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ASSESSMENT OF GENETIC DIVERSITY AMONG KIDNEY BEAN (*Phaseolus vulgaris* L.) CULTIVARS USING EST-SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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ABSTRACT

Genetic variation among kidney bean (*Phaseolus vulgaris* L.) cultivars, procured from the farmers of Kinnaur district of Himachal Pradesh (India), was evaluated using EST-SSR markers. A total of 25 primers were used out of which 19 primers showed amplification. The total n0umber of bands amplified was 26, out of which 24 were polymorphic and 2 were monomorphic. On an average, the number of bands generated primer^{-1} was 1.37 of which 1.26 was polymorphic and 0.11 were monomorphic. Seven primers produced 14 bands (2 bands primer^{-1}) and only one band each was obtained with remaining primers. The number of unique bands were 2 and the size of amplified product ranged from 70 to 800 bp. Similarity between cultivars estimated as per Jaccard's coefficients ranged from 0.17-0.90 indicating substantial diversity in bean cultivars. Maximum similarity coefficient (0.90) was between 'Capsule' and 'Kaju' cultivars while minimum (0.17) between 'Chitra' and 'Luxmi'. Dendrogram grouped cultivars into two major clusters. The PIC value was highest for primer SP5.

Key words: Genetic diversity, *Phaseolus vulgaris,* polymorphism, simple sequence repeat marker

INTRODUCTION

Kidney bean (*Phaseolus vulgaris* L.) seeds are the primary source of protein for human and domesticated animals. They also are a good source of vitamins, minerals and complex carbohydrates (Richardo *et al*., 2010). Crop plants rely on broad genetic base of variation to adjust and adapt themselves to ever-changing environment and pathogens. Characterization of germplasm using molecular marker techniques provides quantitative estimates of genetic diversity and the information is essential for rational use of germplasm in breeding programmes (Khaidizar *et al*., 2012). Breeding programmes become successful only if sufficient variability exists in the selected crop. Therefore, the study on genetic variability and its effective utilization is a preliminary step for crop improvement. A major constraint in exploiting kidney bean diversity for breeding purpose is scanty information on germplasm characteristics. Without enough knowledge about the genetic diversity among genotypes, it is practically impossible to enhance yield and other desirable characters of a crop as selection of improved genotypes depends upon the proportion of genetic variability within the available genotypes.

Many studies have used morphological and agronomical characters to quantify genetic diversity existing in kidney bean genotypes (Galvan *et al*., 2001). Data related to morphological traits are easy to identify but it has some drawbacks as the number of distinctive characteristics is limited,

descriptive, error-prone and affected by environmental and physiological factors. Approaches to assess the diversity at molecular level are more useful than morpho-agronomic characterization. DNA markers developed to study genetic diversity and crop evolution are considered better over morphological markers (Burle *et al*., 2010). They may reveal the differences among various genotypes at DNA level and serve as direct, reliable and efficient tool for germplasm characterization, conservation and management. Genetic diversity in kidney bean have been studied using different molecular markers such as allozymes (Santalla *et al*., 2002), RFLP (Metais *et al*., 2000), AFLP (Lioi *et al*., 2005; Wang *et al*., 2010; Ojuederie *et al*., 2014), RAPD (Filimon *et al*., 2011; Razvi*et al*., 2013), ISSR (Sadeghi *et al*., 2012) and CAPS (Ince and Karaca, 2011). Simple sequence repeats (SSRs), also termed microsatellites, are widely scattered at many different loci throughout the genome (Tautz and Renz, 1984; Kandemir *et al*., 2010). These small repetitive DNA sequences provide a basis for PCR-based multi-allelic codominant genetic marker system (Saghai *et al*., 1994). Polymorphisms revealed by PCR amplification are due to the variation in the number of repeats in a defined region of genome. The utility of SSR markers is due to their abundant distribution and high polymorphism in the whole genome and power to distinguish between closely related genotypes. SSRs have been used to construct a PCR-based genetic map (Blair *et al*., 2003), to identify genetic variability (Arunga *et al*., 2015; Maryrose *et al*., 2015) and to develop multiplex SSR-PCR in kidney bean (Masi *et al*., 2003; Hanai *et al*., 2010; Chen *et al*., 2015). SSRs also represent a rich source of allelic diversity which has been exploited in many types of genetic analysis. SSR markers are locus-specific and highly polymorphic. These markers are co-dominant and allow discrimination between homozygotes and heterozygotes. Fortunately, SSR primers obtained from one species are very often usable in closely related species of the same genus and sometimes even through genera of a family. The present study was aimed to evaluate the level of genetic diversity in local bean selections using EST-SSR markers so that the information gathered may be used in selection and breeding programs of kidney bean.

MATERIALS AND METHODS

Seeds of kidney bean (*Phaseolus vulgaris* L.) cultivars *viz*., Baspa, Capsule, Chitra, Contender, Jawala, Kailash, Kaju, Kanchan, Luxumi and Triloki grown by the farmers of Kinnaur district of Himachal Pradesh (India), were procured from the Vegetables Research Station Kalpa, Kinnaur (India).

Extraction of genomic DNA

Genomic DNA was extracted from the fresh leaves of different kidney bean cultivars by using CTAB method (Doyle and Doyle, 1990) with some modifications. The quality of DNA samples was judged on the basis of the formation of single high molecular weight band or smear on 0.8% agarose gel after electrophoresis. The concentration of DNA was assessed by using picometer (Pico drop Ltd., Cambridge, UK). The ratio of absorbance at 260 and 280 nm was measured to check the contamination of RNA and protein. To make all the DNA samples concentration uniform, they were diluted to a concentration of 40-50 ng μL^{-1} before use.

DNA amplification and electrophoresis of amplified DNA

The DNA samples from different kidney bean cultivars were amplified by polymerase chain reaction (PCR) using the protocol of Williams *et al.* (1990) with a few modifications. The EST-SSR primers of genus *Phaseolus* used in present study are given in Table 1. The amplified DNA was mixed thoroughly with 6X loading dye and then electrophoresed on 1.6% agarose gel. The size of amplified product was determined by co-electrophoresis of 100 bp standard molecular weight marker. DNA profiles were visualized and the images taken by using gel documentation system (Alpha Imager).

Similarity analysis and clustering

Co-migratory bands were considered to represent the same locus while scoring. DNA fragment profiles were scored in binary fashion with '0' indicating the absence and '1' indicating the presence of band. Similarity matrix was generated from binary data using Jaccard coefficient. The similarity matrix generated was used for pair-group method with arithmetic average (UPGMA) using software package NTSYS-PC 2.0 h (Rohlf, 1998). The output data was graphically represented as a dendrogram and used for diversity analysis.

Polymorphic information content (PIC) value

Marker index was calculated for the characterization of the capacity of each primer to detect polymorphic loci among the cultivars under study. It is the sum total of polymorphism information content (PIC) values of all the markers produced by a particular primer. PIC value was calculated using the formula (Nei *et al*., 1983):

$$PIC = 1-\Sigma pi^2$$

Where pi is the frequency of the i^{th} allele.

RESULTS AND DISCUSSION

A total of 25 EST-SSR primers of 21-24 bp length were used in PCR reaction. The optimal annealing temperature and GC contents of each primer was explored separately (Table 1). After primer screening, 19 primers which showed clear, repeatable, amplified bands under optimal conditions were selected and used to analyze the polymorphism among bean cultivars. Out of the 19 primers, only 2 primers (SP2 and SP16) were monomorphic, while the rest 17 primers were polymorphic (Table 2). A varying level of genetic polymorphism was revealed in the banding pattern across 10 bean cultivars (Fig. 1). For a total of 19 primers 26 bands were amplified, of which 24 (92.3%) were polymorphic and 2 (7.7%) were monomorphic across the cultivars. On average, the total number of bands generated $primer^{-1}$ was 1.37 of which 1.26 were polymorphic and 0.11 were monomorphic (Table 3).

Seven primers (SP3, SP4, SP5, SP6, SP10, SP14 and SP20) produced a total of 14 bands (2 bands $primer^{-1}$). Remaining 12 primers (SP1, SP2, SP7, SP8, SP11, SP12, SP16, SP17, SP19, SP22, SP23 and SP25) generated one band each. The size of amplified product ranged from 70 to 800 bp with minimum of 70 and 150 bp for primer 'SP20' and 'SP25', respectively.

The PIC values, a reflection of allele diversity and frequency among all the cultivars, were not uniformly higher for all the SSR loci tested. The PIC values ranged from 0.00 (for primers SP1, SP2, SP7, SP8, SP11, SP12, SP16, SP17, SP19, SP22, SP23 and SP25) to 0.50 (primer SP5). Amplified primers, the number of PCR amplified bands and amplicons obtained in EST-SSR analysis of bean selections are given in Table 3. Arunga *et al*. (2015) used SSR markers to evaluate the genetic diversity among 36 French bean accessions and only 18 primer pairs were found to be informative, producing a mean of 2.17 alleles per SSR locus and a PIC value between 0.17 and 0.41. More than 97% polymorphism was reported among 38 kidney bean landraces grown in Northern Anatolia region using 30 primer pairs (Khaidizar *et al*., 2012). Different levels of polymorphism and PIC value were obtained by several researchers using SSR marker (Blair *et al*., 2003; Hanai *et al*., 2010).

Genetic diversity analysis

The similarity coefficient ranged between 0.17 to 0.90, indicating substantial diversity in the cultivars studied. Maximum similarity coefficient was observed between 'Capsule' and 'Kaju' cultivars while minimum in 'Chitra' and 'Luxmi'. The dendrogram obtained after SSR analysis (Fig. 2) showed that the bean cultivars were broadly divided into two major clusters comprising of 9 (cluster A) and 1 (cluster B) cultivars, respectively, truncated at similarity value of 0.24.

The first major cluster A consisted of 2 sub-clusters A_1 and A_2, merged at 0.47 similarity coefficient. Sub-clusters A_1 and A_2 comprised of 7 and 2 cultivars, respectively. Sub-cluster A_1

Primer name	Sequence 5'-3'	Tm value (°C)	GC contents (%)	Length (bp)
SP1	F- TTCTCCTTCTCCTTCTCCTCCT	60.3	50	22
	R- CGGAATACCCTTTCACTTCTTG	58.4	45	22
SP2	F- TTTTGAGGATTGGGAATATTGG	54.7	36	22
	R- TCAAATGGACTACGATTAACTTGC	57.6	38	24
SP3	F- CAGTCGATCAATTCAGTGGAGA	58.4	45	22
	R- ACAAAACACCTCCACAGTCAAA	56.5	41	22
SP4	F- ATGTCAGCTCGATTGATGAAGA	56.5	41	22
	R- GATTTGGGATTTGATACGGAAA	54.7	36	22
SP5	F- GTACGGGGATTATGTGCAATTT	56.5	41	22
	R- CTCAGCAGGCCTATCTCTCAAC	62.1	55	22
SP6	F- AGAACCTCAAGAGGGGTTTCTC	60.3	50	22
	R- AGGCCATTGTGTTCTTCCTAAAA	56.5	41	22
SP7	F- TGGATTCGAGACATGAGATGAG	58.4	45	22
	R- GATTCGGTTCATCCTCTCCAG	59.8	52	21
SP8	F- TTCGTCTGATTCTATTGGAAGAAA	55.9	33	24
	R- ATGGGGTTGAACTCATCCTAGA	58.4	45	22
SP9	F- ACCACCAGAAAATGGAACAACT	56.5	41	22
	R- ATTAGTAGATTCAGCCACCGGA	58.4	45	22
SP10	F- GATTTGCTTGATCACATTCCCT	56.5	41	22
	R- GAGGTGCCAAAGTATCACATCA	58.4	45	22
SP11	F- GTAGGTATCGGTGGAACTCCTG	62.1	55	22
	R- GAAGCTGCAAACAATACTGCTG	58.4	45	22
SP12	F- ATGATGAGGAAATATTGGTGGC	56.5	41	22
	R- GTGAAGGGGAAGAGAAAAGGTT	58.4	45	22
SP13	F- GAAGAAATCGTCGTTGTTGTGA	56.5	41	22
	R- CAATAGAGATCGTGGGAAGCAT	58.4	45	22
SP14	F- CAACGTCCATAGGTTTCATTCC	58.4	45	22
	R- GATGAAGAGCCTAGTGATTGGG	60.3	50	22
SP15	F- CTGCTGAGATTTTATCGAGGCT	58.4	45	22
	R- CTGCTGTGTTTGTTGGTTCAAT	56.5	41	22
SP16	F- TGTTTGGTTAACAATTCGATGC	54.7	36	22
	R- TTTCAAGCGGGAAAAGAAATAA	52.8	32	22
SP17	F- CAACAATAACCCCGTTTTGAAT	54.7	36	22
	R- GGAGGAGAAGGAGAAGGAGAAG	62.1	55	22
SP18	F- TTTCTTCTTCACACTCCCCATT	56.5	41	22
	R- GACATGGGAACAGCAAAATGTA	56.5	41	22
SP19	F- GCACTGCTAGCGAGATTAGACA	60.3	50	22
	R- AACACCAGCTTTGTCTTCTTCC	58.4	45	22
SP20	F- CAGAAATTCAAGGAAAGGTTGG	56.5	41	22
	R- TGTGCTATTCTTGTTCCATTGC	56.5	41	22
SP21	F- AATAGAAGCAAATGCCACGAAC	56.5	41	22
	R- CTTTGTTCCCCTTTATAACGCC	58.4	45	22
SP22	F- CCCAAATTATCCTCATCCAAAA	54.7	36	22
	R- AAAGCAAATGGTAATTGGTTCC	54.7	36	22
SP23	F- CTTTCTCTTCACTTCTCATTGGC	58.9	43	23
	R- GATGCCGTTGTTGTCAGAAAT	55.9	43	21
SP24	F- GGGAGGAGGTGACTTGTCTTTT	60.3	50	22
	R- ATCACCATAACCACGATCCCTA	58.4	45	22
SP25	F- TTACCCCACAATCCTGCTATCT	58.4	45	22
	R- AGATAGGAACTTGGGAAGGAGG	60.3	50	22

contained two sub-sub clusters comprising of 4 and 3 cultivars, respectively. In this sub-sub-cluster, a maximum similarity of 90% was found between 'Kaju' and 'Capsule'. The cultivar 'Jawala' and 'Triloki' showed 86.8% similarity which, in turn, was 83.60% similar with 'Kaju' and 'Capsule'. Hence, the whole sub-sub-cluster (A_{11}) showed 83.6% genetic similarity among the grouped selections (Fig. 3). Likewise, sub-sub-cluster A_{12} contained 3 cultivars *viz.*, 'Baspa', 'Kanchan' and 'Kailash'. In this sub-cluster, maximum similarity of 81.4% was found among Kanchan and Kailash which in turn showed 75.60% similar with Baspa. Hence, this whole sub-sub cluster (A_{12}) showed 75.60% genetic similarity among the grouped selections (Fig. 3). From the observed parameters, it was observed that 64.68% genetic similarity was obtained among 7 selections grouped under sub-cluster A_1 whereas, 50.60% genetic similarity was found among 2 selections (Contender and Luxmi) grouped under sub-cluster A_2. Therefore, it was reported that cluster A comprising of sub-clusters A1 and A2 showed 47.4% genetic similarity among the 9 cultivars grouped as a whole. Cluster B contained only one selection (Chitra) merged at similarity coefficient of 0.24 (Fig. 3). It revealed that the cluster

Table 2: Total number and size range of amplified fragment and number of polymorphic and monomorphic bands generated by 19 EST-SSR primers in bean selections

Primers	Number of bands				Amplified product range (bp)	No. of amplified fragments	PIC value
	Total	Monomorphic	Polymorphic	Unique			
SP1	1	0	1	-	275	8	0.00
SP2	1	1	0	-	600	9	0.00
SP3	2	0	2	1	280-300	9	0.20
SP4	2	0	2	-	100-200	8	0.38
SP5	2	0	2	-	150-350	15	0.50
SP6	2	0	2	-	320-550	9	0.44
SP7	1	0	1	-	200	4	0.00
SP8	1	0	1	-	120	9	0.00
SP10	2	0	2	-	50-250	14	0.49
SP11	1	0	1	-	235	8	0.00
SP12	1	0	1	-	300	9	0.00
SP14	2	0	2	-	200-240	10	0.32
SP16	1	1	0	-	200	10	0.00
SP17	1	0	1	-	180	9	0.00
SP19	1	0	1	-	300	5	0.00
SP20	2	0	1	1	70-150	8	0.22
SP22	1	0	1	-	200	6	0.00
SP23	1	0	1	-	300	8	0.00
SP25	1	0	1	-	800	4	0.00
Total	26	2	24	2		162	

tree analysis for ten bean cultivars evaluated in present study had 24% similarity with one another and depicted 76% genetic fidelity as a whole. Genetic diversity index among the bean accessions of

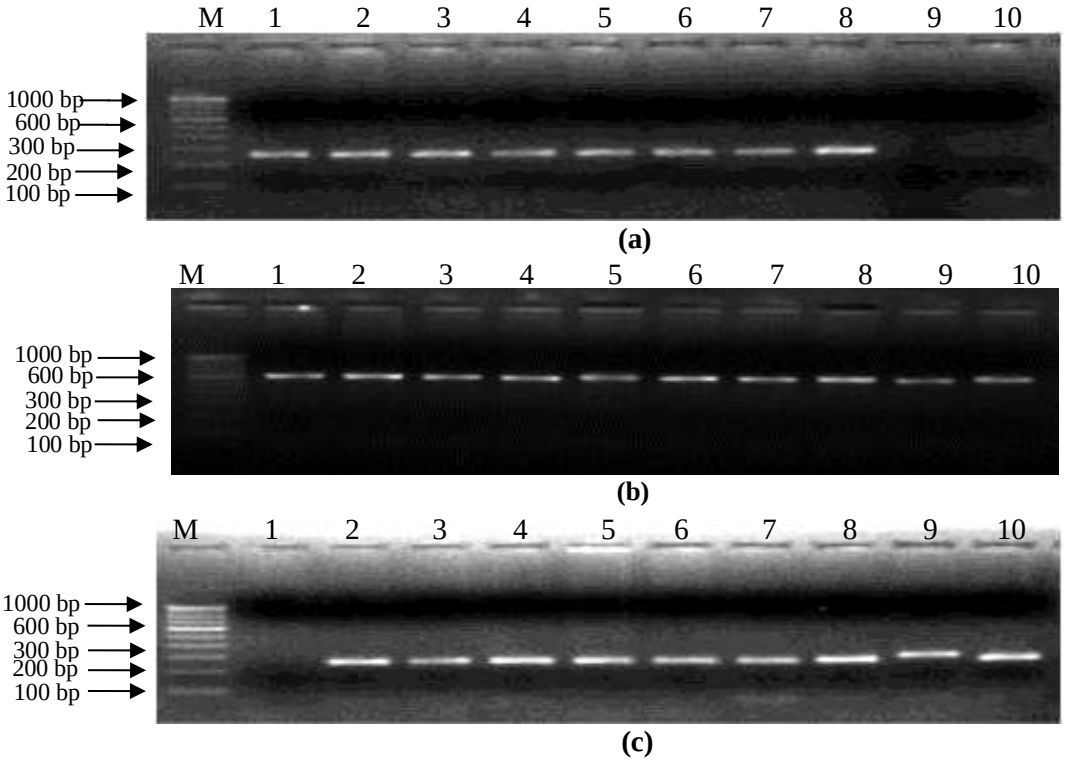

Fig. 1: Banding profile using EST-SSR primers SP1 (a), SP2 (b) and SP3 (c); Lanes are as follow: M- Marker 100 - 1000 bp; 1- Jawala; 2- Triloki; 3- Baspa; 4- Kaju; 5- Contender; 6- Capsule; 7- Kanchan; 8- Kailash; 9- Luxumi; 10- Chitra

Table 3 Summary of EST-SSR amplified products obtained from ten kidney bean selections

Description	EST-SSR
Total number of primers used	25
Total number of informative primers	19
Number of polymorphic primers	17
Total no. of scorable bands amplified by primers	26
Average number of bands per primer	1.37
Total number of polymorphic band	24
Total number of monomorphic bands	2
Average number of polymorphic bands per primer	1.26
Average number of monomorphic bands per primer	0.11
Percentage of total polymorphic bands	92.31

Kenya was found to be 0.1267-0.2377, 0.1475 and 0.1991 among the accessions of Western province, Eastern province and dry lands, respectively (Maryrose *et al.*, 2015). Genetic diversity of a set of 36 Kenyan French bean accessions was evaluated using SSR primers and found to

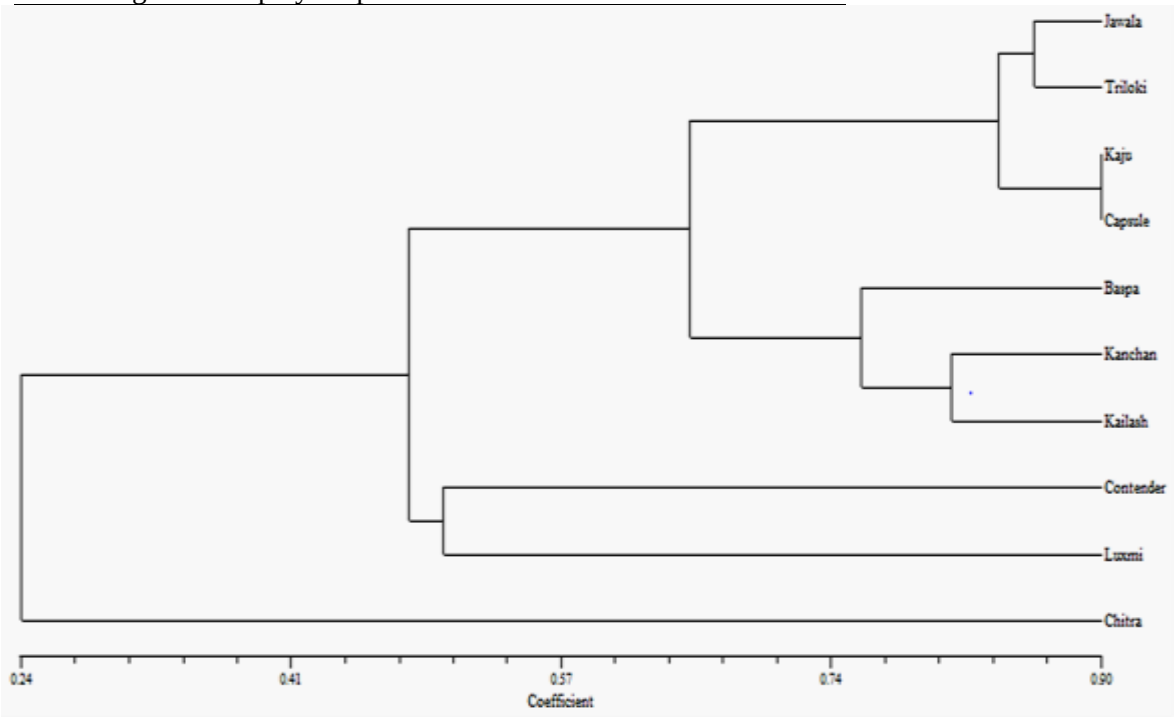

Fig. 2: Dendrogram (Jaccard's coefficient) based on SSR banding patterns showing relationship among 10 bean (*Phaseolus vulgaris* L.) selections

Cluster	Sub-cluster	Sub-sub-cluster		Cultivar		No.	Similarity coefficient (%)				
A	A_1	A_{11}	A_{11a}	Jawala Triloki		2	86.80	83.60	64.68	47.40	24.00
			A_{11b}	Kaju Capsule		2	90.00				
		A_{12}	A_{12a}	(Solitary)	Baspa	1	Truncated at 75.60	75.60			
			A_{12b}	Kanchan Kailash		2	81.40				
	A_2	Contender, Luxmi				2	50.60				
B	(Solitary)	Chitra				1	Truncated at 24.00				

Fig. 3: Clustering observed in 10 bean cultivars based on EST- SSR analysis

found to range between 0.19 and 0.50 (mean 0.36) (Arunga *et al.*, 2015). Similarity coefficient in 13 bean genotypes by using Dice coefficient reportedly ranged from 27.72 to 82.35 (Razvi *et al.*, 2013). Genetic similarity coefficient among 8 kidney bean cultivars was reported to vary from 60 to 96% (Filimon *et al.*, 2011).

Conclusion: The study revealed that remarkable genetic diversity existed in kidney bean cultivars which could be exploited in germplasm conservation, maintenance and selection intensity of desirable types for crop improvement. Among the ten selections of kidney bean maximum genetic divergence was shown by 'Chitra', 'Jwala' and 'Triloki'.

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