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Phosphate Transporter Based Genetic Diversity of *Eleusine coracana* L., Varieties

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Abstract

The present study aimed to characterize finger millet (*Eleusine coracana* L.) varieties on the basis of their phosphate transporter gene which is considered a reliable gene marker for the identification and discrimination in various genotypes. The analysis had 7 different phosphate transporter based SSR markers which were developed and tested against 47 finger millet varieties. Fingerprint analysis showed that germplasm VHC3752 and PRM1 Ragi, PRM 6118 Ragi and PRM 6122 Ragi and, Ragi local Korchara and PRM 5103 were closely located where as VHC 3611 and VHC3972 were very distantly located in the phylogenetic tree. Principle Component Analysis (PCA) of the obtained fingerprint also confirmed genetic diversity among various finger millet varieties. Therefore, this study could be proposed as a reliable tool for characterization of different finger millet varieties in a precise and standard manner.

Keywords: Finger millet, EST, phosphate transporter, SSR, PCR, UPGMA, Genetic fingerprint

Introduction

Millets rank as the world's sixth most important food crops among cereals and are primarily grown in Asian and African countries [1]. Millets are found in the form of major and minor millet. Minor millets are a group of grassy plants with short slender culms and small grains [1]. Finger millet (*Eleusine coracana* L.) an allotetraploid cereal (4× = 36 chromosomes) [2] is an important crop used for food, forage and industrial products [3]. This plant is widely cultivated in arid and semi-arid regions of the world [2]. The nutritional quality is superior to that of rice and is on a par with that of wheat [3]. It is rich in protein (6 to 13 %) and calcium (0.3 to 0.4 %) [4] and serves as an important staple food for rural populations in calcium deficient and anaemic areas [5]. Among 8 minor millets, finger millet is grown globally over more than 4 million hectares and is the primary food source for millions of people in tropical dry land regions. The crop can withstand high saline conditions, is resistant to drought and has pest tolerance as well as a few serious diseases [6] that can be attributed to the genetic diversity of the crop.

DNA marker based finger printing techniques are quite useful for diversity analysis [2]. The most commonly used marker systems are randomly amplified polymorphic DNA (RAPD) [7,8], restricted fragment length polymorphism (RFLP) [9], amplified fragment length polymorphism (AFLP) [10], inter simple sequence repeats (ISSRs) [11], and microsatellite or simple sequence repeat (SSRs) [12]. SSRs are multi-allelic; they have high potential for use in genetic diversity studies in a variety of plant species [13-15]. The nature of SSRs gives them a number of advantages over other molecular markers [16] due to its high transferability between related species, with high allelic diversity, detection of multiple SSR alleles at a single locus by using a simple PCR-based screen, co-dominance, distribution of SSR all over the

genome and semi-automated analysis. Thus, SSR markers allow for rapid generation of genetic data from a relatively small amount of sample, with high reliability and reproducibility. They are particularly useful for genetic mapping, linkage and association studies, as well as for phylogenetic and population studies [17].

It is argued that phosphorus is a limiting factor for plant development in many soils worldwide [18] and inorganic phosphate (Pi) is the primary source of phosphorus for plants. Limitation of Pi ability resulted in the evolution in plants of a range of adaptive morphological, biochemical and symbiotic strategies that increase the acquisition of Pi and/or improve the efficiency of internal utilization of Pi [19]. Transport of Pi between the cytosol and the stroma of the chloroplast is mediated by triose phosphate/Pi translocators [20]. These transporters are localised in the inner membrane of the chloroplast envelope. Phosphate transporter (Pt) genes play an important role in phosphate translocation from soil to provide nutritional supplement to plants and hence growth, development and yield potential of the crop [21,22]. Pt genes based genetic diversity study can be an ideal platform for study of *E. coracana* high and low yielding varieties to know the role and genetic diversity of phosphate transporter genes among different varieties.

We conducted this study to analyze genetic diversity among different varieties of *E. coracana* collected from different agro-climatic regions of India by using phosphate transporter based SSR markers for this purpose.

Materials and methods

Sample collection and genomic DNA extraction

Seeds of 47 different varieties (Breeders collection) were collected from different parts of India (**Table 1**). Collected seeds were sown at the Breeders Seed Production Center (BSPC), G.B.P.U.A & T, Pantnagar. Plant leaf material of each genotype was used for DNA extraction. Plant genomic DNA was extracted from 5 g of fresh plant leaf material [23].

Primer designing and source of genetic information

To determine genetic divergence within and amongst plant species, several molecular markers have been used. A total of 1,742 expressed sequence tags (ESTs) of finger millet along with 25 phosphate transporter genes of rice were retrieved from the NCBI. Homologous sequences were retrieved by BLAST and processed through MegAlign (DNA STAR). SSRs were searched from an input (in the form of multiple FASTA format DNA sequences) by SPUTNIK. Finally SSR-primers were designed using web-based PRIMER3 [24], and the resulting output was specifically analyzed. A total of 25 pairs of SSR primers, targeting the phosphate transporter gene in finger millet were designed.

PCR reactions and electrophoresis

Each 20 µl reaction volume contains 3.0 µl reaction buffer (10×), 1.2 µl $MgCl_2$ (25 mM), 1.2 µl dNTP mixture (2.5 mM), 1.2 µl of each forward and reverse primer (10 µmol/l), 2.0 µl Taq DNA polymerase (3 units/µl) and 0.5 µl of DNA. PCR consists of initial denaturation of 94 °C, 5 min, followed by 94 °C, 15 s; 61 °C, 30 s touchdown 1 °C/cycle decrease (10 cycles); 72 °C, 30 s; 40 cycles of 94 °C, 15 s; 54 °C, 30 s; 72 °C, 30 s, and finally one cycle of 72 °C, 30 min. The PCR product was analyzed by electrophoresis using 1.5 % agarose gel.

Data analysis

A binary matrix was generated for the bands in the gel. The presence or absence of the bands in a same line in the gel was recorded as l (present) and 0 (absent). Jaccard similarity coefficient was used to estimate genetic distances between lines. Simplified representation of genetic distances between lines was obtained by UPGMA (unweighted pair-group method with arithmetic mean) and represented by a dendrogram. Principal Component Analysis (PCA) was also performed with obtained SSR banding pattern.

Table 1 Details of 47 different genotype of finger millet selected in the study.

S. No.	Genotype
1	VHC 3611
2	VHC 3607
3	VHC 3772
4	VHC 3577
5	VHC 4120
6	VHC 3972
7	Khairna 14
8	PRM 3570
9	VL 124
10	Suwalbari 18
11	VHC 4170
12	VHC 4107
13	VL 204
14	VHC 3752
15	VHC 3594
16	PRM 6112 Ragi
17	VHC 3857
18	VHC 3924
19	VHC 3652
20	VHC 4073
21	Khairna 24
22	VHC 4138
23	Suwalbari 9
24	VHC 3680
25	VHC 4001
26	VHC 3602
27	VHC 3887
28	VHC 3749
29	PRM 6101 Ragi
30	PRM 6115 Ragi
31	PRM 1 Ragi
32	Ragi Local (Dingothi)
33	Ragi Local
34	Ragi Local (Dubakoti)
35	Ragi Local (Daargi)
36	Ragi Local (Pali)
37	Ragi Local (Tenga)
38	Ragi Local (Timgri)
39	Ragi Local (Korchhara)
40	PRM 5103
41	Ragi Local (Kechhu)
42	Ragi Local (Gaja)
43	PRM 502 Ragi
44	PRM 6118 Ragi
45	PRM 9802 Ragi
46	PRM 6122 Ragi
47	Ragi Local (Jagdhar)

Table 2 Unique SSR primers used to analyze genetic diversity in different finger millet varieties.

Number	Forward primer(5'-3')	Reverse primer(5'-3')	PIC value
3F/R	AATAATGGTGGCAGCAGGAG	TCTGGCTGTAGAACGCAATG	0.827
4F/R	TACTACTTCAACCCGGCGTC	CGTCATCCCGTACACCTTCT	0.945
6F/R	TGGGTCGCCTTTACTACACC	GAGGATGAGCGTGAATCCAT	0.877
14F/R	GGTCATGTACGCCTTCACCT	AGAAGAACCCGATGACGTTG	0.926
GF/R	AGCAGCACACTCTCCTGTGA	GACGAAGATGAGGGTGGAGA	0.759
HF/R	CACTACACCGCATCATCTCG	AGCCGTGATGCCTACAACTC	0.827
KF/R	GAGTCACGACTCACGAGCAA	CCGTACTTCCGAATCAGACC	0.856

Results and discussion

Genomic DNA was successfully isolated from fourty seven selected varieties and was amplified using twenty-five SSR primers. Out of the 25 pairs, only seven pair of primers showed proper amplification and were used in the present study (**Table 2**). Polymorphism information content (PIC) values for selective primers, ranging from 0.759 (GF/R) to 0.945 (4F/R) (mean = 0.859), indicating significant polymorphism in different varieties of *E. coracana* (**Table 2**). The application of SSR Primer provides a unique accessibility of SSR molecular markers. This is useful for the selection of markers that may be most suitable for specific applications or particular organisms [24,25].

The PCR result with SSR repeats showed significant variation in genomic context between the varieties of *E. coracana*. Varieties 14 and 31 (VHC3752 and PRM1 Ragi), 44 and 46 (PRM 6118 Ragi and PRM 6122 Ragi) and 39 and 40 (Ragi local Korchara and PRM 5103) were found closely related whereas, varieties 1 (VHC 3611) and 6 (VHC3972) were very distantly related with regards to the phosphate transporter gene (**Figure 1**). These results clearly matched with the principal component analysis (PCA) where varieties separated into distinct groups in the PCA ordination. Varieties 14 and 31, 44 and 46 and 39 and 40 were in different quadrates of the biplots as separate clusters A, B and D respectively while variety 6 was in separate cluster E (**Figure 2**). The first 2 principal components accounted for 46.70 % of the total variance and effectively captured the main patterns of variation in the original variables. Percent variance for PC1 and PC2 was 27.99 and 18.79 %, respectively.

SSR primers, targeting phosphate transporter gene provided very useful information for monitoring genetic diversity in different finger millet varieties. Results indicated that not a single primer could categorize all 47 varieties. However, the banding pattern in combination with 7 selected primers identified them. Earlier studies proved that the availability of inorganic phosphate (Pi) in most natural ecosystems limits plant growth [22,26]. Evolution in plants for adaptive morphological, biochemical and symbiotic strategies increase the acquisition of Pi for improved efficiency of internal utilization of Pi [19]. SSR primers (7 pairs) based on phosphate transporter gene can be used to identify the different germplasm of finger millet. This study also provides the information about the genetic variance among the finger millet germplasm in India.

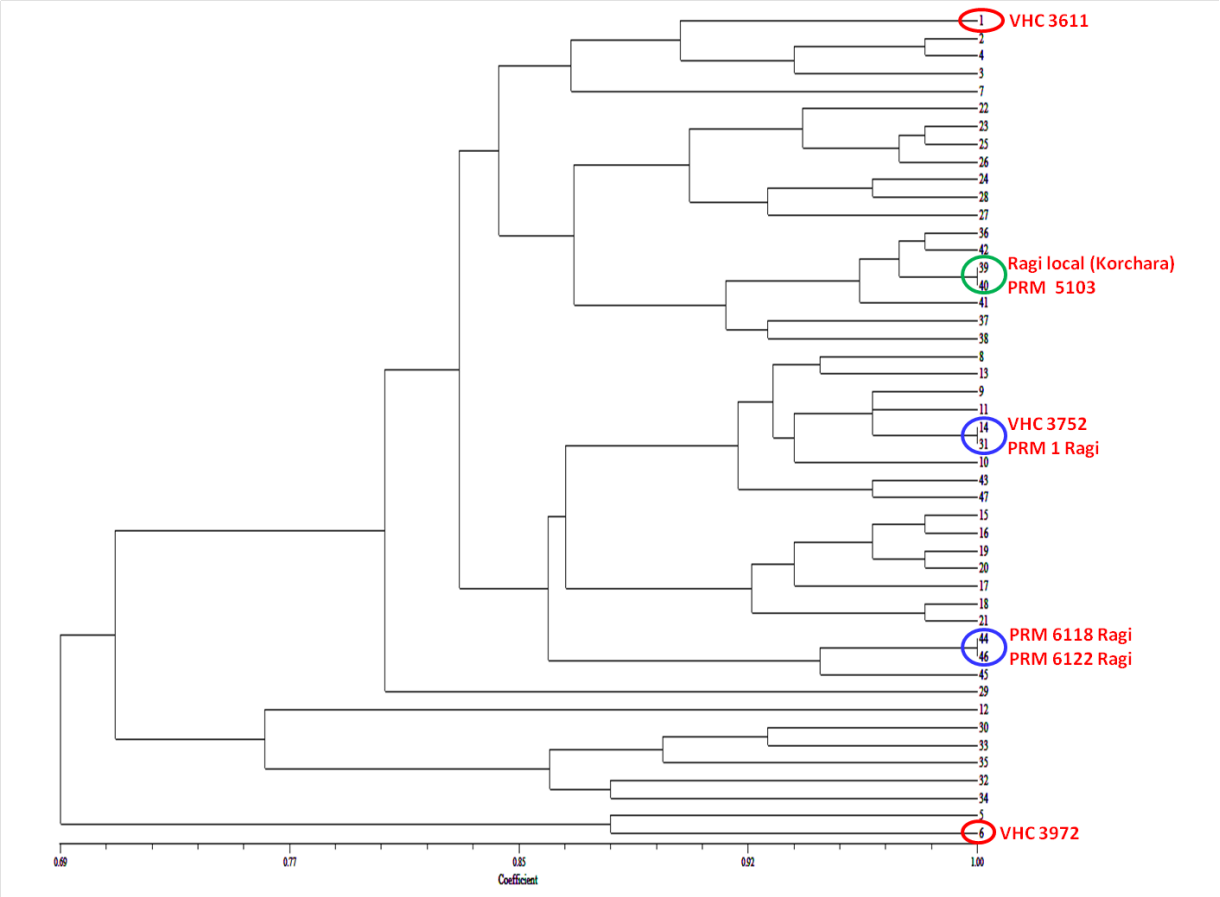

Figure 1 UPGMA cluster analysis from DNA band fingerprints obtained by PCR with specific SSR primers targeting the Phosphate transporter gene. Each variety is defined by a number.

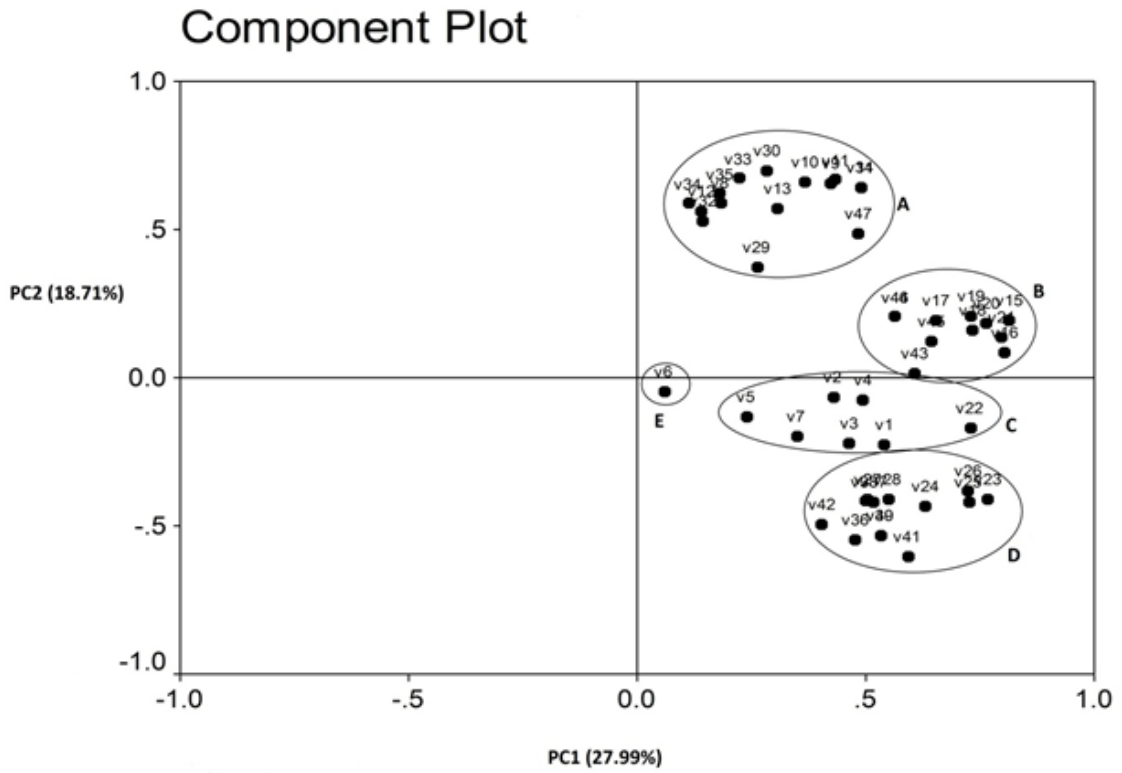

Figure 2 Two-dimensional plot of the PCA performed for the whole dataset. Each variety represented in the figure as a dot is defined by a code (v) followed by a variety number.

Conclusions

In conclusion, it could be stated that SSR markers, based on phosphate transporter gene will help to understand the genetic variation in different varieties of finger millet. This may be used as a tool for easy understanding of genetic similarity and dissimilarity between different varieties, especially in relation to the phosphorus transporter gene. Therefore, in future these selected markers could also be used for expression studies of phosphate transporter genes in finger millet varieties.

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