P. ISSN: 0971-8184 II E. ISSN: 0976-1926
DOI: 10.61949/0976-1926.2024.v37i02.08

Indian Journal of Plant Genetic Resources

RESEARCH ARTICLE

Molecular Characterization of Grapes (*Vitis* spp.) Using EST-derived Simple Sequence Repeat (SSR) Markers

Sharmistha Naik[1], Jagesh K Tiwari[2], Rasna Zinta[2], Narender Negi[3], Rajnish Sharma[1], Gopal Singh[1], and DP Sharma[1*]

Abstract

The aim of this study was molecular characterization of 27 grape accessions maintained at the ICAR – National Bureau of Plant Genetic Resources, Regional Station, Shimla, Himachal Pradesh, India using EST-derived five SSR primer pairs, and also to study the transferability of EST-SSRs across different species, interspecific hybrids, and one non-*Vitis* accession *Parthenocissus quinquefolia*. SSR bands were analyzed on ethidium bromide-stained 2.5% high resolution agarose gels. A total of 31 distinct alleles were scored in 27 grape accessions, and the average number of alleles per locus was 6.2. The mean observed and expected heterozygosity values were 0.103 and 0.749, respectively. The number of effective alleles ranged from 3.415 to 4.766 with an average of 4.050. The polymorphism information content (PIC) ranged from 0.7072 to 0.8064 with a mean of 0.7650. The clustering dendrogram divided 27 grape accessions into 7 clusters, and suggested complex origins of some of the accessions. A good level of transferability of EST-SSRs across different species and interspecific hybrids was observed except in 2 grape accessions *viz*., *Vitis arizonica*/DVIT1269 and *Parthenocissus quinquefolia*/DVIT2400. A database of 5 EST-SSR markers was developed for future use in breeding and molecular studies.

Keywords: EST-SSR, Genetic resources, Microsatellite, Molecular characterization, *Vitis vinifera*.

[1]Dr YS Parmar University of Horticulture and Forestry, Solan–173230, Himachal Pradesh, India.

[2]ICAR-Central Potato Research Institute, Shimla-171001, Himachal Pradesh, India.

[3]ICAR-NBPGR Regional Station, Phagli, Shimla-171004, Himachal Pradesh, India.

***Author for correspondence:**

sharmadp2022@gmail.com

Received: 22/05/2022 **Revised:** 26/04/2024

Accepted: 09/05/2024

How to cite this article: Naik S, JK Tiwari, R Zinta, N Negi, R Sharma, G Singh and DP Sharma (2024) Molecular Characterization of Grapes (*Vitis* spp.) Using EST-derived Simple Sequence Repeat (SSR) Markers. *Indian J. Plant Genet. Resour.* 37(2): 255-262.
DOI: 10.61949/0976-1926.2024.v37i02.08

Introduction

Vitis vinifera L., the commonly cultivated grapevine, is one of the most widely grown fruits in the world (Vivier and Pretorius, 2002). Availability of adequate genetic resources and knowledge about their genetic diversity and relationships is important for recognizing gene pools, and developing effective conservation and management strategies (Khadivi *et al.,* 2019). Traditionally, the morphological method is used to characterize grape genetic resources. The history of grape morphological studies dates back to the 1st century and is still considered an efficient method for characterization (Cangi *et al*., 2006). Morphological evaluations are direct, inexpensive, easy, and do not require expensive technology. A special term "Ampelography" is used for the morphological characterization of grapes. Ampelographic characterization is a scientific method of morphological characterization of grapes and involves phenological, pomological, and morphological characteristics (Ates *et al.,* 2011). The major disadvantages of morphological markers are that they are limited in number, influenced by the plant growth stages, and influenced by various environmental factors (Dettweiler-Miinch, 1993).

With the advent of DNA markers, the characterization of grape varieties and genetic resources using molecular markers has become more popular. Molecular markers are sequences of DNA, which are located at a specific position on the chromosomes or genes, and their presence or absence can easily be used to identify

differences among individuals (Kumar, 1999). Molecular markers display polymorphism, which may arise due to alteration of nucleotide(s) or mutation in the genome loci and make it possible to identify genetic differences between individual organisms or species (Collard *et al.,* 2005). In contrast to ampelography, molecular markers are not influenced by the environmental conditions, growth stage of the plant, pleiotropy, and epistasis. They are highly polymorphic, not limited in numbers, and the results are reproducible. Therefore, molecular characterization is widely accepted for the identification of grape varieties and genetic diversity studies (Thomas *et al.,* 1994; Bowers *et al.,* 1996). Several types of molecular markers are available now and broadly categorized into two classes *viz*., *i*) hybridization-based markers, and *ii*) polymerase chain reaction (PCR)-based markers (Garrido-Cardenas *et al.,* 2018). Among them, simple sequence repeats (SSRs) or microsatellites are markers of choice for the molecular characterization of grapes because of their procedural ease, robustness, polymorphism, reproducibility, and codominant nature (Sefc *et al*., 2001; This *et al*., 2004).

De novo development of SSRs is a costly and time-consuming endeavor. Besides, the SSR primers are frequently species-specific; therefore, SSR markers of one taxon cannot be readily used to analyze another species. This lack of transferability makes interspecific comparisons difficult. To address these associated issues with SSRs, researchers have developed inexpensive gene-based SSR markers from available genomic resources that are more likely to be transferable across taxonomic boundaries. This rapid and inexpensive development of SSRs from expressed sequence tags (ESTs) databases has been shown to be a feasible option for obtaining high-quality EST-derived SSR markers (Gupta *et al*., 2003; Chagne *et al*., 2004). Recent advancements in molecular biology have resulted in the development of large amounts of DNA sequence data and ESTs from several plant species from which EST-SSRs can be quickly developed. (Scott *et al.,* 2000) reported the characterization of the first ten EST-SSR loci and their transferability among grape species. (Decroocq *et al*., (2003) reported other eight EST-SSR loci developed from an enriched cDNA library from the grape root. There are several reports that confirm the significant transferability of EST-SSRs across taxonomic boundaries than traditional SSRs in grapes (Scott *et al.,* 2000; Arnold *et al.,* 2002) as well as in other plant species (Chagne *et al.,* 2004). In view of their potential use, EST-SSRs are being developed in several crop plants (Vidya *et al.,* 2021; Li *et al.,* 2022; Sun *et al.,* 2022).

The objective of the present study was to utilize the known grape EST-SSRs for molecular characterization of grape accessions maintained at the ICAR - National Bureau of Plant Genetic Resources, Regional Station (ICAR-NBPGR-RS), Shimla and to confirm their transferability across different grape species.

Materials and Methods

Young, uniformly-sized and healthy fresh leaves were collected from 27 grape accessions maintained at the ICAR-NBPGR-RS, Shimla, Himachal Pradesh, India (31.097786 N; 77.160409 E; 1876 msl). The leaf samples were packed in aluminium foil and stored in zipper bags at -20 ^{0}C until further use. The accessions contained 16 *Vitis vinifera* cultivars, 6 non-*vinifera* species and their cultivars, 4 inter-specific hybrids, and 1 non-*Vitis* genus (*Parthenocissus quinquefolia)* from within the Vitaceae family (Table 1).

DNA was extracted from the leaf tissues based on a modified CTAB-dichloromethane protocol described by Saghai-Maroof et al. (1984). The yield and purity of extracted DNA were checked by running it on 1% (W/V) agarose gel. Details of the EST-derived SSR markers are given in Table 2. PCR was performed in 25 µl reaction volume containing 2.5 µl of 10x PCR buffer (without $MgCl_2$), 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of 10 µM of each forward and reverse primer, 0.5 µl of 10mM dNTP, 0.2 µl of 5 U/µl of Taq polymerase (Qiagen, Venlo, Limburg, Netherlands), about 50 ng of template genomic DNA and Milli-Q water to make reaction volume to 25 µl. The PCR mixtures were cycled at 94°C for 1 min, 50-55 °C (depending on primer) for 1 min, and 72°C for 1 min repeated for 35 cycles on a Veriti Thermal Cycler (Life Technologies, Carlsbad, CA, USA). The resulting products were resolved and scored manually on ethidium bromide-stained 2.5% high resolution agarose gels. The gels were then visualized and documented on gel doc unit (AlphaImager®HP, USA).

The bands were scored using Alphaimager EC version 3.2.2 (Cell Biosciences, Inc) for data analysis. GenAlEx 6.5 (Peakall and Smouse, 2012) and DARwin 6.0.21 (Perrier *et al.,* 2003) software were used for data analysis. Number of alleles, allele size, absolute frequencies and polymorphic information content (PIC) of markers were calculated for 27 samples. The PIC value of SSR markers was calculated according to the formula: PIC = 1 –Σ (P2i), where Pi is the frequency of the i^{th} allele of a marker detected in accessions (Nei, 1973). Simple matching coefficient of dissimilarity, 100 bootstraps and weighted neighbor joining were used to construct radial tree using DARwin 6.0.21 (Perrier *et al.,* 2003). Factorial analysis was also done using DARwin 6.0.21.

Results and Discussion

Five known EST-SSR primers were used to reveal the allelic composition of 27 grape accessions. Amplified EST-SSR products were recorded in all the accessions except 2 accessions namely PGR-10 (*Vitis arizonica*/DVIT 1269) and PGR-19 (*Parthenocissus quinquefolia*/DVIT2400) in which no amplification was noticed. Representative amplified products from 3 different EST-SSR markers are shown in Fig. 1. All EST-SSRs were polymorphic and the EST-SSR amplification products were detected on agarose gel in

Table 1: Grape accessions used in the study for SSR analysis

S. No.	Genotype code	Accession Number	Name of the accession
1	PGR-01	EC772083	*Vitis riparia x (V. cordifolia x V. rupestris)*/ Malegue 44-53
2	PGR-02	EC772100	*Vitisvinifera*/ Mauzac
3	PGR-03	EC772085	*Vitisvinifera*/ Admirable de Curtiller
4	PGR-04	EC772082	*Vitis riparia x V. rupestris*/Couderc 3309
5	PGR-05	EC732195	*Vitis interspecific cross*/ Black Fredonia Bunch
6	PGR-06	EC772103	*Vitis vinifera*/ Muscat A Petits Grains Blancs
7	PGR-07	EC772092	*Vitis vinifera*/ Chardonnay
8	PGR-08	EC452213	*Vitisamurensis*/ DVIT2005.5
9	PGR-09	EC772096	*Vitis vinifera*/ Hans
10	PGR-10	EC452207	*Vitisarizonica*/ DVIT1269
11	PGR-11	EC452206	*Vitisficifolia*/DVIT1106
12	PGR-12	EC452209	*Vitisberlandieri*
13	PGR-13	EC772086	*Vitisvinifera*/ Aghiorghitiko
14	PGR-14	EC772094	*Vitis vinifera*/ Furmint
15	PGR-15	EC772087	*Vitisvinifera*/ Alvarelhao
16	PGR-16	EC772106	*Vitisvinifera*/ Touriga Nacional
17	PGR-17	EC772095	*Vitisrupestris*/ Goethe 9
18	PGR-18	EC772098	*Vitis vinifera*/ Madeleine Royale
19	PGR-19	EC452215	*Parthenocissus quinquefolia*/ DVIT2400
20	PGR-20	EC772108	*Vitisvinifera*/ Savagnin Rose
21	PGR-21	EC772090	*Vitis vinifera*/ Cabernet Sauvignon
22	PGR-22	EC772101	*Vitis vinifera*/ Merlot
23	PGR-23	EC732197	*Vitis labrusca*/ Niagara Bunch
24	PGR-24	EC772080	*Vitislongii x V. riparia*/ Couderc 1616
25	PGR-25	IC566150	*Vitis vinifera*/ Unknown variety
26	PGR-26	EC772099	*Vitisvinifera*/ Mancin
27	PGR-27	EC772107	*Vitisvinifera*/ Ugni Blanc

Table 2: Information on EST-SSRs used in this study including marker name, repeat, primer sequences, annealing temperature (TA), and expected length

S. No.	*Marker*	*Repeat*	*Forward (F) and Reverse (R) Primer sequences (5′-3′)*	*TA (°C)*	*Expected length (bp)*
1	scu05vv	$(AT)_{13}$	F: CAAGCAGTTATTGAAGCTGCAAGG R: TCATCCATCACACAGGAAACAGTG	51.2	174
2	scu06vv	$(AT)_{8}$	F: CCTAATGCCAGGAAGGTTGC R: CCCTAGTCTCTCTACCTATCCATG	49.7	171
3	scu08vv	$(GGT)_{5}$	F: CGAGACCCAGCATCGTTTCAAG R: GCAAAATCCTCCCCGTACAAGTC	57.7	180
4	scu11vv	$(CTT)_{8}$	F: AATTGATAGTGCCACGTTCTCGCC R: AACGCCGACAAGAATCCCAAGG	57.3	248
5	scu15vv	$(GAA)_{6}$	F: GCCTATGTGCCAGACCAAAAAC R: TTGGAAGTAGCCAGCCCAACCTTC	53.5	195

Source: Scott et al., 2000

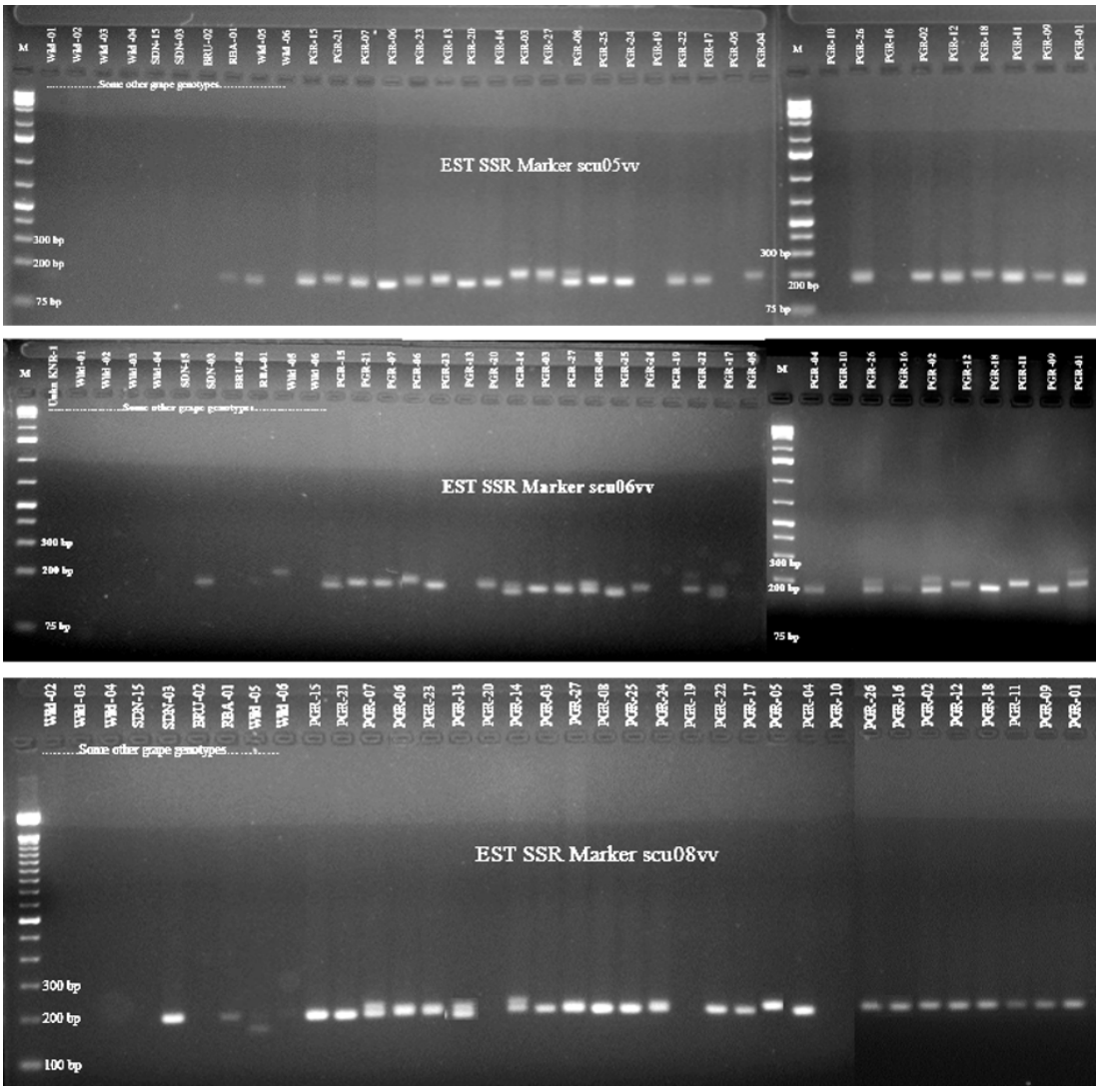

Fig. 1: Amplification of EST-SSR markers (scu05vv, scu06vv and scu08vv) in studied grape accessions. M = DNA ladder. EST-SSR marker names are shown on respective images

Table 3: Allele variations and polymorphic information content of EST-SSR markers used in the analysis of grape accessions

S. No.	*Primer name* [a]	*Est-ssr alleles*		*Pic* [c]
		Number	*Size (bp)*[b]	
1	Scu05vv	8	120(6), 142(6), 160(1), 169(3), 183(1), 188(1), 189(1), 193(6)	0.8064
2	Scu06vv	6	156(7), 171(8), 177(3), 186(2), 192(4), 196(3)	0.7929
3	Scu08vv	5	190(1), 200(5), 212(8), 226(9), 233(4)	0.7435
4	Scu11vv	8	227(4), 248(10), 252(5), 260(2), 263(2), 270(1), 274(1), 284(1)	0.7751
5	Scu15vv	4	194(5), 200(10), 210(7), 214(3)	0.7072
	Total	31		3.8251
	Average	6.2		0.7650

[a]Source of EST-SSR markers: Scott et al. (2000)
[b]Value in parentheses represents the actual number of accessions (out of total 27) where allele amplified.
[c]PIC: Polymorphic Information Content

Table 4: A database of summary of ETS-SSR allelic variations in 27 grape genotypes

S. No.	Genotype	EST-SSR marker and allele size (bp)					Total allelic frequency
		scu05vv	*scu06vv*	*scu08vv*	*scu11vv*	*scu15vv*	
1	PGR-01	183, 189	192, 196	212	248	210	7 (5.38%)
2	PGR-02	193	171, 196	200	248	210	6 (4.62%)
3	PGR-03	169	156	226	248	200	5 (3.85%)
4	PGR-04	160	177	226	227	210	5 (3.85%)
5	PGR-05	0	0	212	0	194	2 (1.54%)
6	PGR-06	120	186	226	252	200	5 (3.85%)
7	PGR-07	120	171	212, 233	263	200	6 (4.62%)
8	PGR-08	120, 169	156, 171	226	248, 270	200	8 (6.15%)
9	PGR-09	193	171	212	248	210	5 (3.85%)
10	PGR-10	0	0	0	0	0	0 (0.00%)
11	PGR-11	193	192	212	227	210	5 (3.85%)
12	PGR-12	193	192	200	248	210	5 (3.85%)
13	PGR-13	188	0	190, 200	252	214	5 (3.85%)
14	PGR-14	120	156	226, 233	252	200	6 (4.62%)
15	PGR-15	142	171	212	260, 284	200	6 (4.62%)
16	PGR-16	0	186	200	0	194	3 (2.31%)
17	PGR-17	142	156	226	227	194	5 (3.85%)
18	PGR-18	193	177	212	248	210	5 (3.85%)
19	PGR-19	0	0	0	0	0	0 (0.00%)
20	PGR-20	120	171	0	252	200	4 (3.08%)
21	PGR-21	142	171	212	263	200	5 (3.85%)
22	PGR-22	142	192	226	227	194	5 (3.85%)
23	PGR-23	142	171	226	260, 274	200	6 (4.62%)
24	PGR-24	120	156	233	248	214	5 (3.85%)
25	PGR-25	142	156	226	248	214	5 (3.85%)
26	PGR-26	193	177, 196	200	248	194	6 (4.62%)
27	PGR-27	169	156	233	252	200	5 (3.85%)
Total allelic frequency		25 (19.23%)	27 (20.77%)	27 (20.77%)	26 (20.00%)	25 (19.23%)	130 (100.00%)

25 out of 27 grape accessions indicating that there was a good level of interspecific transferability of the EST-SSRs. The observed amplification in genetically distant grape accessions was within the arbitrary expected size range that is 100 bp above or below the original *V. vinifera* fragment (Arnold *et al*., 2002). Non-detection of EST-SSR products in 2 genotypes on agarose gels may be due to non-amplification of DNA or the DNA bands were too faint to be scored. It has also been reported by Arnold (2002) that the average EST-SSR cross-transferability is about 51.1% within Vitaceae. Thus, it may be possible that the used *V. vinifera*-derived EST-SSRs are not transferable in 2 distant relatives *viz*., PGR-10 (*Vitis arizonica*/DVIT 1269) and PGR-19 (*Parthenocissus quinquefolia*/DVIT2400). Secondly, in EST-SSRs, the primers flanking them are derived from relatively conserved DNA sequences, however, if the cDNA from which EST-SSRs are derived lacks introns, unrecognized intron splice sites disrupt priming sites resulting in failed amplification (Rungis *et al.,* 2004).

A summary of EST-SSR polymorphism including allele number, size, absolute allele numbers, and PIC values is presented in Table 3. EST-SSR analysis showed 31 alleles ranging from 4 alleles per locus in scu15vv to 8 alleles per locus in scu05vv and scu11vv with average of 6.2 alleles per

Table 5: Summary of genetic variation statistics for EST-SSR loci in 27 grape accessions

EST-SSR locus	*Allelic parameters*					
	Number of accessions (N)	*No. of Alleles (Na)*	*No. of Effective Alleles (Ne)*	*Observed Heterozygosity (Ho)*	*Expected Heterozygosity (He)*	*Shannon's Information Index (I)*
scu05vv	23	8.000	4.766	0.087	0.790	1.724
scu06vv	23	6.000	4.560	0.174	0.781	1.637
scu08vv	24	5.000	3.646	0.125	0.726	1.386
scu11vv	23	8.000	3.861	0.130	0.741	1.600
scu15vv	25	4.000	3.415	0.000	0.707	1.299
Mean	-	6.200	4.050	0.103	0.749	1.529
SE	-	0.800	0.262	0.029	0.016	0.080

SE: Standard Error

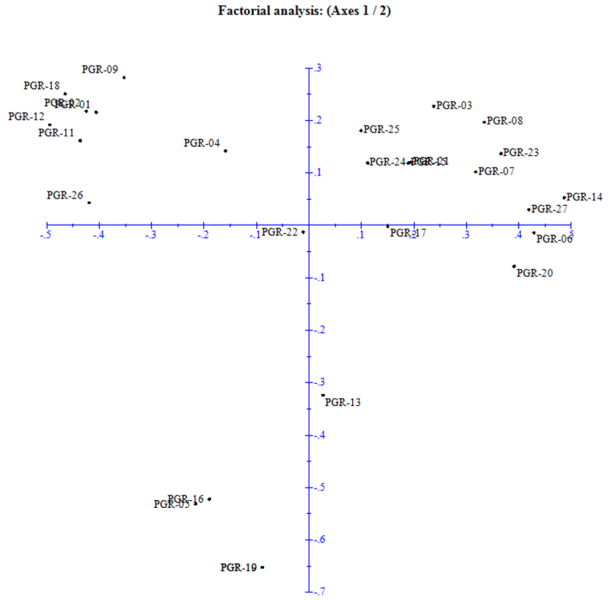

Fig. 2: Factorial analysis of 27 grape accessions using EST-SSR profiles and DARwin software

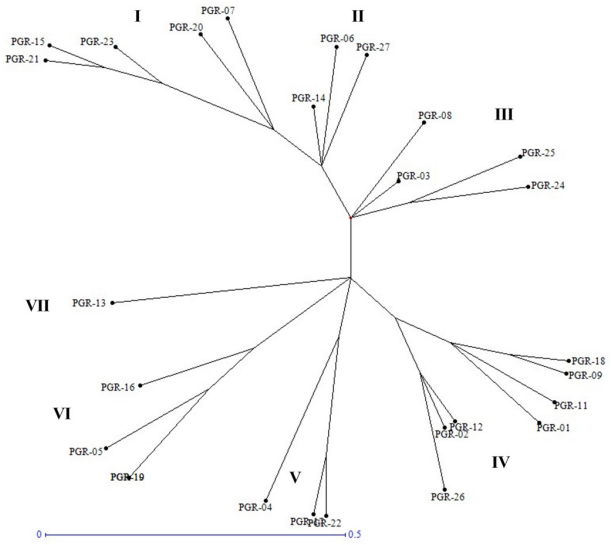

Fig. 3: Diversity analysis of 27 grape accessions using DARwin software and bootstrap value of 100

locus. The number of alleles amplified by each of the EST-SSR in our study was more than those reported by Scott *et al.* (2000) in same EST-SSRs. The number of alleles per locus in EST-SSRs in the present study was, however, lower than those obtained with traditional SSRs in the same grape accessions (unpublished). Similar results were obtained by Tabbasam *et al.* (2014), who reported that the traditional SSRs amplified a higher number of alleles compared to the EST-SSRs in cotton. This type of amplification behaviour of these markers can be attributed to their origin. SSR markers are distributed throughout the genome, while, EST-SSRs are specific and located within functional genes only. The PIC value indicates the informativeness of the SSR locus and its potential in detecting the differences among the genotypes based on genetic relationships. The PIC values in our study ranged from 0.7072 in scu15vv to 0.8064 in scu05vv with a mean of 0.7650 (Table 3). Relatively high PIC values obtained in the present investigation demonstrated that the used EST-SSRs can be useful for grape germplasm characterization. Based on PIC values, the most informative and polymorphic locus was scu05vv.

Absolute allele numbers varied from a minimum of 1 in scu05vv (160, 183, 188 and 189 bp), scu08vv (190 bp), and scu11vv (270, 274 and 284 bp)] to a maximum of 10 in scu15vv (200 bp) and scu11vv (248 bp) (Table 3). A total of 130 allelic bands were detected in 27 grape accessions that ranged from 0 (in PGR-10 and PGR-19) to 8 (in PGR-08). A database of EST-SSR markers prepared based on the

scored PCR amplification products is summarized in Table 4. Marker-wise total allele numbers in descending order were: 27 (20.77%) in scu06vv and scu08vv; 26 (20.00%) in scu11vv; and 25 (19.23%) in scu05vv and scu15vv. A high genetic variation in terms of number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and Shannon's Information Index (I) was evident in 27 grape accessions (Table 5). The factorial analysis conducted using DARwin software indicated that 51.37% of the total variance was explained by the first three components 22.22%, 16.81%, and 12.34%, respectively. A factorial graph depicting the distribution of grape accessions is shown in (Fig. 2). Significant genetic diversity among studied grape accessions was also exhibited by the dendrogram generated using DARwin software (Fig. 3). Except for PGR-10 and PGR-19, in which no amplification was achieved, all other 25 accessions could be successfully discriminated. The dendrogram exhibited 7 clusters. Cluster I, II, and VII contained nine *V. vinifera* cultivars; cluster III had three *V. vinifera* cultivars and *V. amurensis*; cluster IV contained four *V. vinifera* cultivars, *V. ficifolia*, *V. berlandieri*, and one interspecific hybrid; cluster V was comprised in one *V. vinifera* cultivar, *V. rupestris*, and one interspecific hybrid; while, cluster VI contained two *V. vinifera* cultivars, and 2 accessions (*V. arizonica*, and *Parthenocissus quinquefolia*) in which no amplification was observed. Though the findings indicate relative relationships among studied grape accessions, they also suggest complex origins of some of the cultivars, for which further research is needed.

In conclusion, the polymorphic and highly transferable EST-SSRs are a viable approach for molecular characterization in grape. Although only 5 known EST-SSRs have been tested in present study, their potential utility has been illustrated. There is a need to discover more such EST markers for extensive molecular characterization and phylogenetic studies in grape genetic resources.

Acknowledgments

We thank the Director, ICAR-National Research Centre for Grapes, Pune, and the Director, ICAR-Central Potato Research Institute, Shimla for supporting the research.

References

Arnold C, M Rossetto, Y McNally and RJ Henry (2002) The application of SSRs characterized for grape (*Vitis vinifera*) to conservation studies in Vitaceae. *Am. J. Bot.* 89(1): 22–28.

Ates F, H Coban, Z Kara and A Sabir (2011) Ampelographic characterization of some grape cultivars (*Vitis vinifera* L.) grown in south-western region of Turkey. *Bulg. J. Agric. Sci.*17: 314-324.

Bowers JE, GS Dangl, R Vignani and CP Meredith (1996) Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* 39: 628-633.

Cangi R, H Çelik and B Kose (2006) Determination of ampelographic characters of some natural foxy grape (*Vitis labrusca* L.) types grown in Northern Turkey (Ordu and Giresun Province). *Int. J. Bot.* 2: 171-176.

Chagne D, P Chaumeil, A Ramboer, C Collada, A Guevara, MT Cervera, G Cervera, G Vendramin, JMF Garcia, C Echt, T Richardson and C Plomion (2004) Cross-species transferability and mapping of genomic and cDNA SSRs in pines. *Theor. Appl. Genet.* 109: 1204–1214.

Collard BCY, MZZ Jahufer and ECK Pang (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assissted selection for crop improvement: The basic concepts. *Euphytica* 142: 169-196.

Decroocq MG, L Fave, LHagen and L Bordenave (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. *Theor. Appl. Genet.* 106: 912-922.

Dettweiler-Miinch E (1993) Influence of climate on morphologic characteristics of grapevines. *Vitis* 32: 249-253.

Garrido-Cardenas JA, C Mesa-Valle and F Manzano-Agugliaro (2018) Trends in plant research using molecular markers. *Planta* 247: 543-557.

Genetic Structure Analysis of *Psathyrostachys juncea* Nevski. *Front. Plant Sci.* 13: 837787.*doi: 10.3389/fpls.2022.837787*

Gupta PK, S Rustgi, S Sharma, R Singh, N Kumar and HS Balyan (2003) Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. *Mol. Genet. Genom.* 270: 315–323.

Khadivi A, A Gismondi and A Canini (2019) Genetic characterization of Iranian grapes (*Vitis vinifera* L.) and their relationships with Italian ecotypes. *Agrofor. Syst.* 93: 435–447.

Kumar LS (1999) DNA markers in plant improvement: an overview. *Biotechnol. Adv.* 17: 143–182.

Li Z, L Yun, Z Gao, T Wang, X Ren and Y Zhao (2022) EST-SSR Primer Development and Genetic Structure Analysis of *Psathyrostachys juncea* Nevski. *Front. Plant Sci.* 13: 837787. *doi: 10.3389/fpls.2022.837787*

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*.70: 3321-3323.

Peakall R. and PE Smouse (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Perrier, X, A Flori, and F Bonnot (2003) Data analysis methods. In: P Hamon, M Seguin, X Perrier and JC Glaszmann (eds) *Genetic Diversity of Cultivated Tropical Plants*. Enfield, Science Publishers. Montpellier. pp 4 -76.

Rungis D, Y Berube, J Zhang, S Ralph, CE Ritland, BE Ellis, C Douglas, J Bohlmann and K Ritland (2004) Robust simple sequence repeat markers for spruce (*Picea* spp.) from expressed sequence tags. *Theor. Appl. Genet.* 109: 1283–1294.

Saghai-Maroof MA, KM Soliman, RA Jorgensen and RW Allard (1984) Ribosomal DNA sepacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci.* 81: 8014–8019.

Scott KD, P Eggler, G Seaton, M Rossetto, EM Ablett, LS Lee and RJ Henry (2000) Analysis of SSRs derived from grape ESTs. *Theor. Appl. Genet.* 100: 723–726.

Sefc KM, F Lefort, MS Grando, KD Scott, H Steinkellner and MR Thomas (2001) Microsatellite markers for grapevine: A state of the art. In: KA Roubelakis-Angelakis (ed) *Molecular Biology & Biotechnology of the Grapevine Springer* Netherlands. pp 433–463.

Sun M, Y Zhao, X Shao, J Ge, X Tang, P Zhu, J Wang and T Zhao (2022) EST–SSR Marker Development and Full-Length Transcriptome Sequence Analysis of Tiger Lily (*Lilium*

lancifolium Thunb). *Appl. bionics biomech*. 2022: Article ID 7641048, *https://doi.org/10.1155/2022/7641048*

Tabbasam N, Y Zafar and Mehboob-ur-Rahman (2014) Pros and cons of using genomic SSRs and EST-SSRs for resolving phylogeny of the genus *Gossypium*. *Plant Syst. Evol.* 300: 559–575.

This P, A Jung, P Boccacci, J Borrego, R Botta, L Costantini, M Crespan, G Dangl, C Eisenheld, F Ferreira-Monteiro, S Grando, J Ibanez, T Lacombe, V Laucou, R Magalhaes, C Meredith, N Milani, E Peterlunger, F Regner and E Maul (2004) Development of a common set of standard varieties and standardized method of scoring microsatellites markers for the analysis of grapevine genetic resources. *Theor. Appl. Genet*. 109: 1448–1458.

Thomas MR, P Cain and NS Scott (1994) DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. *Plant Mol. Biol.* 25: 939–949.

Vidya V, D Prasath, M Snigdha, R Gobu, C Sona and CS Maiti (2021) Development of EST-SSR markers based on transcriptome and its validation in ginger (*Zingiber officinale* Rosc.). *PLoSOne* 16: e0259146.

Vivier MA and IS Pretorius (2002) Genetically tailored grapevines for the wine industry. *Trends Biotechnol.* 20: 472–478.