**Short communication**

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# ASSESSMENT OF GENETIC VARIABILITY AMONG SELECTED SPECIES OF APOCYNACEAE ON THE BASIS OF *rps* 11 GENE

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## ABSTRACT

The importance of genetic variation for sustaining biological diversity and evolutionary processes is widely accepted. The present study aims to investigate the phylogenetic relationships among five selected species of Apocynaceae collected from various regions of Pakistan using chloroplast gene (*rps*11) which encode ribosomal protein of smaller subunit11. For this purpose *rps*11 gene was amplified, purified, sequenced from each species and analyzed with MEGA5. Phylogenetic analysis revealed an overall narrow genetic background (0.5 %) among selected species. *Nerium oleander*, *Rhazya stricta* and *Vinca major* were the most similar with a bootstrap value of 24-44 % while *Catharanthus roseus* and *Nerium oleander* showed distant relationship. Divergence value was observed within a range of 0.023-9.994 on the basis of pair wise distance calculation. The study further confirmed the use of *rps*11 gene as a potential candidate for phylogenetic analysis.

**Key words:** Apocynaceae, *rps*11, Genetic Variation.

## INTRODUCTION

Apocynaceae is considered as one of the largest family of angiosperms containing 375 genera and over 5100 species around the globe (Endress *et al.*, 2007). Most species include lianas, herbs, shrubs, trees frequently found in rainforests, tropics and subtropics but some can grow in tropical dry and xeric environments (Endress and Bruyns, 2000). Modern classification of Endress *et al.* (2007) divided the family into five subfamilies i.e Asclepiadoideae, Secamonoideae and Periplocoideae are from the former Asclepiadaceae and Apocynoideae, Rauvolfioideae are from Apocynaceae. Pakistan is hosting 19 genera and 26 species (Nazimuddin *et al.*, 1983) mostly found in North Punjab, Azad Kashmir, Hazara, Rawalpindi, Attock, Swat, Bajaur, Waziristan and Salt range area (Ali, 1983).

The family carries considerable importance in the field of medicine because of its extensive use in Cancer Chemotherapy, skin diseases, Diabetes, Diarrhea and Malaria (Middleton, 2007). Besides its importance in economy and construction, the family serve as a potential source for vast amount of chemicals like Alkaloids, Steroids, Triterpenoids, Phenolics from *Alstonia* spp. (Singh and Singh, 2003) and Terpenoids Indole Alkaloids (TIA), Vincristine, Vinblastine, Catharanthine from *Catharanthus roseus* species (Gueritte and Fashy, 2005). Similarly reports of Shazly *et al.* (2005) confirmed the presence of Cardiotonic glycoside ‘Neeriifolin’ which has insecticidal property and Thevetin that work as heart stimulant.

Preservation and successful management of natural flora is deeply associated with the accurate assessment of genetic diversity, which in turn is of paramount importance for possible adaptation and consequently for prolong survival of a species (Hanski and Ovaskainnen, 2000). A series of technique and genetic marker system has been developed for estimation and analysis of genetic variability at inter and intraspecific level. A wide range of molecular approaches has been used by numerous research groups to find the natural relationship within various groups of Apocynaceae (Potgieter and Albert, 2001; Rapini *et al.*, 2003; Rapini *et al.*, 2006; Livshultz *et al.*, 2007; Simoes *et al.*, 2007; Livshultz, 2010). A recent review by Nazar *et al.* (2013) has significantly emphasized on the systematics and taxonomy of Apocynaceae.

Chloroplast DNA can serve as a main source of phylogenetic analysis (Small *et al.*, 2005) at different systematic level in various genera of plant kingdom (Kallersjo *et al.*, 1998). In past, chloroplast genome has been assessed for phylogenetic study of Apocynaceae in genus *Asclepias* (Mark *et al.*, 2011), *Hoya* (Livia *et al.*, 2011), *Diplolepis* (Hechem *et al.*, 2011) and *Pachypodium* (Burg *et al.*, 2013), subfamily Secamonoideae plus Asclepiadoideae (Tatyana *et al.*, 2010), sub tribe Metastelmatinae (Silva *et al.*, 2012) and Rauvolfioideae (Mahadani *et al.*, 2013) by various research groups. The present study aims to investigate the genetic variability within selected species of Apocynaceae using *rps*11 gene and to use this information for better management and phylogenetic analysis.

## MATERIALS AND METHODS

**Plant Collection:** Five species of Apocynaceae were collected from different regions of Pakistan (Table 1) and were identified in the National Herbarium of Pakistan, Department of Plant Sciences, Quaid-i-Azam University, Islamabad.

**DNA Extraction:** DNA was extracted using CTAB (Cetyltrimethyl ammonium bromide) method (Richards, 1997) with little alteration (Mahmood *et al.*, 2011a) and its quality was checked on 1% agarose gel via gel documentation system (Dolphin-Doc Plus, Wealtech).

**Primer Designing and PCR:** A set of primers and PCR conditions were used as given by Mahmood *et al.* (2011a) with slight modification. Annealing temperature was 56 $C^0$ with 25 ul of reaction mixture, which contain 12.5 µl PCR Master Mix (2X), (MBI Fermentas), 1 µl (25 pmol) of forward and reverse primer each, 1 µl template and 9.5 µl of nanopure water. PCR (Multigene, Labnet) was used for amplification and products were checked on 1.5 % agarose gel.

**Sequencing:** PCR products were purified by JET quick PCR Product Purification Spin Kit (Genomed), resolved on 2% agarose gel and sequenced by Macrogen (Korea).

**Phylogram Construction:** Sequences were analyzed using Molecular Evolutionary Genetics Analysis (MEGA5) and dendrogram was constructed by neighbour-joining (NJ) method to check the phylogeny and genetic variability. Pair wise distance analysis was conducted to calculate evolutionary divergence using MEGA5.

## RESULTS AND DISCUSSION

**Phylogenetic Analysis:** Phylogenetic Analysis divided the species into two clusters. Cluster 1 consists of three species showing overall bootstrap value from 24-44 % while cluster 2 includes only one species. Values of evolutionary divergence ranged from 0.023-9.994 among selected species (Table 2). Lowest divergence value was observed between *Alstonia scholaris* and *Rhazya stricta* while highest divergence value was observed between *Catharanthus roseus* and *Nerium oleander*. *Catharanthus roseus* emerged as the most primitive species forming an out group (Fig. 1). Previously genetic variability was assessed among various species of Apocynaceae using RAPD markers, CAPS technique and sequences of *rps*11 region (Mahmood *et al*., 2010; 2011 a, b, c). According to our knowledge no earlier work has been reported on selected species using *rps*11 as a marker for estimation of genetic variability. Overall the results are an indicator of change in the chloroplast DNA during the course of evolution. The present study showed narrow genetic background among selected species with 0.5% genetic diversity which is lower than already reported results of 15%, 34% and 42% (Mahmood *et al*., 2011a, b, c) which is due to variation in marker selection and species number. Dendrogram divided the species into two clusters which is accordance to the result already reported by Mahmood *et al.* (2011a) but contradictory to the reported results of Mahmood *et al.* (2011b) which divided the species into three clusters which may be attributed to different marker system (RAPD) used for phylogenetic studies. Earlier work of Mahmood *et al.* (2011b) placed *Catharanthus roseus* and *Alstonia scholaris* in separate cluster while in this study they appear as an out group to the remaining species and are considered as the most primitive and common ancestor. *Nerium Oleander* (Apocynoideae) revealed maximum similarity (44%) with *Rhazya Stricta* (Rauvolfioideae) which is contradictory to the reported results of Endress and Bruyns (2000) based on morphological data such results indicated a common lineage history for both species.

In future, the close relationship between *Nerium oleander* and *Rhazya stricta* and distant relationship of *Catharanthus roseus* require further confirmation not only at molecular but also at morphological and biochemical level using enormous number of species and markers.

**Table 1. List of selected species along with their subfamily, order, longitude, latitude and area of collection**

| S.No | Species Name | Subfamily | Order | Site name | Longitude and latitude |
|---|---|---|---|---|---|
| 1 | *Nerium oleander* | Apocynoideae | Gentianales | Islamabad | 33° 42' N and 73° 10' E |
| 2 | *Catharanthus roseus* | Rauvolfioideae | Gentianales | Islamabad | 33° 42' N and 73° 10' E |
| 3 | *Alstonia scholaris* | Rauvolfioideae | Gentianales | Islamabad | 33° 42' N and 73° 10' E |
| 4 | *Vinca major* | Rauvolfioideae | Gentianales | Swat | 34° 51' N and 72° 33' E |
| 5 | *Rhazya stricta* | Rauvolfioideae | Gentianales | Nowshehra | 34° 0' N and 71° 58' E |

**Table 2. Estimation of evolutionary divergence among *rps* 11 gene sequences for five species of family Apocynaceae**

| **S.No.** | **Species** | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| **1** | S1 | | | | | |
| **2** | S2 | 9.994 | | | | |
| **3** | S3 | 0.042 | 3.742 | | | |
| **4** | S4 | 0.479 | 4.410 | 0.487 | | |
| **5** | S5 | 0.046 | 4.345 | 0.023 | 0.500 | |

**S1:** *Nerium oleander*, **S2:** *Catharanthus roseus*, **S3:** *Alstonia scholaris*, **S4:** *Vinca major*, **S5:** *Rhazya stricta*

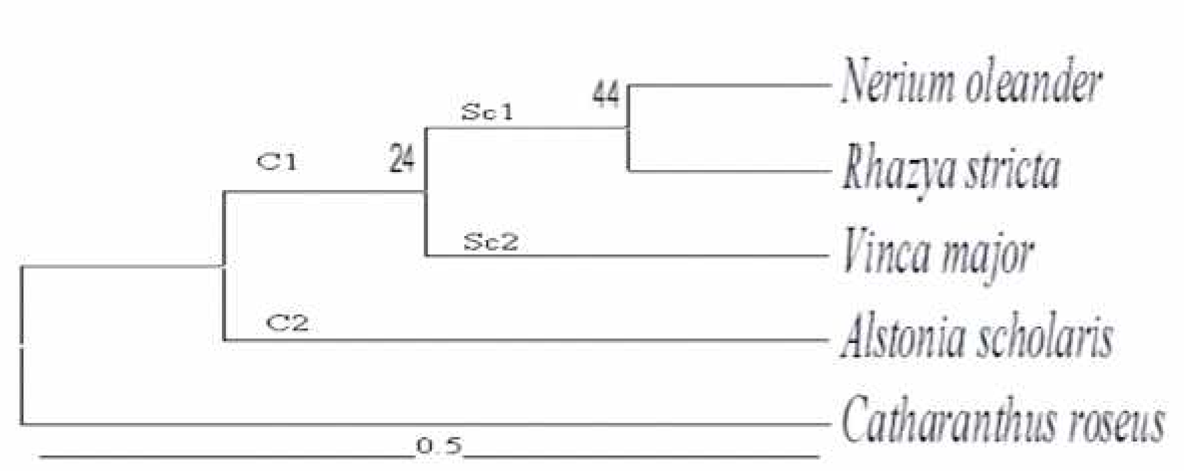

**Fig. 1.** Phylogram produced by MEGA5 for *rps* 11 gene sequences representing genetic relationship among selected species of Apocynaceae**.** C= Cluster, Sc= Sub cluster

**Conclusion:** The study confirmed the use of *rps*11 as a source for assessing genetic variation in Apocynaceae showing distant relationship of *Catharanthus roseus* (Rauvolfioideae) with *Nerium oleander* (Apocynoideae) and close genetic relationship between *Nerium oleander* and *Rhazya stricta*.

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