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Original article

Assessment of genetic diversity in 29 rose germplasms using SCoT marker

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ABSTRACT

Roses are well known for commercial floriculture and greatly used in the field of perfumery, soap, cosmetics, jams & jelly and essential oil production. Due to interspecific hybridization, a large number of hybrids and cultivated varieties of rose are recognized which reveals distinguishable features in flowers such as size, shape, and color. Apart from this, the geographical distribution and polyploidy also make *Rose* genus more complex. Therefore, the present study was undertaken for the identification and characterization of genetic variation within 29 rose accessions through Start codon targeted polymorphism (SCoT) markers. Out of 36 primers, 32 revealed polymorphic amplification profile in 29 rose accessions with amplification ranging from 150 bp to 1.2 kb. A total of 299 polymorphic amplicons were obtained, ranging from 4 to 19 amplicons with an average of 9.34 amplicons per primer. The polymorphic information content (PIC) ranged from 0.42 to 0.92 with an average of 0.78. The dendrogram was constructed to establish genetic relationship among 29 different accessions using Neighbor-joining and Nei-Li matching coefficient. The distinguishable genetic background and a high degree of variation in the rose genotypes successfully exhibited by the SCoT markers may serve as a valuable aid in Rose improvement strategy.

1. Introduction

Rose is considered as "Queen of the Flowers", belonging to the *Rosaceae* family. It is woody perennial flowering plant grown all over the globe, especially sub-tropical and temperate regions of the northern hemisphere (Werlemark and Nybom, 2010). Rose flowers are large & showy, vary in size & shape and are being utilized for commercial perfumery, essential oils production, commercial cut flower, as landscape plant, for hedging and other utilitarian purposes (Akond et al., 2012). Generally, the rose petals are used for rose oil production. About 3000 kg of rose petals can produce one kg of rose oil (Baser, 1992; Baydar and Baydar, 2005). In addition to rose oil, some important base materials for the cosmetic industry such as rose concrete, absolute and rose water are also obtained from *Rosa chinensis* and *Rosa canina* (Baydar et al., 2004). Rose hip seed oil is employed in various skin care and cosmetic products. Apart from its beautification application, there are several uses of rose viz. Rose hips of *Rosa canina* is used in making soup, jam, and jelly because of its high vitamin-C content. *Rosa chinensis* is used for stomach problems as well as in controlling cancer growth (www.Pfaf.org).

Through interspecific hybridization, a large number of hybrids and cultivated varieties have been developed which differ in color ranging from yellow and white to many shades of red and pink with single or double blooms. Due to the allopolyploidization and hybridizations, the number of rose varieties has reached to approximately 25,000 which make it difficult to classify '*Rosa*' genus and wild type of some modern roses (Azeem et al., 2012; Zhang and Gandelin, 2003). Although, *Rosa* genus has a broad and overlying territory of morphological deviations that are influenced by the environmental circumstances, so the classification based on morphological data only, is not sufficient. Many researchers classify rose varieties on the basis of their morphological characteristics like flower weight, flower diameter, peduncle length,

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number of petals, number of stamens and oil content (Kaul et al., 2009; Panwar et al., 2010; Riaz et al., 2011; Tabaei-Aghdaei et al., 2007; Zeinali et al., 2010).

Moreover, biochemical markers as chemotaxonomic analyses of roses based on a vast range of polyphenolic compounds have also been reported in relation to identify rose taxonomy amongst the different rose species (Mikanagi et al., 1993; Okuda et al., 1992; Raymond et al., 1995; Sarangowa et al., 2014). While, Isozyme based marker studies have also been used by some research groups for the identification and classification of *Rosa* genus (Kim and Byrne, 1996; Walker and Werner, 1997). However, the usage of isozyme markers is limited because of a small number of constantly resolvable loci (Kim and Byrne, 1994). For assessing genetic diversity which is vital for species survival, molecular markers with the aid of modern computing facilities are best suited as they offer fast, cheap and highly discriminating properties between species and within species or varieties (Azeem et al., 2012). Information to enrich our knowledge on genetic diversity is obtained from factors such as morphological, biochemical and molecular markers which lead us to a more meaning taxonomical classification (Gonçalves et al., 2009; Mohammadi and Prasanna, 2003; Sudré et al., 2007).

DNA based markers have been used very commonly in ecological, taxonomical, comparative biology, diversity, conservation, phylogenic and genetic studies amongst plant species (Haq et al., 2014). After the advent of PCR several advancement and introduction of new concepts were employed in the improvement of various types of molecular marker technologies like, amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSRs), simple Sequence Repeats (SSR), single sequence polymorphism (SNP). For distinct genetic applications amongst diverse plant species different markers have been used by various researchers in rice (Huang et al. 1997), bread wheat (Gupta et al., 2003), barley (Varshney et al., 2007), *Jatropha curcas* (Khurana-Kaul et al., 2012), Tomato Cultivars (Nawaz et al. 2015), Poaceae plants (Haq et al., 2016), *Citrullus colocynthis* (Verma, 2017) etc.

A novel molecular marker known as Start Codon Targeted (SCoT) polymorphism targets on short ATG start codon in plant genes has been reported (Collard and Mackill, 2009). It has several advantages over RAPD, ISSR and AFLP, as it is more stable, produce more reproducible and reliable bands and can be used effectively for population studies, genetic mapping in different plants and in the marker assisted selection programs. Similar to RAPD and ISSR markers, SCoTs are important markers which could be used for different genetic application such as, to assess genetic diversity and structure, in bulk segregation analyses, quantitative trait loci (QTL) mapping and DNA fingerprinting. These markers are directly involved in relation of gene function and can be utilized in genotyping and to explore polymorphism (Gorji et al., 2011; Poczai et al. 2013). SCoT markers have been successfully practiced for diversity analysis and diagnostic finger-printing in mango (Luo et al., 2010), peanut (Xiong et al., 2011), grape (Guo et al., 2012), *Jatropha* (Mulpuri et al., 2013), orchard grass (Zeng et al., 2014), *Dendrobium* species (Feng et al., 2015), kalmegh (Tiwari et al., 2016), cowpea (Igwe et al. 2017), plantago (Rahimi et al., 2018) and taxus (Hao et al., 2018). The aim of present study was to evaluate the effectiveness of SCoT markers to determine genetic polymorphism and diversity amongst 29 rose germplasms.

2. Materials and methods

2.1. Plant material

A set of 29 different cultivars of genus *Rosa* were collected from different location of Jaipur District, Rajasthan, India and were used for genetic polymorphism, diversity and phylogenetic relationships amongst them using SCoT markers. All the cultivars represented distinguishable morphological characteristics (Fig. 1) and differed in flower color, stem height, bloom shape and plant habit (Table 1).

2.2. DNA extraction and purification

Total genomic DNA was extracted from young leaves according to the CTAB method (Doyle and Doyle, 1990). Leaf samples were crushed using 5 ml of pre-heated extraction buffer [1% PVP, 1 M

Fig. 1. Pictorial views of twenty-nine rose accessions.

Table 1
List of the *Rosa* accessions evaluated in this study.

S.no.	Cultivar Name	Class	Bloom Color	Bloom Shape	Stem Height	Collection Site
1	Lovers Meeting	Hybrid tea	Orange blend	Double tea shaped	60–90 cm	Durgapura nursery, Jaipur
2	Careless Love	Hybrid tea	Pink blend, stripes	Double cupped	50–90 cm	Durgapura nursery, Jaipur
3	Avalanche	Floribunda	White color with hint of green around petals	Double bloom	90–120 cm	Janta store circle rajendra marg Jaipur
4	Black Lady	Hybrid tea	Deep red	Double bloom	30–50 cm	Durgapura nursery, Jaipur
5	Eiffel Tower	Hybrid tea	Medium pink	Double tea shaped	120–180 cm	Durgapura nursery, Jaipur
6	Sunset Blend	–	Lavish orange center warm coral pink	–	25–70 cm	Ram newas bagh nursery jaipur
7	Gold Strike	Floribunda	Lemon yellow	Star-shaped bloom	50–70 cm	Ram newas bagh nursery Jaipur
8	Candy Stripe	Hybrid tea	Pink blend	–	90–120 cm 121–180 cm	Ram newas bagh nursery Jaipur
9	Tajmahal	Hybrid tea	Red	–	90–150 cm	Janta store circle Jaipur
10	Claude Monet	Hybrid tea	White medium yellow red blend	Semi double	90–120 cm	Ram newas bagh nursery Jaipur
11	Paradise	Hybrid tea	Mauve and mauve blend	Double bloom	90–120 cm	Ram newas bagh nursery Jaipur
12	Double Delight	Hybrid tea	Red blend	Double tea shaped	90–120 cm 90–180 cm	Ram newas bagh nursery Jaipur
13	Kaiser Wilhelm I	Hybrid perpetual	Purple red Violet shading	Double bloom	90–180 cm	Ram newas bagh nursery Jaipur
14	Yellow Patio	–	Rich yellow	Double bloom	50 cm	Ram newas bagh nursery Jaipur
15	Strawberry Romance	Hybrid tea	Pink blend Bi color	High centered	90–120 cm	Ram newas bagh nursery jaipur
16	All Gold	Floribunda	Golden or Vibrant yellow	Semi double cupped	60 cm	Ram newas bagh nursery Jaipur
17	Pleasure	Cluster flowered (incl. Floribunda & Grandiflora)	Medium pink	Double bloom	90–120 cm	Ram newas bagh nursery Jaipur
18	Love	Grandiflora	Red reversed silvery white	Fully Double	90–120 cm	Ram newas bagh nursery Jaipur
19	Tangerine Jewel	Hybrid Bracteata	Orange blend	Single to Semi double	60–90 cm	Ram newas bagh nursery Jaipur
20	William Shakespeare	English rose	Velvety crimson or medium red	Double Cupped	90–120 cm	Ram newas bagh nursery Jaipur
21	Henry Hundson	Hybrid rugosa	White blend	Double flat bloom	60–120 cm	Ram newas bagh nursery Jaipur
22	Black Baccara	–	Black tinged burgundy red/ Dark red	Double tea shaped	120–150 cm	Ram newas bagh nursery Jaipur
23	Pope john paul II	Hybrid tea	White	–	120–150 cm	Durgapura nursery, Jaipur
24	Radnectar	Grandiflora	Apricot	–	120–150 cm	Durgapura nursery, Jaipur
25	Baimir/Kashmir	Shrub	Dark Red	Double tea shaped	90–120 cm	Durgapura nursery, Jaipur
26	Apricot nectar	Floribunda	Apricot or apricot blend	Cupped bloom	60–120 cm	Durgapura nursery, Jaipur
27	First red	Hybrid tea	Classic Red	–	90–120 cm	Durgapura nursery, Jaipur
28	Mundi	Old garden (Gallica)	Crimson	Semi-double bloom	75–120 cm	Ram newas bagh nursery Jaipur
29	Mozart	Hybrid Musk	Deep Pink, white center	Continuous	80–150 cm	Ram newas bagh nursery Jaipur

TrisHCl pH 8.0, 5 M NaCl, 2% CTAB, 0.5 mM EDTA, 200 μl βME] and incubated for 1 h at 65 °C. Then it was treated with equal volume of Chloroform: Isoamylalcohol mixture (24:1; v/v). DNA was pelleted with double volume of ice cold Isopropanol and washed twice with 70% ethanol. The isolated DNA was air dried and stored at −20 °C in TE buffer. The dissolved nucleic acid was treated with RNase solution and incubated at 37 °C for 1 h. Followed by, purification was carried out using Phenol: Chloroform: Isoamylalcohol (25:24:1v/v) solution and upper aqueous phase was collected after centrifugation. After that 3 M Sodium acetate (0.1v) and 500 μl absolute alcohol were added into mixture, which was followed by pellet washing with 70% ethanol then air dried and dissolved in TE buffer. DNA was stored at −20 °C and concentration was adjusted via spectrophotometric method.

2.3. PCR amplification for SCoT markers

Total 36 sets of primers were custom synthesized by Operon Technologies (Almeda, USA) according to Collard and Mackill (Collard and Mackill, 2009) (Table 2). These were 18-mer primers having GC content between 50% and 72%. All PCR reactions were carried out within a total volume of 10 μl in 96 well plate's thermal cycler (Bio-Rad.UK) for SCoT primers. Each reaction contains 25 ng template DNA (1 μl), 1.0 μl of primer (10 pmole/μl), 0.3 μl of 100 mM of dNTPs, 0.5 unit of Taq DNA polymerase, 1.2 μl of 10× PCR buffer (Bangalore Genei, India). Amplification was performed in the following conditions – an initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, annealing for 1 min and extension at 72 °C for 2 min with a final extension at 72 °C for 5 min. The PCR conditions mainly for annealing temperatures (varying from 50 °C to 58 °C) were standardized for each primer and amplified products were stored at 4 °C. All amplified products were resolved on 1.5% high resolution agarose gel made in 0.5× TBE buffer then preformed electrophoresis for 3.5 h at 70 V and visualized with ethidium bromide (10 mg/mL). The image of banding patterns was captured under UV light using gel documentation system (Bio-Rad).

2.4. Genetic diversity analysis

All PCR amplified SCoT fragments were detected on gels and scored as binary data, for their presence (1) or absence (0) by visual observation. In order to ensure credibility only reproducible and well defined bands were scored. Smeared and weak bands were excluded. Polymorphic and monomorphic bands were determined for each SCoT primer. The dendrogram was constructed based on neighbor-joining and Nei and Li similarity matrix through Free tree/Tree view software's (Pavlicek et al., 1999). The genetic diversity displayed among different genotypes were based on their similarity matrix which were obtained from binary data existence. Bayesian clustering was conducted to infer population structure and assign individuals to populations based on SCoT genotypes using STRUCTURE 2.3.4 software (Pritchard et al., 2000; Falush et al., 2003) with different values of the number of clusters (K). To obtain the optimum K value, the length of the burning period was 100,000 iterations followed by 200,000 Monte Carlo Markov Chain replicates. Each *K* value was run 10 times with values ranging from K 1 to K 10, it was plotted against the mean estimate of

Table 2
Sequence of SCoT primers and GC content (%).

Primer	Sequences (5′–3′)	%G/C	Primer	Sequences (5′–3′)	%G/C
SCoT1	CAACAATGGCTACCACCA	50	**SCoT19**	ACCATGGCTACCACCGGC	67
SCoT2	CAACAATGGCTACCACCC	56	**SCoT20**	ACCATGGCTACCACCGCG	67
SCoT3	CAACAATGGCTACCACCG	56	**SCoT21**	ACGACATGGCGACCCACA	61
SCoT4	CAACAATGGCTACCACCT	50	**SCoT22**	AACCATGGCTACCACCAC	56
SCoT5	CAACAATGGCTACCACGA	50	**SCoT23**	CACCATGGCTACCACCAG	61
SCoT6	CAACAATGGCTACCACGC	56	**SCoT24**	CACCATGGCTACCACCAT	61
SCoT7	CAACAATGGCTACCACGG	56	**SCoT25**	ACCATGGCTACCACCGGG	67
SCoT8	CAACAATGGCTACCACGT	50	**SCoT26**	ACCATGGCTACCACCGTC	61
SCoT9	CAACAATGGCTACCAGCA	50	**SCoT27**	ACCATGGCTACCACCGTG	61
SCoT10	CAACAATGGCTACCAGCC	56	**SCoT28**	CCATGGCTACCACCGCCA	67
SCoT11	AAGCAATGGCTACCACCA	50	**SCoT29**	CCATGGCTACCACCGGCC	72
SCoT12	ACGACATGGCGACCAACG	61	**SCoT30**	CCATGGCTACCACCGGCG	72
SCoT13	ACGACATGGCGACCATCG	61	**SCoT31**	CCATGGCTACCACCGCCT	67
SCoT14	ACGACATGGCGACCACGC	56	**SCoT32**	CCATGGCTACCACCGCAC	67
SCoT15	ACGACATGGCGACCGCGA	67	**SCoT33**	CCATGGCTACCACCGCAG	67
SCoT16	ACCATGGCTACCACCGAC	56	**SCoT34**	ACCATGGCTACCACCGCA	61
SCoT17	ACCATGGCTACCACCGAG	67	**SCoT35**	CATGGCTACCACCGGCCC	72
SCoT18	ACCATGGCTACCACCGCC	67	**SCoT36**	GCAACAATGGCTACCACC	56

log probability of the data *L(K)*. The real number of sub-population was identified using the maximum *L(K)* value. Population structure was calculated with ΔK using STRUCTURE Harvester, based on the second-order rate of change of likelihood distribution mean L ("K") and with respect to *K* estimated (Evanno et al., 2005).

3. Results

The genetic polymorphism, diversity and phylogenetic relationships were established amongst 29 distinct cultivars of *Rosa* genus through SCoT markers or polymorphism in short conserved region flanking the ATG start codon. The cultivars were collected from different location of Jaipur, Rajasthan, India which displayed certain dissimilarity in their morphological traits. Each rose cultivar had distinct characteristic differences amongst bloom color (deep red, lavish orange, white blend etc.), bloom shape (double cupped, double flat bloom or double tea shaped) and stem height which range from 25–190 cm.

A sum of 36 SCoT primers was examined for PCR optimization, characterization, and their amplification within 29 different *Rosa* germplasms. Most of the primers revealed polymorphic and reproducible amplification profiles and yielded 299 unblurred and bright bands ranging from 150 bp to 1.2 kb in size. While, the numbers of bands ranged from four to nineteen, with an average of 9.34 bands per primer and SCoT21 displayed maximum number (19) of banding patterns and primers while SCoT17 and SCoT22 showed least number (4) of banding profiles (Table 3). However, primers SCoT7, SCoT9, SCoT27, and SCoT32 were unable to produce any amplification with Rose genomic DNA. These results indicated that a high polymorphism could be disclosed by SCoT markers in *Rosa* accessions. Furthermore, the amplification profile generated by SCoT1, SCoT2, SCoT21 and SCoT28 primers has been presented in Fig. 2.

The binary matrix was constructed from the amplified bands obtained from SCoT primers extension. This formulated matrix was used to unveil genetic affinity and genetic diversity among 29 *Rosa* germplasms. In the present study, the highest similarity coefficient was observed between *Rosa Black Baccara* & *Rosa Lovers Meeting* followed by *Rosa Careless Love* & *Rosa Black Baccara* and *Rosa Pope John Paul II* and *Rosa Black Baccara* also showed close occurrence. The lowest similarity was observed between *Rosa Baimir* and *Rosa Apricot Nectar* with the value of 0.24 coefficients (Supplementary Table 1). Thus these results unveiled the closeness of *Rosa Black Baccara* to *Rosa Lovers Meeting*, *Rosa Careless Love* and *Rosa Pope John Paul II*, while *Rosa Baimir* and *Rosa Apricot Nectar* showed dissimilarity between them.

A dendrogram was constructed amongst 29 distinct rose genotypes using binary data that was based on Nei-Li similarity coefficient/Neighbor-joining through free-tree/tree view software. It was observed that the dendrogram grouped all the cultivars into two major cluster I and II. While, cluster I included only *Rosa Strawberry Romance* and cluster II was subdivided into two groups A and B. Group A was further divided into two subgroups, IIa and IIb. The subgroup IIa revealed 14 different rose cultivars in relation to their closeness and subgroup IIb was with two cultivars i.e. *Rosa*

Table 3
Percent amplification (PA), Total number of bands (TNB), Polymorphism information content (Pic) obtained by SCoT primers in *Rosa* accessions.

Primer No.	PA	TNB	Pic Value
1	86.66	10	0.8344
2	86.66	14	0.894706
3	80	8	0.5275
4	43.33	7	0.8206
5	83.33	5	0.4289
6	86.66	10	0.7715
8	23.33	8	0.779
10	40	5	0.6113
11	73.33	15	0.89792
12	43.33	9	0.8568
13	63.33	9	0.7953
14	80	11	0.868508
15	90	14	0.912109
16	86.66	7	0.6821
17	66.66	4	0.5899
18	83.33	9	0.8402
19	76.66	13	0.89504
20	63.33	10	0.8686
21	86.66	19	0.929811
22	93.33	4	0.6549
23	83.33	6	0.77
24	40	7	0.7343
25	53.33	7	0.7678
26	80	8	0.8192
28	90	14	0.875743
29	73.33	7	0.7043
30	86.66	10	0.7954
31	66.66	10	0.8339
33	43.33	11	0.901844
34	83.33	9	0.8056
35	93.33	11	0.876509
36	90	8	0.8237
Total average	**72.49**	**299 (9.34)**	**0.7864**

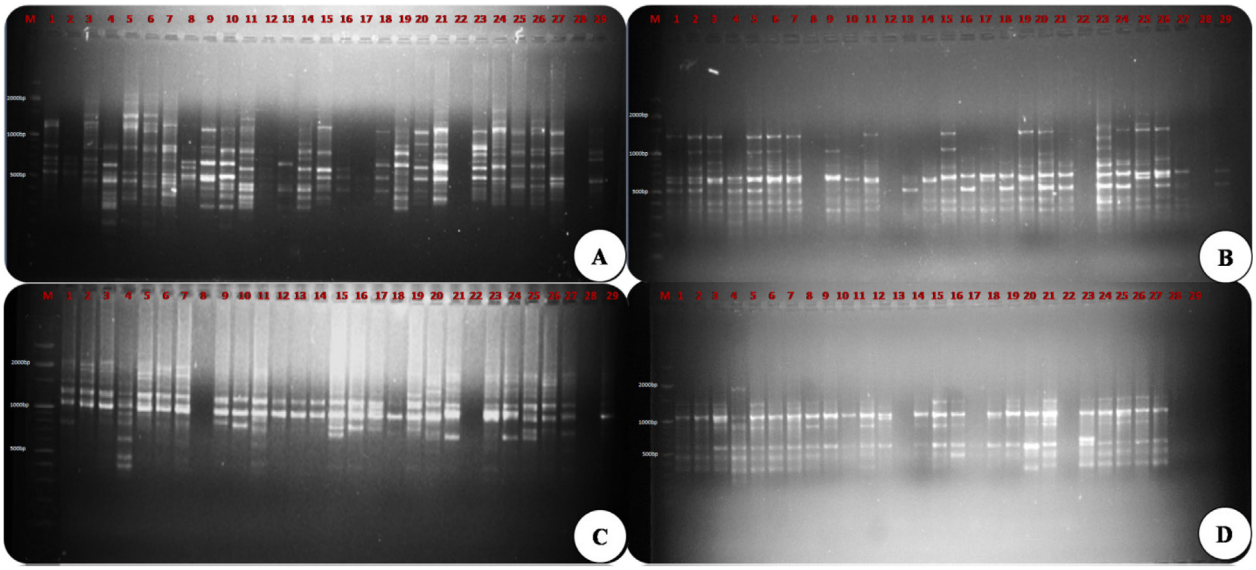

Fig. 2. SCoT Amplification Profile of Primer (A) SCoT21 (B) ScoT2 (C) SCoT28 And (D) SCoT1 amongst the 29 *Rosa* accessions. Lanes marked as 1 to 29 which represent the accessions according to serial numbers in Table A.1 and M represents 100 bp molecular weight ladder.

Paradise and *Rosa Tajmahal* which were distinct from subgroup IIa and cluster I cultivar. As well, group B was with 12 different rose cultivars and divided into two subgroups namely; IIc and IId. While, the IIc was with only one cultivar (*Rosa Tangerine jewel*) and IId consisted 11 distinct Rose cultivars (Fig. 3). Bayseian clustering conducted was used to assign the 29 *Rosa* germplasms with reference to their population structure. The estimated membership fraction ranged from K 1 to K 10 and the most reliable probabilities obtained at K = 3 (Fig. 4). The highest ad hoc measure of delta K was observed at K = 3 (Fig. 5) which indicates the 29 germplasms are divided into three subgroups. The membership probability threshold score was observed 0.78 (Fig. 6). The 29 germplasms were separately assigned to each subgroup and fractions less than 0.22 score were considered to be admixed.

4. Discussion

Molecular markers are considered to be the best for characterization of genetic polymorphism at DNA level, germplasm characterization, genetic diversity analysis, parentage determination, genetic distance estimation, gene mapping, and marker-assisted selection (Gupta and Rustgi, 2004; Varshney et al., 2005; Agarwal et al., 2008; Madhumati, 2014). Till date, a variety of molecular markers technologies have been developed through innovations of different new principle and approaches in molecular markers system. On account of these, different marker technologies have been investigated in *Rosa* genus for their genetic characterizations using distinct types of molecular markers such as simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) (Akond et al., 2012; Azeem et al., 2012; Panwar et al., 2015).

The present study, reports the use of utilitarian marker such as SCoT primers for comparing genetic diversity and for establishing genetic relationships among 29 *Rosa* germplasms. SCoT marker technique used in the current study is simple, low cost, fast, effective and highly reproducible with requirement of small amount of DNA in addition to no prior information of DNA sequence. These markers are very easy to design based on ATG context that is conserved region surrounding the translation initiation codon, thus the SCoT marker technique correspond to functional genes and their correlating characters (Xiong et al., 2011). Disparate from RAPD, AFLP and ISSR marker system, SCoT is gene targeted marker with multilocous nature and it can generate more information correlated with biological traits and helpful in high genetic polymorphism. Evaluation of SCoT markers in diversity analysis and diagnostic fingerprinting has already been established in Mango (Luo et al., 2012), Orchid (Bhattacharyya et al., 2013), Date palm (Al-Qurainy et al., 2015), *Diospyros* (Deng et al., 2015), *Elymus sibiricus* (Zhang et al., 2015), *Vigna unguiculata* (Igwe et al., 2017) and taxus (Hao et al., 2018).

In the present investigation, a set of 36 SCoT primers were used to examine genetic polymorphism, out of total, 32 SCoT primers produced unambiguous and reproducible banding profile with 150–1200 bp product size but 4 SCoT primers failed to amplify the rose genomic DNA. High polymorphism (100%) as reported in the present study is in compliance with earlier investigations in rose (Henuka et al., 2015) who reported (98.54% polymorphism) with RAPD markers. Panwar et al. (2015) who observed 94% of genetic polymorphism with ISSR markers then Prasad et al. (2006) who reported 87.5% of polymorphism in fragrant rose cultivars with RAPD markers. Our study is comparable to high SCoT based polymorphism obtained in several other plant species also viz. 97.10% in *Atriplex halimus* (Elframawy et al., 2016), 96.68% in *Diospyros* (Deng et al., 2015), 95.71% in *Quercus brantii* (Alikhani et al., 2014), and 93% in Grape (Guo et al., 2012).

The present study is first report to have shown significant genetic polymorphism amongst various *Rosa* germplasm using SCoT markers. A total of 299 scorable bands were identified through the amplification of 32 SCoT primers in 29 diverse rose germplasms. The amplification ranged from 4 bands to 19 bands with an average of 9.42 bands per primer which is nearly comparable with earlier study by Panwar et al. (2015) who reported an average of 11 bands per primer in 32 rose cultivars with ISSR primers while polymorphism generated by RAPD marker is not comparable to our work as it gave only 6.5 bands per primer (Henuka et al., 2015). Moreover, an average PCR amplification found to be 72.49% and polymorphic information content (PIC) ranged from 0.42 (SCoT-5) to 0.92 (SCoT-21) with an average of 0.78 which exhibit similarity to PIC value obtained in previous studies such as, 0.72 by SSR marker (Ghose et al., 2013), 0.88 by STMS marker system (Fernández-Romero et al., 2009), while in RAPD a value (0.38) was obtained by Henuka et al. (2015). Thus,

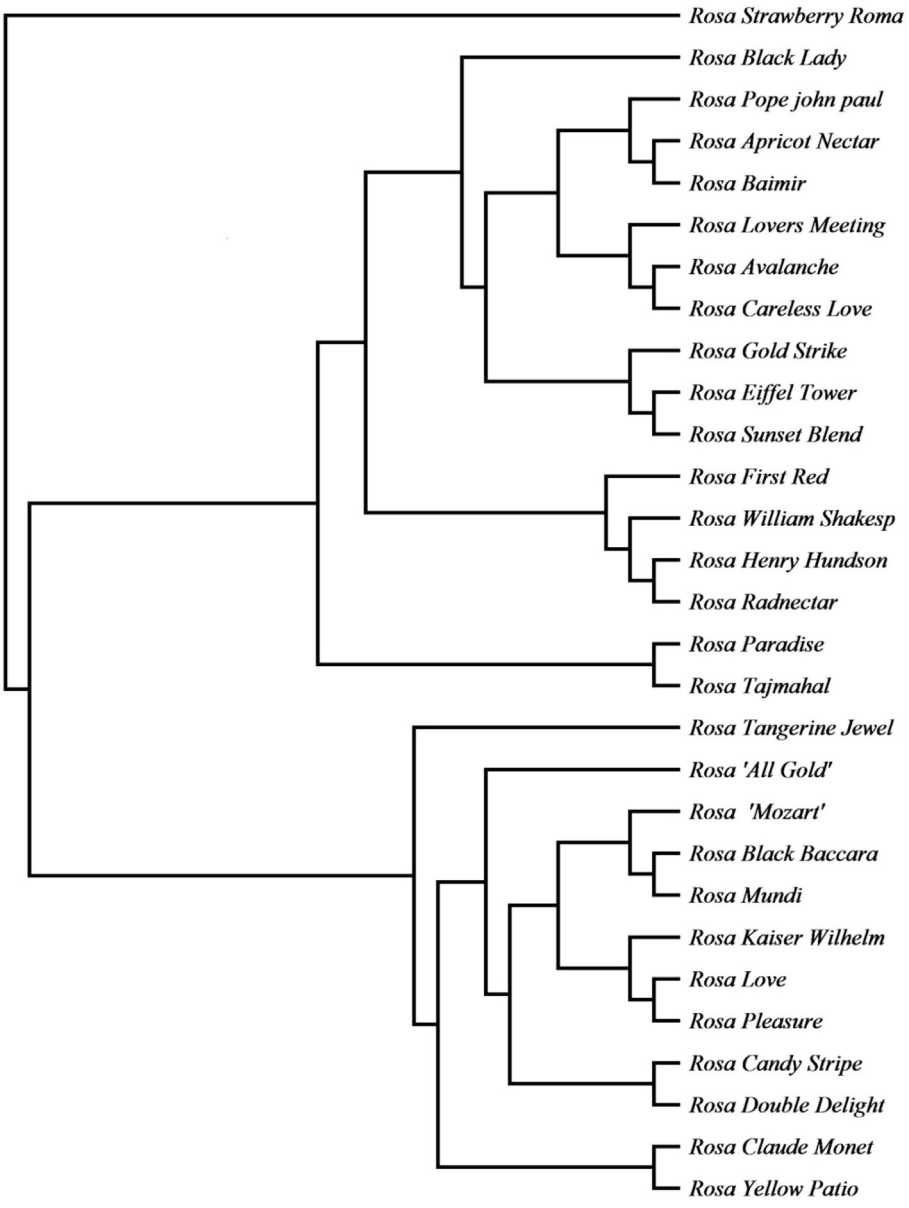

Fig. 3. A dendrogram revealed genetic relationships among 29 different *Rosa* accessions using SCoT marker.

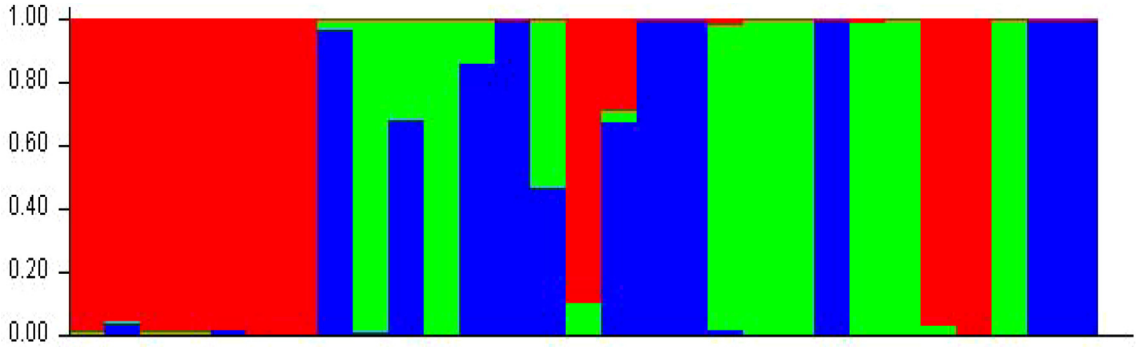

Fig. 4. Pattern of variation of 29 accessions with 32 SCoT markers, bar length representing the membership probability of accessions belonging to different subgroups at $K = 3$.

the parameters such as primer polymorphism and polymorphic information content (PIC) used in the present study are found to be very supportive to examine markers for their usefulness in the fingerprinting process.

The binary matrix from the PCR amplified product was used for similarity index/coefficient calculation that was based on Nei-li matching coefficient. The maximum similarity was identified between *Rosa Careless* Love & *Rosa Black Baccara*, and between *Rosa*

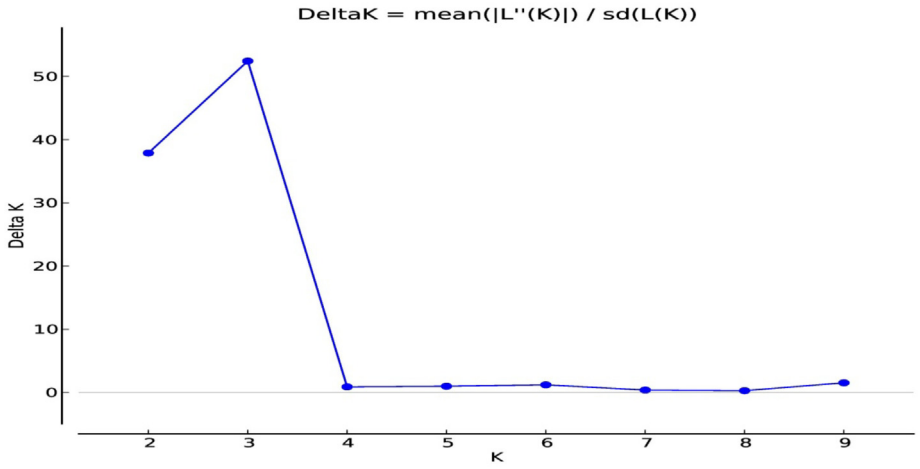

Fig. 5. Graph of estimated membership fraction for $K = 3$.

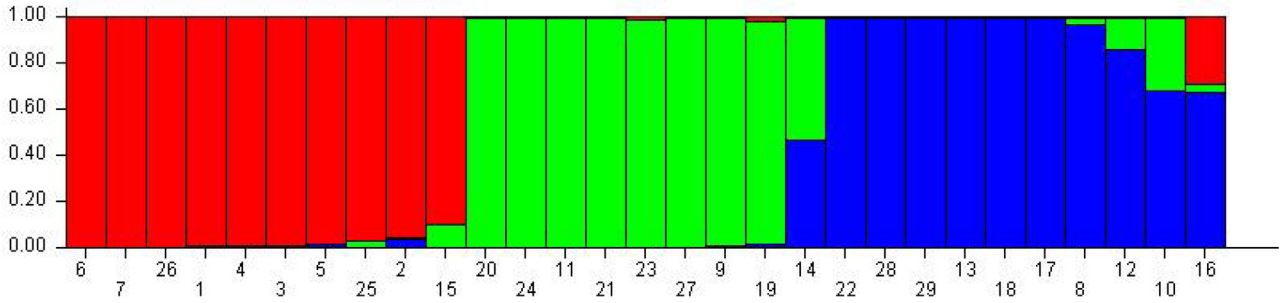

Fig. 6. Population structure of 29 accessions arranged based on inferred ancestry.

Pope John Paul II & *Rosa Black Baccara*. Lowest similarity was seen between *Rosa Baimir* and *Rosa Apricot Nectar*. Furthermore, a genetic relationship amongst 29 distinct rose accessions was evaluated by the construction of dendrogram using data of 32 SCoT markers amplification. Several distinct clusters of rose accessions were recognized which fell down into various edges of dendrogram that might be possible of their differences in genetic constitutions which represent distinct morphological characters and variations amongst them. Further, this grouping is also supported by Bayseian clustering algorithm using STRUCTURE software which was conducted amongst different 29 Rosa germplasm (Evanno et al. 2005). The Inference of best K is explained by using the delta K method which was found to be the best at K = 3 that clearly separated the rosa germplasm into major three groups. This method recognize the true number of clusters (K) in the given individual collections using an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values (Evanno et al., 2005). Moreover, Bayesian clustering approach has the ability to use genetic information to determine the population membership of individuals without assuming predefined populations. They allocate either members or portion of their genome to a number of clusters based on multilocus genotypes (Chen et al., 2007). Vukosavljev et al. (2013) also tried to classify garden rose in three groups- wild, old garden and modern garden rose on the basis of several morphological parameters. Several earlier studies have established the genetic relationship amongst rose accessions based on several morphological traits such as number of petals, stem width, number of side shoots, or rose hips to diversify rose genotypes (Gitonga et al., 2014; Riaz et al., 2007). Diverse studies have been conducted to analyze genetic relationship based on different marker technologies in rose, like STMS (Fernández-Romero et al., 2009), AFLP (Pirseyedi et al., 2005), SSR (Akond et al., 2012; Ghose et al., 2013; Nadeem et al., 2014; Samiei et al., 2010; Vukosavljev et al., 2013), ISSR (Mirali et al., 2012; Panwar et al., 2015; Zhou et al., 2009) and RAPD (Agaoglu et al., 2000; Henuka et al., 2015; Panwar et al., 2015).

5. Conclusion

This is first report of genetic polymorphism on 29 different *Rosa* germplasm using SCoT markers technique. It is single primer PCR based amplification methods which depend on gene-targeted formulation and as a better alternative to RAPD, ISSR, AFLP, SRAP, and TRAP techniques. Some attributes like higher polymorphism, high reproducibility, low cost, easy to access, and time saver nature made SCoT method more reliable and suitable to study genetic relationship amongst various *Rosa* germplasm. Our study identified the polymorphic nature of SCoT marker without facilitating functional validation of genes and established genetic diversity among different rosa germplasms. Therefore, this practice will be useful for planning conservation strategies and also helpful in rose improvement programs such as linkage mapping, QTL mapping, genotype identification, gene pyramiding and marker assisted selection.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jksus.2018.04.022.

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