSR primers used for molecular analysis, twenty primers showed polymorphism among forty ragi genotypes. The twenty polymorphic primers generated a total of 101 alleles. The number of alleles produced by different primers ranged between three and ten with an average of 5.05 alleles per primer. The percentage of amplification ranged from 33 (ISSR 20) to 85 (ISSR 29). Forty genotypes grouped into thirteen clusters (SSR) and five clusters (ISSR), respectively in UPGMA analysis.
Figure 1 SSR marker profile of forty ragi genotypes generated by RM 2

Figure 2 Marker profile of forty ragi genotypes generated by ISSR 30

Polymorphic Information Content (PIC)

Polymorphism Information Content (PIC) value was calculated for ten SSR primers and given in the (Table 1). PIC value was highest for the primer RM 440 (0.729) followed by RM 492 (0.726) while, the lowest PIC value recorded by the primer RM 244 (0.32). The mean PIC value for ten SSR primers was 0.323 which was quite lower than the other reports (0.196 (UGEP107) to 0.834 (UGEP12) with an average of 0.530 in finger millet by Bharathi, 2011; 0.064 (RM 274) to 0.72 (RM 580) with an average of 0.46 in rice by Seetharam et al., 2008). The higher PIC value indicated the informativeness of the primer pairs. Hence, the primer pairs RM 440, RM 492 and RM 2 are considered to be worth in future studies in the field of taxonomical and genetic resource management.

Polymorphism Information Content (PIC) was calculated for twenty ISSR primers (Table 2). PIC value was highest for the primer ISSR 44 (0.761) followed by primer ISSR 99 (0.756) while, the lowest PIC value was recorded by the primer ISSR 58 (0.345). The mean PIC value for ten ISSR primers was 0.588. Of all the primers, the primer ISSR 45 generated a total of ten alleles which was recorded to be the highest, which was quite lower than the results

Table 1 List of SSR primers used and the level of polymorphism detected

| S. No. | Marker code | PIC value | Number of markers | Percentage of amplification |
|-------|-------------|-----------|-------------------|-----------------------------|
| 1     | RM 2        | 0.606     | 4                 | 78                          |
| 2     | RM 244      | 0.320     | 2                 | 70                          |
| 3     | RM 341      | 0.000     | 1                 | 62                          |
| 4     | RM 411      | 0.000     | 1                 | 72                          |
| 5     | RM 440      | 0.7292    | 4                 | 90                          |
| 6     | RM 492      | 0.7263    | 5                 | 65                          |
| 7     | RM 570      | 0.432     | 2                 | 58                          |
| 8     | RM 584      | 0.000     | 1                 | 80                          |
| 9     | RM 3351     | 0.000     | 1                 | 92                          |
| 10    | RM 6378     | 0.424     | 2                 | 76                          |
| Total |             |           | 23                |                             |
| Mean  |             | 0.323     | 2.3               |                             |
Table 2 List of ISSR primers used and the level of polymorphism detected

| S. No. | Marker code | PIC value | Number of markers | Percentage of amplification |
|--------|-------------|-----------|-------------------|-----------------------------|
| 1      | ISSR 20     | 0.486     | 3                 | 33                          |
| 2      | ISSR 23     | 0.647     | 6                 | 65                          |
| 3      | ISSR 24     | 0.634     | 3                 | 82                          |
| 4      | ISSR 25     | 0.675     | 5                 | 55                          |
| 5      | ISSR 29     | 0.378     | 5                 | 85                          |
| 6      | ISSR 30     | 0.487     | 7                 | 53                          |
| 7      | ISSR 44     | 0.761     | 9                 | 76                          |
| 8      | ISSR 45     | 0.623     | 10                | 62                          |
| 9      | ISSR 47     | 0.545     | 3                 | 57                          |
| 10     | ISSR 49     | 0.726     | 6                 | 79                          |
| 11     | ISSR 50     | 0.570     | 7                 | 49                          |
| 12     | ISSR 51     | 0.468     | 3                 | 67                          |
| 13     | ISSR 53     | 0.565     | 4                 | 37                          |
| 14     | ISSR 54     | 0.581     | 3                 | 78                          |
| 15     | ISSR 55     | 0.5517    | 5                 | 55                          |
| 16     | ISSR 58     | 0.345     | 3                 | 60                          |
| 17.    | ISSR 60     | 0.734     | 6                 | 56                          |
| 18.    | ISSR 78     | 0.705     | 4                 | 53                          |
| 19     | ISSR 94     | 0.516     | 3                 | 64                          |
| 20     | ISSR 99     | 0.756     | 6                 | 63                          |
| Total  |             |           | 101               |                             |
| Mean   |             | 0.588     |                   | 5.05                        |

obtained by the Gupta et al (2010) in finger millet. The higher PIC value indicated the informativeness of the primer pairs. Hence, the primer pairs ISSR 49, ISSR 44 and ISSR 45 can be of use in future studies in the field of taxonomical and genetic resource management.

**Cluster analysis**

In the present study, 40 genotypes were subjected to cluster analysis for assessing the molecular diversity based on UPGMA analysis. Using SSR data the 40 genotypes were grouped into thirteen clusters (Table 3) at genetic similarity of 72%, which is quite higher than the earlier reports. Babu et al (2007) in his study grouped 32 finger millet genotypes into two clusters; Das et al (2007) grouped 29 finger millet genotypes into two clusters and Bharathi, (2011) grouped 959 accessions into six clusters. Among the different clusters, Cluster I and cluster II was constituted by only one genotype namely Co 9 and MS 2927, respectively. Cluster III had the highest number of genotypes (18). Genotypes AF 459, Malavi 2028, GS 112 and GS 69 constitute the cluster IV and MS 9272, TNAU 4833, GS 159 and GE 333 coming under cluster V. Cluster VI consists of TNAU 21; cluster VII had genotype TNAU 487; cluster VIII had the genotypes MS 8068 and TNAU 44/2; cluster IX had genotypes Malavi 1446 and TNAU 30; cluster X had genotypes MS 2944 and MS 8070; cluster XI had genotypes GEC 417 and GEC 520; cluster XII had genotype TNAU 5 and cluster XII had genotype Malavi 1876.

Using ISSR data the 40 genotypes were grouped into five clusters (Table 4) at 60 per cent of similarity level. Among the different clusters, Cluster II had the highest number of genotypes (31) followed by cluster I had four genotypes namely Co 9, Malavi 1876, MS 8068, MS 2927. Cluster III had three genotypes namely AF 3055, GE 333 and GEC 417; cluster IV and V had only one genotype each, TNAU 44/2 and MS 2944 respectively.

The genotypes AF 459, AF 269, TNAU 1008 and GS 159, which showed a higher level of salinity tolerance at both 3000 and 6000 ppm for one or several seedling
Table 3 Cluster composition of forty ragi genotypes based on SSR analysis using UPGMA

| Cluster number | Number of genotypes | Name of the genotypes |
|----------------|---------------------|-----------------------|
| I              | 1                   | Co 9                  |
| II             | 1                   | MS 2927               |
| III            | 18                  | GE 527, GEC 539, GEC 534, MS 8100, TNAU 1008, GE 522, TNAU 193, GS 133, GS 261, TNAU 72, AF 329, GE 346, GE 2510, AF 269, Co 14, GS 431, IE 3297/1, AF 3055 |
| IV             | 4                   | AF 459, Malavi 2028, GS 112, GS 69 |
| V              | 4                   | MS 9272, TNAU 4833, GS 159, GE 333 |
| VI             | 1                   | TNAU 21               |
| VII            | 1                   | TNAU 487              |
| VIII           | 2                   | MS 8068, TNAU 44/2    |
| IX             | 2                   | Malavi 1446, TNAU 30  |
| X              | 2                   | MS 2944, MS 8070      |
| XI             | 2                   | GEC 417, GEC 520      |
| XII            | 1                   | TNAU 5                |
| XII            | 1                   | Malavi 1876           |

Table 4 Cluster composition of forty ragi genotypes based on ISSR analysis using UPGMA

| Cluster number | Number of genotypes | Name of the genotypes |
|----------------|---------------------|-----------------------|
| I              | 4                   | Co 9, Malavi 1876, MS 8068, MS 2927 |
| II             | 31                  | All other genotypes   |
| III            | 3                   | AF 3055, GE 333, GEC 417 |
| IV             | 1                   | TNAU 44/2             |
| V              | 1                   | MS 2944               |

characters came under the cluster II formed by the study using 30 ISSR markers. However, the above genotypes were not present in the one cluster formed by the study using 10 SSR markers.

**Material and Methods**

The material for this study included 40 different genotypes of ragi (Table 5). The materials were obtained from department of millet, Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The

Table 5 List of finger millet genotypes used in the study

| S. No. | Genotypes | S. No. | Genotypes |
|--------|-----------|--------|-----------|
| 1      | Co 9      | 21     | AF 459    |
| 2      | GE 527    | 22     | GE 522    |
| 3      | GEC 534   | 23     | GEC 539   |
| 4      | GS 133    | 24     | GS 69     |
| 5      | GS 261    | 25     | GS 112    |
| 6      | Malavi 1446 | 26  | Malavi 2028 |
| 7      | MS 2944   | 27     | MS 8100   |
| 8      | MS 8070   | 28     | TNAU 21   |
| 9      | TNAU 30   | 29     | TNAU 193  |
| 10     | TNAU 72   | 30     | TNAU 1008 |
| 11     | AF 329    | 31     | AF 269    |
| 12     | Co 14     | 32     | AF 3055   |
| 13     | GE 346    | 33     | GE 333    |
| 14     | GE 2510   | 34     | GEC 417   |
| 15     | GS 431    | 35     | GEC 520   |
| 16     | IE 3297/1 | 36     | GS 159    |
| 17     | MS 9272   | 37     | Malavi 1876 |
| 18     | TNAU 5    | 38     | MS 2927   |
| 19     | TNAU 487  | 39     | MS 8068   |
| 20     | TNAU 4833 | 40     | TNAU 44/2 |
young leaf ten days old plant were collected and DNA was extracted by CTAB (mini-prep) method. The extracted DNA was purified for RNA contamination by RNAase treatment. The extracted and purified DNA were then quantified and quality assessed by agarose gel-electrophoresis. Based on intensity of bands produced the DNA were diluted to appropriate concentration for use of molecular analysis.

**SSR amplification**
SSR amplification reaction were carried out in a volume of 15 μL containing 50 ng of genomic DNA, 0.5 units of Taq DNA polymerase, 0.1mM each dNTP, 3 mmol/L Primer (Forward and Reverse), 1.5 μL Taq buffer and amplification was performed in PTC thermal (AB PCR). Amplification conditions were, initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 45°C~60°C for 1 minute and extension 72°C for 1 minute and a final extension at 72°C for 10 minutes.

**ISSR amplification**
ISSR amplification reaction were carried out in a volume of 15 μL containing 50 ng of genomic DNA, 0.5 units of Taq DNA polymerase, 0.1 m mol/L each dNTP, 3 mmol/L Primer (Forward and Reverse), 1.5 μL Taq buffer and amplification was performed in PTC thermal (AB PCR). Amplification conditions were, initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C~60°C for 1 seconds and extension 72°C for 2 minute and a final extension at 72°C for 10 minutes.

PCR amplified products were subjected to gel electrophoresis in a 3% agarose gel in 1XTBE at 90 V for 3 hours using gel electrophoresis unit. The ethidium bromide stained gels were visualized under UV and documented using Alpha Imager 1200.

**Statistical analysis for construction of dendrogram using molecular data**
The SSR and ISSR gels were scored and represented by their allele sizes as allelic data. Using the DARwin-5.0 software package (Perrier and Jacquemoud-Collet, 2005), a simple matching dissimilarity index was calculated from the allele-size data set with 100 bootstraps, and this matrix was then subjected to UPGMA analysis.

Dissimilarity between units i and j, \( d_{ij} = \frac{1}{L} \sum_{l=1}^{L} \frac{m_l}{\pi} \)

Where, \( L = \) number of loci; \( \pi = \) ploidy; \( m_l = \) number of matching alleles for locus \( l \).

Cluster analysis was performed using UPGMA strategy to obtain a dendrogram.

**Polymorphic Information Content (PIC) value**
Polymorphic Information Content (PIC) values were calculated for SSR markers in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. It is the sum total of polymorphism information content values of all the markers produced by a particular primer. PIC value was calculated using the formula PIC = 1 - \( \sum p_i^2 \), where, \( p_i \) is the frequency of the ‘\( i \)’th allele (Smith et al., 1997).

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