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Original Article

Variation in DNA Barcode of Rose Varieties

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ABSTRACT

The DNA barcode of a species is expected to vary only 2-7%, among different varieties of the species. There are more than 4000 morphologically different varieties of roses that evolved due to the selection pressure of the aesthetic choices of humans. The extensive use of roses in decoration, bouquet, as well as in medicine and food also increase the value of their species identification from forensic trace samples. DNA barcoding-based identification of species is emerging as a robust forensic technique. Here we have determined the DNA barcode of a few prominent varieties of rose and estimated variation among them as well as their phylogenetic relationships. The rbcl gene does not vary significantly among these rose varieties, at most, it can identify the class/group of a rose variety.

Keywords: DNA barcoding, rbcl gene, Rubisco, Rose cultivars, *Rosa species*, Rose phylogenetics, Identification of roses

Abbreviations: DNA: Deoxyribonucleic acid; **PCR:** Polymerase chain reaction; **rbcL:** Ribulose-1, 5-bisphosphate carboxylase/oxygenase; **matK:** Maturase-K; **CTAB:** Cetyl trimethyl ammonium bromide; **EDTA:** Ethylene diaminetetraacetic acid; **PVP:** Polyvinyl pyrrolidone

INTRODUCTION

Overall biodiversity is decreasing day by day, mainly due to anthropogenic activities. But humans have also increased diversity in specific genera like roses and dogs, using artificial selection and breeding according to their aesthetic choice and utility [1]. The selection and artificial breeding of roses by humans have created hundreds of varieties of this genus [2]. The "theplantlist.org" includes 4,389 scientific plant names of the species rank for the genus *Rosa*, of these 366 are accepted species names [3]. The lineage and geographical origin of many species of rose are contentious. There are significant contentions about various varieties being separate species or a variety of existing species names [4]. Rose garden of Chandigarh has 1600 species of roses as per 2014 directory of the world federation of rose societies [5]. Chandigarh hosts a rose festival every year in the month of February [6]. The roses are mostly propagated vegetatively so there is a lot of genetic diversity expected due to a lack of gene flow between small subpopulations. Some varieties are developed by grafting the stem of one species on other species. It can cause a change in morphological features while retaining the genetic makeup the same as the parent species. Recently genetic markers like *Maturase* K

(matK) and ribulose bisphosphate carboxylase (rbcl) are becoming very popular for species identification [7]. The makK and rbcl genes are present on the chloroplast genome. Therefore MatK and rbcl markers are inherited maternally. Rbcl is more diverse than MatK. Genetic phylogeny using RAPD (rapidly amplified polymorphic DNA) [8], microsatellite analysis [9], and AFLP (amplified fragment length polymorphism) have been used for roses earlier [10]. But enriching databases of standardized DNA barcoding markers shall be very useful in precise identification, specifically in forensic cases where there may be no clue about the genus of a specimen. Earlier studies have used nuclear marker GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and chloroplast marker trnL-F and psbA-trnH to study respectively, reticulate relationships and first phylogenetic relationships of all representative wild varieties. Due to hybridization in roses, the genetic phylogeny is not always consistent with traditional taxonomy [11]. The genetic diversity in *Rosa* on these genetic loci is also very low therefore extensive data on various varieties of *Rosa*, for standard DNA barcode markers like rbcl and matk can be very useful for the identification of the precise biogeographical origin of forensic samples. Rbcl, ITS [12], and matk [13] genes have been used previously as well for the study of diversity and phylogenetic relationships among rose varieties. The ITS2 marker is very effective for species identification in the Rosaceae family except for its genus *Rosa* [14].

This study is focused on the cultivated varieties in India, rather than a broad phylogeny encompassing wild varieties. The 10 varieties studied here are given in Table 1 along with their lineage, name of the breeder, literature reference, and Genbank accession number of the DNA sequence determined by us. The *Rosa Anusuia*, included

Table 1: List of Rose varieties DNA barcoded along with their GenBank accession numbers (BOLD process ID), Breeder name, lineage, and literature reference

Rose cultivar/variety (class)	GenBank accession numbers (BOLD process ID)	Breeder	Lineage	Reference
Rosa Grand Mogul (Hybrid Tea)	MT412378 (SDP850037-20)	Georges Delbard	Sultane × Chic Parisien	[15]
Rosa Anusuia (Hybrid Tea)	MT412379 (SDP850040-20)	Dr. Kalyan Chakraborti	Not disclosed	[16]
Rosa Tiara (Hybrid Tea)	MT412380 (SDP850036-20)	Eugene S. “Gene” Boerner	Chic x Demure seedling (white)	[17, p.12]
Rosa Paradise (Hybrid Tea)	MT412381	O.L. “Ollie” Weeks	Swarthmore × seedling of Angel Face	[18]
Rosa Grand mugul orange (Hybrid Tea)	MT412382	Georges Delbard	Sultane × Chic Parisien	[15]
Rosa Grandiflora Gold Medal (floribunda)	MT412383	Unknown	Hybrid Teas x floribunda roses.	[19]
Rosa Ingrid Bergmann (Hybrid Tea)	MT412384	L. Pernille Olesen	Precious Platinum × Else Poulsen	[20]
Rosa Queen Elizabeth (Hybrid Tea)	MT412385	Dr. Walter E. Lammerts	Charlotte Armstrong × Floradora	[21]
Rosa floribunda var angel face (Hybrid Tea)	MT412386	Unknown	Hybrid Teas roses × polyantha roses	[22]
Rosa Floribunda Var Zorina (Hybrid Tea)	MT412387	Unknown	Hybrid Teas roses with polyantha roses	[22]

in this study, is very popular in the markets of India. *Rosa grand-mogul* and *Rosa Paradise* are often found in India. *Rosa Tiara* is very popular in Europe. The barcoding of such roses can have forensic significance in case of dispute in the supply of the spurious herbal adulterated product, or identification of cultivar of a commercial lot of flowers, or tracing the source of evidence found at the crime site.

MATERIAL AND METHODS

DNA Extraction

The DNA from leaves was isolated using the DNAzol® kit, following the protocol described by the manufacturer of the kit [23]. Leaves or petal sample was homogenized in DNAzol and the genomic DNA was precipitated from the lysate with ethanol. Following an ethanol wash, DNA was solubilized in 8 mM NaOH. Fresh leaf and petal samples of plants were collected from the Rose Garden (Sector 16, Chandigarh, India, GPS location 30.748148, 76.784284).

DNA Amplification

The DNA extracted from the leaves of *roses* has been used as a template to amplify the DNA barcode sequence for the respective cultivars. The rbcl gene has been successfully amplified for DNA barcoding, using a universal primer pair for rbcl. The forward primer sequence is TAAAACGACGGCCAGTATGTCACCAC AAACAGAGACTAAAGC and the reverse primer sequence is CAGGAAACAGCTATGACGTAAAA TCAAGTCCACCRCG(which also has an M13 tag included at 5' for easy sequencing of PCR product). As expected, the amplified PCR product was around 600 bp long, as observed in agarose gel electrophoresis. The DNA amplification reaction mixture had 25 µl master mix, 1.5 µl forward and reverse primer, 5 µl template DNA, and 20 µl nuclease-free water. Reaction mixtures were placed in a thermocycler and the program steps were: (1) 95 °C for 6 min for 1 cycle (2) 95 °C for 30 sec. for 30cycles (3) 64 °C for 60 sec. for 30 cycles (4) 72 °C for 45 sec. for 30 cycles (5) 72 °C for 5min for 1 cycle for the last cycle. Then agarose gel Electrophoresis of the PCR solution was performed to check the amplification of DNA. The electrophoresis buffer contained 4.8 gm Tris buffer, 0.7 gm EDTA and 1.2 ml Glacial Acetic Acid in 1 L distilled water. DNA fragments were stained with Ethidium Bromide and observed under 254 nm UV light to verify the correct range of size of PCR product (~600 bp). The amplified PCR products were sent to a commercial vendor, AgriGenome (https://www.aggenome.com/), for Sanger DNA sequencing. The PCR products were sequenced using M13 forward and reverse primer pairs.

Phylogenetic Analysis

The easy-to-use web server tool phylogeny.fr/ was used to make phylogenetic trees from DNA sequences of rbcl markers of various samples [24]. The default parameters of the webserver in its “one-click” option were used to make the phylogenetic tree. EMBOSS software suite was also used to convert sequence format into fasta and to verify the phylogenetic analysis [25].

RESULTS AND DISCUSSION

There has been extensive selection by humans on various morphological features of roses which lead to the development of more than 4000 varieties. There have been attempts to classify and decipher the phylogeny of Indian roses, using morphological features [26]. There had also been attempts to decipher the phylogenetic relationship among roses in India using Molecular markers [27]. Here, we have selected a few very popular rose cultivars from the Rose garden of Chandigarh, including few Indian origin cultivars. We have tested the utility of rbcl markers for the identification of these rose cultivars and deciphering their phylogenetic relationship. We also successfully deposited 3 of these DNA barcodes in the Barcode of Life Database (BOLD), and others in the GenBank (GenBank accession number and barcode process are mentioned in Table 1). DNA barcoding-based identification is gaining popularity in forensics for the identification of trace evidence of plant origin. Rose plant

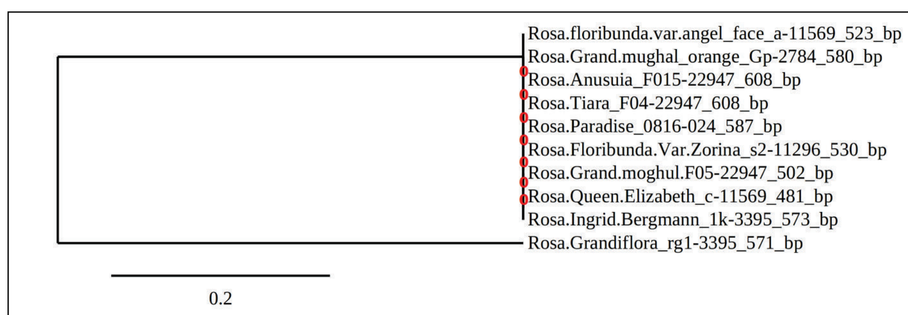

Figure 1: Phylogenetic tree of rose varieties drawn from their partial rbcl gene sequence

can be very useful in forensics as it is very often found in households and its sticky pollen can stick to a culprit present at the crime site. Rose can also solve some crimes related to the adulteration of herbal medicine and food products. Various forensic cases may require identification of plant material at the level of species or biogeographical variety/cultivar, or even at the level of the individual plant. Genetic markers can be very useful for the identification of plant trace evidence at various levels of identification [28]. The DNA sequences determined were 481-608 base pairs long, as mentioned in the phylogenetic tree in Figure 1. The multiple sequence alignment is common for these sequences for 450 base pairs. From the phylogenetic tree given in Figure 1, it can be inferred that the 9 out of 10 cultivars of rose are identical to each other in their rbcl gene's barcoding region sequence. The only cultivar which is different in its rbcl sequence from the other 9 is *Rosa Grandiflora*. It is only 0.67% different from the other 9 cultivars. Out of 450 base pairs of MSA, the *Rosa Grandiflora* differs in 3 nucleotides only from the MSA consensus sequence. Therefore the rbcl gene does not have enough divergence to identify separate cultivars, although the cultivars are clearly identifiable from their morphological features. The *Rosa Grandiflora* which is having different DNA barcodes at rbcl locus, from the other 9 cultivars is the only floribunda class of rose whereas the other 9 cultivars are from rose class hybrid tea. Therefore maybe we can identify broadly the class of a rose plant from its rbcl gene sequence. Other DNA- barcoding locus used for plants such as psbA-trnH spacer, trnL intron, trnL-F spacer, trnS-G spacer, trnG intron [11], ITS2,[14], rpl16, trnL-F, and atpB-rbcl locus [29], may give a more specific identification of the rose varieties/cultivars. Some highly variable locus in the microsatellite DNA region such as RAPD markers (Random Amplified Polymorphic DNA) can even associate an individual plant with a forensic sample [27].

AUTHORS' CONTRIBUTIONS

Rajinder Kaur, Navpreet Kaur, Geetanjali Vij, Lakshay Kalra, Lalhruaitluangi, Kavita Dhiman, Poonam, and Priyanka Sareen determined the DNA barcode of the plant using the leaf as a template DNA source. Jagdish Rai designed experiments and wrote the manuscript.

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REFERENCES

[1] Akey JM, Ruhe AL, Akey DT, *et al.* Tracking footprints of artificial selection in the dog genome. National Academy of Sciences. 2010;107:1160–1165.

[2] Raymond O, Gouzy J, Just J, *et al.* The Rosa genome provides new insights into the domestication of modern roses. Nature Genetics. 2018;50:772–777.

[3] Crawler.Gbif.Org. The Plant List with literature [Internet]. GBIF Secretariat; 2016 [cited 2020 May 1]. Available from: http://www.gbif.org/dataset/dedf296e-7bc6-48cf-9225-9cd600a0d2ef.

[4] Smulders MJM, Arens P, Bourke PM, *et al.* In the name of the rose: a roadmap for rose research in the genome era. Hortic Res [Internet]. 2019 [cited 2020 May 1];6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6499834/.

[5] World Federation of Rose Societies, 2014 Directory [Internet]. [cited 2020 May 1]. Available from: https://archive.org/stream/WorldFederationOfRoseSocieties2014/World%20Federation%20of%20Rose%20Societies%2C%202014_djvu.txt.

[6] Pal BP. The rose in India. New Delhi: Indian Council of Agricultural Research; 2011.

[7] Ratnasingham S, Hebert PDN. BARCODING: bold: The Barcode of Life Data System (http://www.barcodinglife.org): BARCODING. Molecular Ecology Notes. 2007;7:355–364.

[8] Jan CH, Byrne DH, Manhart J, *et al.* Rose Germplasm Analysis with RAPD Markers. Horticulture Science. 1999;34:341–345.

[9] Scariot V, Akkak A, Botta R. Characterization and Genetic Relationships of Wild Species and Old Garden Roses Based on Microsatellite Analysis. Journal of the American Society for Horticultural Science. 2006;131:66–73.

[10] Koopman WJM, Wissemann V, De Cock K, *et al.* AFLP markers as a tool to reconstruct complex relationships: A case study in Rosa (Rosaceae). American Journal of Botany, 2008;95:353–366.

[11] Fougère-Danezan M, Joly S, Bruneau A, *et al.* Phylogeny and biogeography of wild roses with specific attention to polyploids. Annals of Botany. 2015;115:275–291.

[12] Wissemann V, Ritz CM. The genus Rosa (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and atpB-rbcL intergenic spacer (IGS) versus conventional taxonomy. Botanical Journal of the Linnean Society. 2005;147:275–290.

[13] Wu S, Ueda Y, He H, *et al.* Phylogenetic Analysis of Japanese Rosa Species Using matK Sequences. Breed Science. 2000;50:275–281.

[14] Pang X, Song J, Zhu Y, *et al*. Applying plant DNA barcodes for Rosaceae species identification. Cladistics. 2011;27:165–170.

[15] Grand Mogul Rose [Internet]. [cited 2020 Apr 29]. Available from: https://www.helpmefind.com/rose/l.php?l=2.21595.0.

[16] Full text of The Indian Rose Annual XXXI, 2015 [Internet]. [cited 2020 Apr 29]. Available from: https://archive.org/stream/TheIndianRoseAnnualXXXI2015/The++Indian+Rose+Annual+XXXI%2C+2015_djvu.txt.

[17] Young MA, American Rose Society. Modern roses 12: the comprehensive list of roses in cultivation or of historical or botanical importance. Shreveport, LA: American Rose Society; 2007.

[18] Phillips R, Rix M. The quest for the rose. 1st U.S. ed. New York: Random House; 1993.

[19] rose | Description & Major Species | Britannica [Internet]. [cited 2020 Apr 29]. Available from: https://www.britannica.com/plant/rose-plant#ref247259.

[20] Society AR, editor. Handbook for Selecting Roses A Rose Buying Guide from the American Rose Society. American Rose Society; 1980.

[21] Moody M. Mary Moody's roses: more than 200 of the world's most beautiful and hardy roses. Sydney: Murdoch Books; 2006.

[22] Phillips R, Rix M. The ultimate guide to roses: a comprehensive selection. New York: Macmillan; 2004.

[23] Chomczynski P, Mackey K, Drews R, *et al.* DNAzol ®/ : A Reagent for the Rapid Isolation of Genomic DNA. BioTechniques. 1997;22:550–553.

[24] Dereeper A, Guignon V, Blanc G, *et al.* Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research. 2008;36:W465-469.

[25] Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet TIG. 2000;16:276–277.

[26] Gaurav A, Banyal N, Raju DVS, *et al.* Genetic characterization of Rosa species using morphological markers. Indian Journal of Agriculture Science. 2018;88:1396–1402.

[27] Rai H, Raju D, Kumar Mb A, *et al.* Characterization and analysis of genetic diversity among different species of rose (Rosa species) using morphological and molecular markers. Indian Journal of Agriculture Science. 2015;85:240–245.

[28] Wallinger C, Juen A, Staudacher K, *et al.* Rapid Plant Identification Using Species- and Group-Specific Primers Targeting Chloroplast DNA. PLOS ONE. 2012;7:e29473.

[29] Liu C, Wang G, Wang H, *et al.* Phylogenetic Relationships in the Genus Rosa Revisited Based on rpl16, trnL-F, and atpB-rbcL Sequences. Horticulture Science. 2015;50:1618–1624.

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