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Anatomical variation and molecular characterization of *Catharanthus roseus* through RAPD analysis

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Key words: *Catharanthus roseus* L., Anatomical character, Genetic diversity, RAPD, Cluster analysis.

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Abstract

Catharanthus roseus is one of the most interesting groups of ornamental plants in the world with high medicinal value and member of the family Apocynaceae. The goal of this study was to investigate the anatomical and molecular characterization of five types (White yellow, White red, Pink yellow, Pink red, and Red yellow) of *C. roseus* plant. The anatomical study was made by cutting transverse sections of the materials and stained with double stained techniques and observed data with the help of high-powered compound microscope. Molecular analysis was done by using RAPD molecular markers with four primers. Anatomical analysis indicated that the epidermis was recorded as a single layer. Bi-collateral vascular bundle was present and frequently arranged in radial symmetry in the stem. The results of RAPD markers revealed a total of sixty-nine (69) amplified bands, forty-six (46) of them were monomorphic and twenty-three (23) of them were polymorphic from using four primers. The result of dendrogram separated the five types of *C. roseus* into two major clusters (C1 and C2). Cluster C1 is represented by a single type Red yellow indicating that it is distinct from other four types, and Cluster C2 is represented by the other four types of *C. roseus*. The dendrogram and linkage distance revealed that the highest similarity was 70% between S1 (White yellow types) and S2 (White red types). However, the lowest similarity was 41.7% between S3 (Pink yellow types) and S5 (Red yellow types). This study could provide a key platform for further crop improvement at molecular level for genetic variability and cross breeding.

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Introduction

Catharanthus roseus (L.) is a perennial tropical plant. *C. roseus* also referred to as “Nayantara” or “Sadabahar”. It is also referred to as *vinca,* bright-eyes, old-maid, periwinkle, pink periwinkle, rose periwinkle, and madagascar periwinkle. The word *Catharanthus* derives from the Greek language meaning "pure flower." While, *roseus* means red, rose or rosy. Although *C. roseus* is native to Madagascar, it has been grown as an ornamental plant for centuries in the tropics and occasionally in the subtropics. As a result, it has naturalized in many places. Madagascar, an island in the Indian Ocean, is home to *C. roseus*. *C. roseus* is simultaneously an ornamental and a medicinal plant species. It is a member of the Apocynaceae family, which has 411 genera and 4650 species, many of which have ornamental and therapeutic uses (Simpson, 2006).

It is one of the most interesting groups of ornamental plants in the world, considerable variation of different colored flowers can be observed including purple, red, pink, or white corolla (Plaizier, 1981). According to Kumar *et al*., (2013) and Nejat *et al*., (2015), there are five variations of flower’s color, namely white-yellow, white-red, pink-red, pink-white, and red white.

C. roseus is an important medicinal plant. It has been used in folkloric remedies for the treatment of many different disease including diabetes (Ahmed *et al*., 2010) malaria (Gathirwa *et al*., 2007) insect bites diarrhea (Sukumar and Osmani, 1981), skin, eye and throat inflammations, indigestion, toothache, fever and lung congestion (Nejat *et al*., 2015) menstrual disorder (Kumar *et al*., 2013) enhances kidney and liver functions (Adekomi, 2010) vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia (Lucas *et al*., 2010). Antimicrobial, antitumor, Anti-proliferative activity of extracts from the plant has been proved (Patil and Ghosh, 2010; Vega-´ Avila, 2012).

It contains flavonoids, saponins, tannins, and several anticancer alkaloids namely vinblastine (VLB), vincristine (VCR) and leurosin (Jaleel *et al*., 2008; Pandiangan, 2012; Nejat *et al*., 2015). The leaves of this plant are used as alkaloid-producing therapeutic plant components (Renault *et al*., widely 1999). Several studies revealed that the leaves of this medicinal plant can treat various diseases.

The leaves are infused and used to treat menorrhagia. To treat wasp stings, the leaf juice is externally applied. Alkaloids are abundant throughout the entire plant, with the root bark containing the highest concentrations, especially during flowering. All parts of the plant are also said to have hypoglycemic and antioxidant properties. This plant has long been used as a remedy for hypertension, diabetes mellitus, malaria, constipation, and diuretics.

The assessment of the genetic diversity of *Catharanthus* species and cultivars was accomplished by secondary metabolites, according to the literature. Inter simple sequence repeat (ISSR), amplified fragment-length polymorphism (AFLP), random-amplified polymorphic DNA (RAPD), and other techniques. (Kalpana *et al*., 2004; Arif *et al*., 2010; Leal *et al*., 2010; Chaudhary *et al*., 2012; El Domyati *et al*., 2012). They provide quick results by revealing genetic variations without regard to stage, physiological factors, or surroundings. It is anticipated that biochemical genetic markers would be crucial in characterizing the genotypes of therapeutic plants (Tharachand *et al*., 2012). RAPD is widely used to study the genetic diversity of many plants (Shaw *et al*., 2009; El-Domyati *et al*., 2012; Lal *et al*., 2011), as this method is straightforward, quick, and affordable in comparison to other kinds of DNA-based approaches. Kim *et al*., (2007) Shaw *et al*., (2009) Vardhan *et al*., (2012) and Prasad (2014) discovered low- moderate to high genetic variation among *Catharanthus* species and cultivars.

The aim of this study to investigate the internodes, leaf midrib, and petiole of *Catharanthus roseus* to determine their gross anatomical characteristics and anatomical quantitative and qualitative traits as well as to ascertain the genetic diversity and relationships between the various types of *C. roseus*.

Materials and methods

Materials

The study involved five types of *C. roseus* (such as: White yellow, White red, Pink yellow, Pink red, and Red yellow) differing in petal color, flower eye and center color (Table 1 and Figure 1).

The Stem and leaf were collected from each type of *C. roseus* and used as plant material for determining the anatomical and genetical variability. The material for the present investigation was collected from the Rajshahi region.

Anatomical study

Section of stem and leaf

Stems and leaves were collected from five types of *C. roseus* plant. The collected plant materials were preserved in FAA (Formalin Acetic Acid and Alcohol). Free hand transverse sections of leaves and stems were cut and thin sections were separated with the help of compound microscope. Then the sections were stained with double stained technique and made permanent slide. For sectioning, every time fresh materials were taken.

The permanent slides with section were studied under a research microscope fitted with digital camera. Microphotographs were taken from various regions of the sections using high and low power of the microscope.

Data analysis

The observed data of leaf and stem anatomical characters were analyzed descriptively and used to describe and determine the differences in leaf and stem anatomical characters. Quantitative data including epidermal cell length radial, epidermal cell length tangential, epidermis area, collenchyma cell length radial, collenchyma cell length tangential, collenchyma area, vascular bundle area, xylem area and phloem area were subjected to analysis Variance (ANOVA), followed by a Lest Significant Difference Test (LSD) at a 5% confidence level to see the variability of the characters and the difference between their means.

Genetically Analysis

Collection of samples

Fresh young leaves were collected from five types of *Catharanthus roseus* plant and stored at 4°C in sealed plastic bags till further process.

DNA extraction

Total genomic DNA was extracted from fresh leaves of *C. roseus* by CTAB method. Finally, DNA was dissolved in 0.1× TE (Tris-Ethylene Di-amine Tetra Acetic Acid) buffer (pH 8.0). The DNA was quantified by spectrophotometer and purity was checked by running it on 1% agarose gel prepared in 0.5×TAE buffer (Tris Acetate Ethylene Diamine Tetra Acetic Acid).

PCR Amplification

A set of primers from OPW series was used for amplification in RAPD analysis. PCR conditions used for amplification were pre PCR denaturation at 95°C for 3 min followed by 42 cycles of denaturation at 95°C for 30sec, annealing at 32°C for 25sec and a final period of extension at 72°C for 60 sec. Final cycle was the same except extension for 5 min at 72°C. After PCR, the contents were held at 4°C till use. About 25µl of the PCR mixture contained 10-20 pmol of primer, 12.5µl of 5× PCR master mixes, 9.5 µl of nuclease free water and 25-65 ng of genomic DNA. Amplification products were run on 1.5% agarose gel prepared in 0.5× TAE buffer, stained with ethidium bromide and visualized under UV light.

RAPD data analysis and dendrogram construction

RAPD data were scored for the presence (1), or absence (0) and bands with the same molecular weight and mobility were considered as a single locus. These data were then used to UPGMA (unweighted pair-group method with arithmetic means) analysis to generate dendrograms using "Origin pro" computer software.

Results

Anatomical features of leaf and stem

In the present study the anatomy of the leaf and stem were taken to observe the features. The anatomy of

leaf in figure 2 and anatomy of the stem in figure 3 showed no considerable difference in their anatomy except with slight variation in midrib shape. The exterior protective epidermal layer and its corresponding tissues made up the gross transverse anatomy of stem. The single layered epidermis is made up of cells that were tangentially flattened yet had roughly identical radial lengths. The inner periclinal wall was thinner than the outer periclinal wall. On the exterior of the epidermis, there was a cuticle deposit. The outer periclinal wall of the epidermal cells seems significantly thicker because cuticle covers its outside. Multicellular epidermal hairs were detected as epidermal outgrowths.

Table 1. Names, flower petal colors and flower eye or center colors of the five types *C. roseus*.

Code	Name of the types	Petal colour	Eye color
A	White yellow	White	Yellow
B	White red	White	Red
C	Pink yellow	Pink	yellow
D	Pink red	Pink	red
E	Red yellow	Red	Yellow

A significant amount of the cortex is made up of collenchyma, which is occasionally interrupted by patches of assimilatory (chlorenchyma) tissue that extend to the epidermis. Just below the epidermis, the hypodermis is made up of 6-7 layers of collenchyma cells. The area's hypodermis (cortex) is made up of 5-7 layers of parenchyma cells. The cells have intercellular gaps and thin isodiametric walls. Endodermis was visible. The cortex and the vascular cylinder's endodermis served as their limiting layers. Next to endodermis there was a complete ring of sclerenchyma fibre tissue known as pericycle.

Table 2. Mean with standard error of quantitative characters of stem of the five types of *C. roseus* (A= White yellow, B= White red, C= Pink yellow, D= Pink red and E= Red yellow).

Types of *C. roseus*	Epidermal cell length radial (mm)	Epiderm-al cell length tangenti-al (mm)	Epidermi-s Area (mm²)	Collen- chyma cell length radial (mm)	Collen–chyma cell Length tangential (mm)	Collen- hyma area (mm²)	Vascu–lar bundle area (mm²)	Xylem area (mm²)	Phloem area (mm²)
A	1.42±0.12b	1.98±0.04a	2.94±0.35a	3.32±0.19b	3.22±0.22a	11.70±2.73b	12.80±1.35a	8.60±1.02a	4.20±0.37a
B	1.18±0.02d	1.70±0.12c	2.00±0.12e	3.46±0.20a	4.24±0.27a	14.78±1.57a	11.50±1.41a	7.80±1.34a	3.70±0.30a
C	1.96±0.02a	1.98±0.02b	3.88±0.07b	3.56±0.23a	4.40±0.24a	15.64 ±1.28a	17.50±2.30a	12.60±1.20a	5.30±1.06a
D	1.78±0.12a	2.50±0.13a	4.39±0.22a	3.40±0.24a	3.82±0.09a	12.92±0.72a	13.26±1.22a	8.90±0.92a	4.36±0.44a
E	1.16±0.04b	1.82±0.09b	2.10±0.09b	3.48±0.14a	3.70±0.12a	12.85±0.53a	11.12±0.94b	7.56±0.28b	3.56±0.23b

a, b, c, d and e indicate significant difference at 5% level. Number followed by the same letters in the same column are not significantly different.

The vascular bundles, which are made up of xylem and phloem, are located inside the pericycle. The bi-collateral vascular bundles are arranged in a ring-like pattern near the periphery. The phloem is present both outside and inside the xylem. Vascular bundles have a longer radial length than tangential length. Sieve tubes and sieve plates make up phloem. Companion cells were observed in interacting with sieve tubes. There was a significant amount of phloem parenchyma present. A considerable amount of vascular tissue is made up of the inner phloem. The pith is composed of parenchymatous tissue in the center. Pith parenchyma cells were larger than cortical parenchyma cells. They have prominent intercellular gaps and thin walls. The pith's central cells are larger than the peripheral ones, which are smaller. The upper epidermis of the five types of *C. roseus* leaf (Figure 2) is made up of a single layer of tiny epidermal cells, as can be seen in the transverse section of the leaf.

Table 3. Mean with standard error of quantitative characters of leaf of five types of *C. roseus* (A= White yellow, B= White red, C= Pink yellow, D= Pink red and E= Red yellow).

Types of *C. roseus*	Epidermal cell length radial (mm)	Epiderm-al cell length tangenti-al (mm)	Epidermi-s area (mm²)	Collen- chyma cell length radial (mm)	Collen -chyma Cell length Tangential (mm)	Collen- chyma area (mm²)	Vascu –lar bundle area (mm²)	Xylem area (mm²)	Phloem area (mm²)
A	1.64±0.13a	1.82±0.09b	2.94±0.19a	3.40±0.18b	3.40±0.24a	11.60±1.12b	7.96±0.56c	5.22±0.35c	4.20±0.37a
B	1.72±0.09a	1.92±0.04a	3.30±0.21a	3.08±0.25a	3.16±0.16c	9.84±1.26c	7.76±0.67c	4.90±0.41b	3.70±0.30a
C	1.70±0.10b	1.96±0.04b	3.12±0.12c	2.96±0.05c	3.66±0.06b	11.00±0.22b	7.56±0.41c	4.80±0.37b	5.30±1.06a
D	1.88±0.11a	1.96±0.04c	3.68±0.24b	2.40±0.24c	2.76±0.26d	6.52±0.62d	9.20±0.80c	6.40±0.50b	4.36±0.44a
E	1.12±0.04b	1.88±0.04a	2.11±0.13b	2.76±0.11c	2.98±0.04c	8.21±0.68c	7.50±0.44d	4.96±0.45d	3.56±0.23b

a, b, c, d and e indicate significant difference at 5% level. Number followed by the same letters in the same column are not significantly different.

The leaf in *C. roseus* is dorsiventral. The lower epidermis is made up of a single layer of tiny, lignified cells that are cuticle-covered by newly emerging epidermal hairs. After the upper epidermis, 8 layers of parenchyma and 7-8 layers of collenchyma are present in the midrib area. In the area of the midrib, there is a well-developed bicollateral vascular bundle that resembles a crescent. The outer and inner phloem are made up of sieve tube cells, companion cells, and phloem parenchyma.

The metaxylem in the lower portion and the protoxylem in the higher portion of lignified xylem are separated by xylem parenchyma. Above the lower epidermis, 4-5 layers of collenchyma and 10-11 layers of parenchyma are present in the midrib region.

Table 4. RAPD primers with corresponding bands scored, their size range, number of monomorphic and polymorphic bands, polymorphism and number of band per types in five types of *C. roseus*.

Primer Codes	Size ranges (bp)	Total no. of bands	No. of Monomor- phic bands	No. of Polymor-phic bands	Polymor- phisms (%)	No. of bands per variety
OPW-04	850-1500	10	5	5	50%	2
OPW-09	120-1500	33	25	8	24.24%	6.6
OPW-10	290-1400	18	11	7	38.88%	3.6
OPW-16	200-1500	8	5	3	37.5%	1.6
Total		69	46	23	33.33%	
Average		17.25	11.5	5.75		

Quantitative analysis of leaf and stem

For the quantitative analysis of leaf and stem of the five types of *Catharanthus roseus* quantitative characters such as epidermal cell length radial (mm), epidermal cell length tangential (mm), epidermis area (mm²), collenchyma cell length radial (mm), collenchyma cell length tangential (mm), collenchyma area(mm²), Vascular bundle area (mm²), xylem area (mm²) and phloem area (mm²) were measured and showed in table 2 and table 3. In the stem (see table 2), epidermal cell length radial was the highest (1.96±0.02a) in stem of pink yellow(C) and the lowest was found (1.16±0.04b) in stem of red yellow (E). Epidermal cell length tangential was the highest 2.50±0.13a in Pink red (D) and the lowest 1.70±0.12c was in White red (B). Epidermis area was the highest 4.39±0.22a in Pink red (D) and the lowest 2.00±0.12e was observed in White red(B). Similarly, the highest collenchyma cell length radial, collenchyma cell length tangential, collenchyma area, vascular bundle area, xylem area and phloem area were measured in Pink yellow(C) and the lowest collenchyma cell length radial, collenchyma cell length tangential, collenchyma area was found in White yellow (A), and the lowest vascular bundle area, xylem area and phloem area were observed in red yellow (E). Here a, b, c, d and e indicate significant difference at 5% level.

Number followed by the same letters in the same column are not significantly different.

Table 5. Summary of linkage distances values for different types of pairs of *C. roseus*. (S1=White yellow, S2=White red, S3=Pink yellow, S4=Pink red, S5=Red yellow).

	S1	S2	S3	S4	S5
S1	0	30.00	45.82	36.05	50.00
S2	30.00	0	44.72	44.72	56.57
S3	45.82	44.72	0	44.72	58.30
S4	36.05	44.72	44.72	0	44.72
S5	50.00	56.57	58.30	44.72	0

In the leaf (see table 3), epidermal cell length radial was the highest (1.88±0.11a) in leaf of pink red(D) and the lowest was found (1.12±0.04b) in leaf of red yellow(E). Epidermal cell length tangential was the highest 1.96±0.04b in Pink yellow(C) and Pink red (D) and the lowest 1.82±0.09b was in White yellow (A). Epidermis area was the highest 3.68±0.24b in Pink red (D) and the lowest 2.11±0.13b was observed in Red yellow (E). Similarly, the highest collenchyma cell length radial, collenchyma area was found in White yellow (A), and Collenchyma cell length tangential, and phloem area was highest in Pink yellow(C), and the highest vascular bundle area, xylem area was measured in Pink red (D). On the other hand, the lowest collenchyma cell length radial, collenchyma cell length tangential, collenchyma area was found in Pink red (D), and the lowest vascular bundle area was in Red yellow(E), the lowest xylem area was measured in Pink yellow(C), and the lowest phloem area were observed in White red (B).

Here a, b, c, d and e indicate significant difference at 5% level. Number followed by the same letters in the same column are not significantly different.

Fig. 1. Photographs of flowers of the five types of *Catharanthus roseus* (A= White yellow, B= White red, C= Pink yellow, D= Pink red and E= Red yellow).

Overall results indicated that quantitative characters were diversify of the all items such as leaf and stem among five types of *C. roseus*.

Genetical analysis

In this work four primers of OPW series are used to RAPD analysis to determine genetic variation among the five types of *C. roseus* (see Figure 4 and Table 4). The size of amplification products was calculated by comparing the migration of each amplified fragment with that of a known size fragments of molecular weight marker (1kbp DNA ladder). Among the four primers produced comparatively maximum number of high intensity bands with minimal smearing and they exposed band sizes that ranged from 120bp to 1500bp. (Figure 4). Primer OPW-04 ranged from 850bp to 1500bp, primer OPW-09 ranged from 120bp to 1500bp, primer OPW-10 ranged from 290bp to 1400bp, primer OPW-16 ranged from 200bp to 1500bp. All distinct bands or fragments (RAPD marker) were thereby given identification numbers according to their position on the gel and scored visually based on their presence (1) or absence (0), separately for each individual and each primer. Using the Thermal Cycler (Genius, Techne) and 1% agarose gel electrophoresis, four primers produced 69 bands

from the five types of *C. roseus*, ranging in size from 120bp to 1500bp. Figures (4) show representative electropherograms for the primers OPW-04, OPW-09, OPW-10, and OPW-16, respectively. Four primers produced a total of 69 bands, ranging in size from 8 to 33. (Table 4). The primer OPW-16 amplified the fewest bands (8), while the primer OPW-09 amplified the greatest number of bands (33). 46 of the 69 bands were monomorphic, whereas 23 of the bands were polymorphic.

The primer OPW-09 amplified the greatest number of bands—6.6 per type. The primer OPW-04 showed a higher level of polymorphism 50% (Table 4).

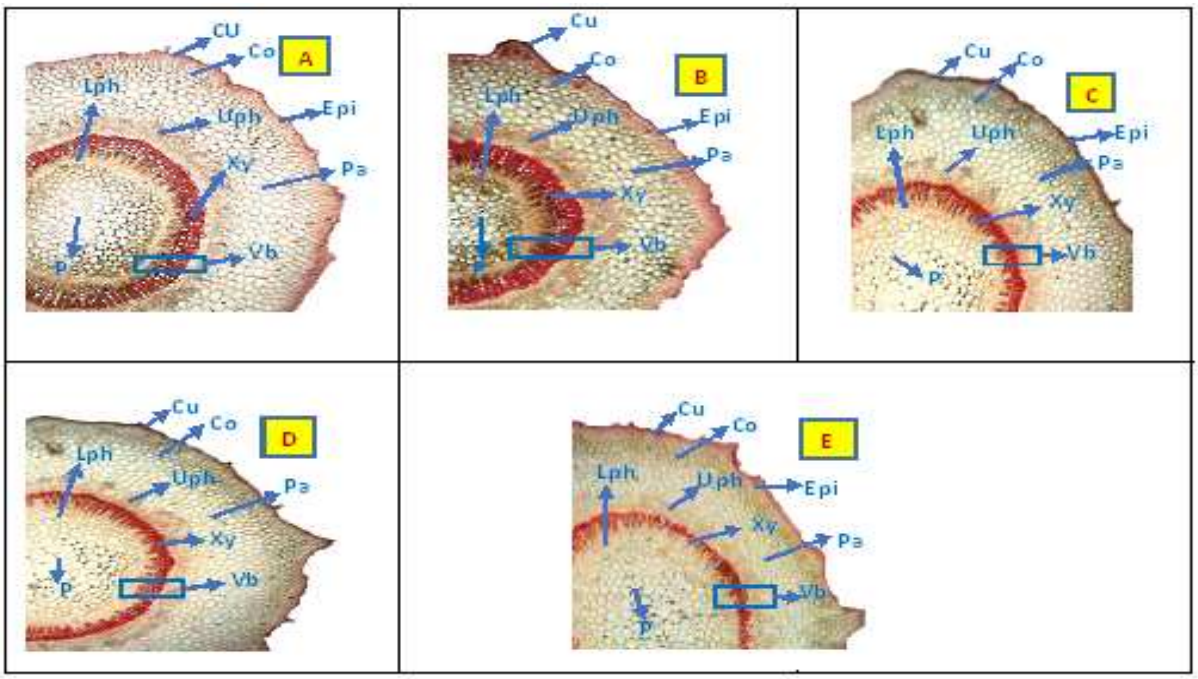

Fig. 2. Transverse section of stem of the five types of *Catharanthus roseus* (A= White yellow, B= White red, C= Pink Yellow, D= Pink red and E= Red yellow) in 10x magnification; showing Cu= Cuticle, Epi= Epidermis, Co= Collenchyma, Pa= Parenchyma, Uph= Upper phloem, Lph= Lower phloem, Xy= Xylem, Vb= Vascular bundle and P= pith.

The genetic distance and cluster analysis among the five types of *C. roseus* are shown in Table 5 and Figure 5. From the combined data for the four primers, linkage distance values for pair-wise comparisons between types were calculated using the software program "Origin Pro," and they ranged from 30.00 to 58.30. (Table 5). Pink yellow vs. Red yellow *C. roseus* types pairs had the largest linkage distance (58.30). White yellow vs. White red types of pairs were determined to have the lowest linkage distance (30.00). Unweighted pair group method with arithmetic mean (UPGMA) was used to determine the genetic relationships among the five types of *C. roseus* based on RAPD data (Nei's, 1978). Two large clusters (C1 and C2) are shown in Figure 5 based on the average distance between the genetic links among the *C. roseus* type. From the dendrogram there were two clear clusters (C1and C2). Cluster C1 was represented by a single type (Red yellow) indicating that it is distinct from all other *C. roseus* types. And cluster C2 was divided into two nodes; one of them had Pink yellow type and the other node further sub divided into sub clusters. One sub cluster comprised of Pink red type.

The other sub cluster comprised of two types, White yellow and White red with similarity value from 70%. The dendrogram shows a close relationship between White Yellow and White Red types. However, these two types are distinct from the Pink yellow, Pink red, and Red yellow types. Compared to all other *C. roseus* types, Red yellow is unique.

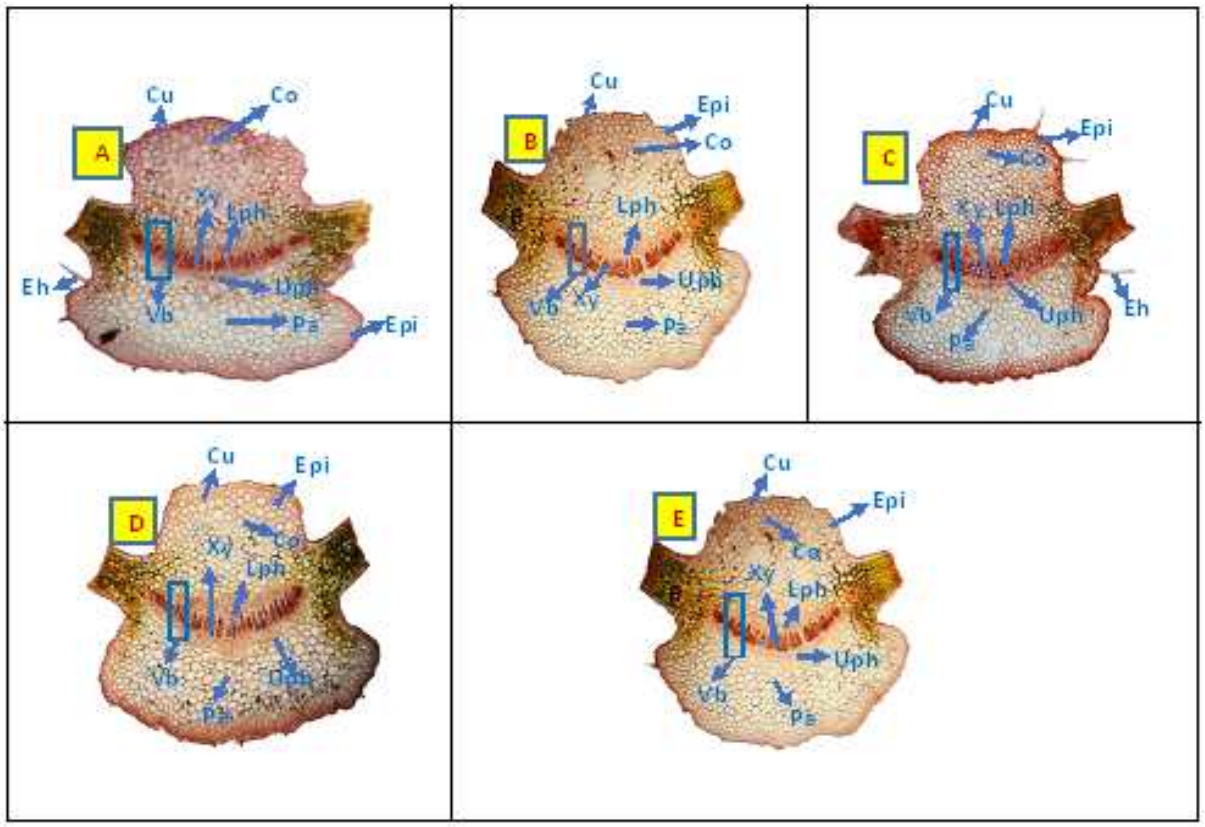

Fig. 3. Transverse section of leaf of the five types of *Catharanthus roseus* (A= White yellow, B= White red, C= Pink Yellow, D= Pink red and E= Red yellow) in 10x magnification; showing Cu= Cuticle, Epi= Epidermis, Co= Collenchyma, Pa= Parenchyma, Uph= Upper phloem, Lph= Lower phloem, Xy= Xylem, Vb= Vascular bundle and Eh= Epidermal hair.

Discussion

The Apocynaceae family, which includes the attractive plant *Catharanthus roseus,* is one of the most fascinating plant families in the world and has tremendous medical potential. (Papon *et al*., 2005). But few work has been done in the past and in recent years on anatomy. This work didn't provide enough description of the anatomy of *C. roseus*. Anatomical character is one approach used to help solve taxonomic problems that are morphologically difficult to separate or still doubtful. Therefore, the present study was undertaken to investigate the gross anatomy of leaf and stem of five types of *C. roseus* of the family Apocynaceae, significant anatomical traits were measured, quantitatively and have been presented in this study. The cortex of the stem of dicotyledonous plants is made up of parenchyma, collenchyma/sclerenchyma, or both. In rare circumstances, the hypodermis is made up of chlorenchyma tissue, a region of the cortex with specialized functions for photosynthesis (Fahn, 1997; Esau, 1965). In our present study materials cortex consists of chlorenchyma/parenchyma and collenchyma tissue. According to reports, collenchyma supports the stem mechanically (Fahn, 1997; Esau, 1965). The cortex's collenchyma either forms a continuous cylinder or can be found as a distinct strip (Fahn, 1997). Collenchyma provides mechanical support of the stem as reported (Fahn, 1997; Esau, 1965). The collenchyma in the cortex forms a continuous cylinder or may be present in the form of separate strip (Fahn, 1997). Vascular tissues of *C. roseus* are bicollateral (Watson and Dallwitz, 1992). In all this investigation, the secondary growth phase indicated a complete ring structure of an open circulatory system in the stems, whereas the mid-ribs and petioles exhibited a vascular arc structure (Wahua *et al*., 2013). Quantitative measurements of different components of the anatomy of leaf and stem indicated clear difference exists among the types.

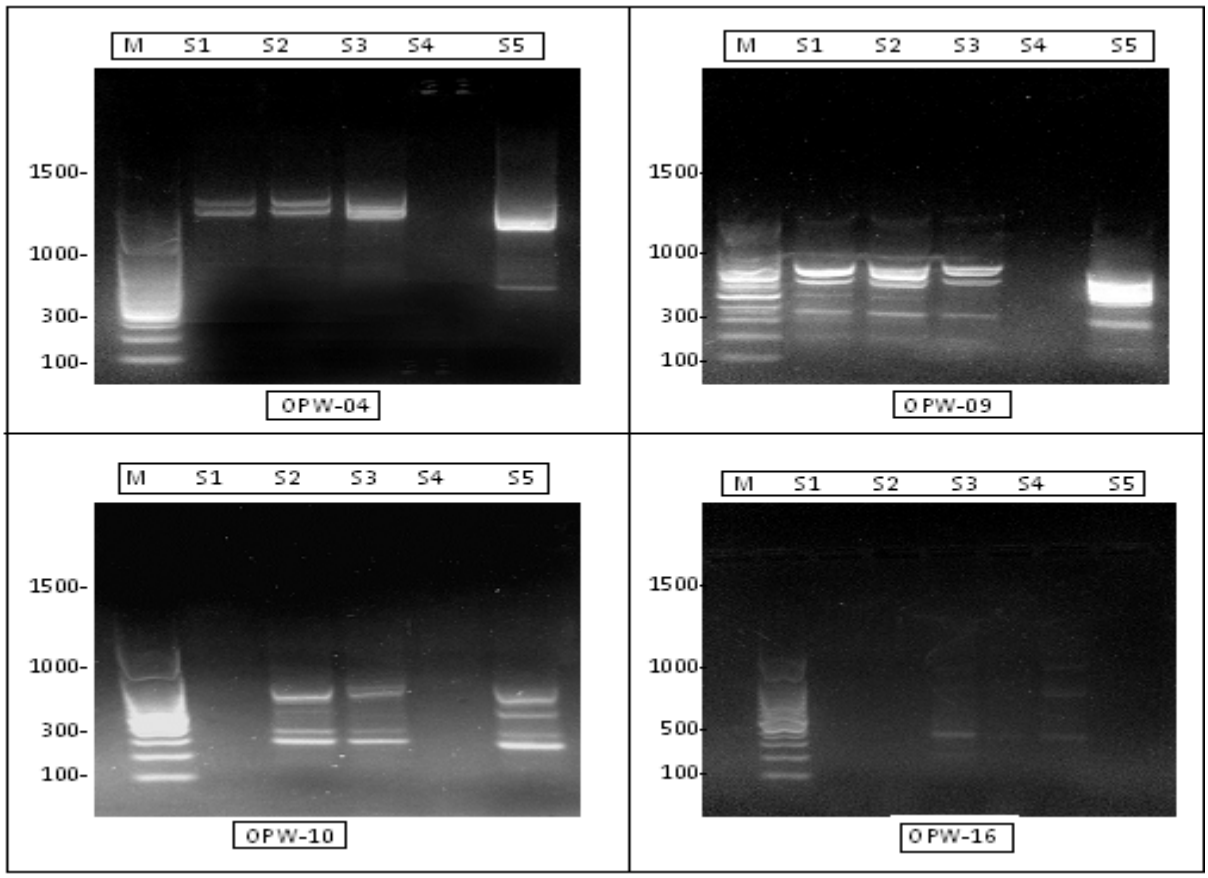

Fig. 4. RAPD profiles of OPW-04, OPW-09, OPW-10 and OPW-16 primers generated from five different types *C. roseus* (S1= White yellow, S2= White red, S3=Pink yellow, S4=Pink red and S5= Red yellow) M: denotes DNA ladder (Markers).

The tangential cells of the epidermis are greater in diameter compared to radial length. Per vascular bundles, Xylem area was consistently bigger than Phloem area. It can be because of mechanical support as well as mineral and water conduction between various plant sections. Evaluation of genetic diversity is crucial for managing and utilizing genetic resources, identifying possible parents in germplasm, and managing genetic resources. Molecular markers (RAPD) are useful tools for analyzing genetic relatedness, identifying, and choosing acceptable genotypes for crosses, and conserving germplasm in gene banks. Additionally, the polymorphism generated by these markers is one of the important characteristics for studying populations and comprehending their genetic variations (Zietkiewicz *et al.*, 1994 and Alvarez *et al.*, 2007). The four RAPD primers OPW series exposed bands with diameters ranging from 120 bp to 1500 bp and produced the greatest number of high intensity bands with the least amount of DNA fingerprint smearing. Four RAPD primers were used to create a total of 69 bands, 23 of which were polymorphic. Each primer produced an average of 5.75 polymorphic bands.

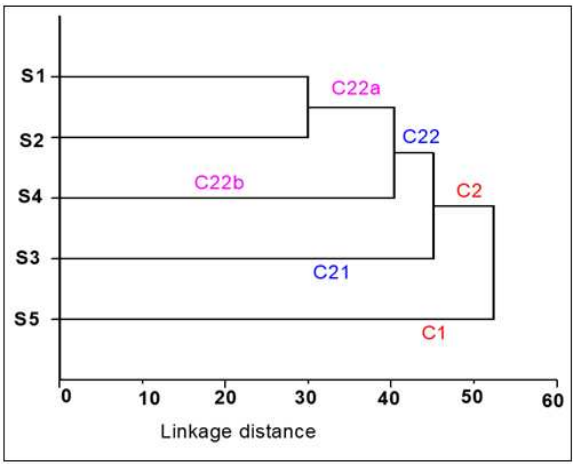

Fig. 5. Cluster analysis by Unweighted Pair Group Method of Arithmetic Means (UPGMA) of five types of *C. roseus* based on four RAPD markers. (S1=White yellow, S2=White red, S3=Pink yellow, S4=Pink red, S5=Red yellow).

The highest number of polymorphic bands 8 were found in the primer OPW-09. Dendrogram based on linkage distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the five *C. roseus* types into two main cluster C1and C2 at linkage distance 53. Type Red yellow is separate Cluster C1 in the Dendrogram which indicates it is different than all other types. In this investigation, RAPD markers were successfully used to differentiate five types of *C. roseus* from each other. Thus, based on RAPD, the findings of this study are like observations of Rajaseger *et al.*, (1997 and 1999). Our results also agree with findings of Loh *et al.*, (1999) who used AFLP markers to study genetic diversity in Caladium bicolar.

Conclusions

The anatomical variation and genetic relationships among five types of *C. roseus* based on the molecular markers can help to decide which types of *C. roseus* are highly variable. Therefore, the present investigation demonstrated that the four RAPD markers were able to identify genetic diversity and relationship among the five types of *C. roseus* through UPGMA cluster analysis. RAPD analysis revealed that a high percentage of polymorphism among the five types of *C. roseus*, which indicates that genetic variation has been preserved. These results will be useful to establish and maintain a germplasm collection of *C. roseus* and may guide us in designing strategies for breeding programs that maximize the utility of *C. roseus* genetic resources. And this study also provides a bench mark for future studies.

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References

Ahmed MF, Kazim SM, Ghori SS, Mehjabeen SS, Ahmed SR, Ali SM, Ibrahim M. 2010. Antidiabetic Activity of *Vinca rosea* Extracts in Alloxan-Induced Diabetic Rats. International Journal of Endocrinology 1-6**.**

Adekomi DA. 2010. Madagascar periwinkle *(Catharanthus roseus)* enhances kidney and liver functions in Wistar rats, European Journal of Anatomy **14,** 111-119.

Arif IA, Bakir MA, Khan HA, Al Farhan AH. 2010. A brief review of molecular techniques to assess plant diversity. International Journal of Molecular Sciences **11,** 2079-2096**.**

Chaudhary AA, Yadav D, Hemant Jamil SS, Asif M. 2012. Real time sequence characterized amplified region (RTSCAR) marker: Development and its application for authentication and quantification of *Catharanthus roseus* L. Donald Journal for Medicinal Plants **6,** 4748-4754**.**

El-Domyati FM, Ramadan AM, Gadalla NO, Edris S, Shokry AM, Hassan SM, Hassanien SE, Baeshen MN, Hajrah NH, Al-Kordy MA, Abuzinadah OA, AlHajar ASM, Akoh CC, Bahieldin A. 2012. Identification of molecular markers for flower characteristics in *Catharanthus roseus* producing anticancer compounds. Life Science Journal **9,** 5949-5960**.**

Gathirwa JW, Rukunga GM, Njagi ENM. 2007. In vitro anti-plasmodial and in vivo antimalarial activity of some plants traditionally used for the treatment of malaria by the Meru community in Kenya, Journal of Natural Medicines **61,** 261-268**.**

Jaleel CA, Gopi R, Manivannan P, Panneerselvam. 2008. Soil salinity alters the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents. Eurasian Journal of Biosciences **2,** 18-25.

Kumar OA, Tata SS, Kumar KP. 2013. Analysis of Several Popular Cultivars of Madagascar Periwinkle (*Catharanthus roseus* (L.) G. Don.) using Biochemical Markers. Notulae Scientia Biologicae **5,** 458-461**.**

Kalpana J, Warude PC, Bhushan P. 2004. Molecular markers in herbal drug technology. Current Science **87,** 159-165.

Kim SW, Ban SH, Jeong SC. 2007. Genetic discrimination between *Catharanthus roseus* cultivars by metabolic fingerprinting using 1H NMR spectra of aromatic compounds. Biotechnology and Bioprocess Engineering **12,** 646- 652.

Lucas DM, Still PC, Pérez LB, Grever MR, Kinghorn AD. 2010**.** Potential of plant-derived natural products in the treatment of leukemia and lymphoma. Curr. Drug. Targets **11,** 812822**.**

Leveque D, Wihlm J, Jehl F. 1996. Pharmacology of *Catharanthus* alkaloids. Bull. Cancer **83,** 176–186.

Leal AA, Mangolin CA, do Amaral ATJ, Goncalves LS. 2010. Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines. Genetics and Molecular Research **9,** 9-18.

Lal S, Mistry KN, Shah SD, Thaker R, Vaidya PB. 2011. Genetic diversity assessment in nine cultivars of *Catharanthus roseus* from Central Gujarat (India) through RAPD, ISSR and SSR markers. Journal of Biological Research **8,** 667-675**.**

Loh JP, Kiew R, Kee A, Gan LH, Gan YY. 1999. Amplified Fragment Length Polymorphism (AFLP) provides molecular markers for the identification of Caladium biocolor cultivars. Annals of Botany **84,** 155-161.

Mishra P, Kumar S. 2000. Emergence of periwinkle *Catharanthus roseus* as a model system for molecular biology of alkaloids: phytochemistry, pharmacology, plant biology and in vitro and in vivo cultivation. Journal of Medicinal and Aromatic Plant Sciences **22,** 306–337.

Nejat N, Maziah M, Valdiani A, Tan YH, Cahill D, Abiri R. 2015. Ornamental exterior versus therapeutic interior of Madagascar periwinkle *(Catharanthus roseus):* the two faces of a versatile herb. Review Article. The Scientific World Journal ID 982412.
http://dx.doi.org/10.1155/2015/982412

Papon N, Bremer J, Vansiri A, Andreu F, Rideau M, Creche J. 2005. Cytokinin and ethylene control indole alkaloid production at the level of the MEP/terpenoid pathway in *Catharanthus roseus* suspension cells. Planta Medicine **71,** 572-4.

Pandiangan D. 2012. The morphological and anatomical changes on tryptophan-treated callus of *Catharanthus roseus*. Journal of Biologos **2(1),** 45-49.

Patil PJ, Ghosh JS. 2010. Antimicrobial activity of *Catharanthus roseus*-A Detailed Study. British Journal of Pharmacology and Toxicology **1,** 40-44**.**

Plaizier AC. 1981. A revision of *Catharanthus roseus* (L.) G. Don (Apocynaceae). Meded Landbouwhogeschool **81(9),** 1-12.

Pandey-Rai S, Mallavarupa GR, Naqvi AA, Yadav A, Rai SK, Srivastava S. 2006. Volatile compounds of leaves and flowers of periwinkle *Catharanthus roseus* (L.) G. Don from New Delhi. Flavour and Fragrance Journal **21,** 427–430.

Prasad MP. 2014. Detection of genetic diversity of *Catharanthus* species using RAPD PCR. Indian Journal of Advanced Botany **1,** 24.

Renoult J, Nuzillard J, Mare L, Le Crouerour G, Thepeiner P, Harnot Z. 1999. Isolation of indol alkaloids from *C. roseus* by centrifuged partition chromatography.

Rajaseger G, Tan HTW, Turner IM, Kumar PP. 1997. Analysis of genetic diversity among Ixora cultivars (Rubiaceae) using random amplified polymorphic DNA. Annals of Botany **80,** 355-361.

Rajasegar G, Tan HTW, Turner IM, Kumar PP. 1999. Random Amplified Polymorphic DNA variation among and within selected Ixora (Rubiaceae) populations and mutants. Annals of Botany **84,** 253-257.

Simpson MG. 2006. Plant systematics. Elsevier, Amsterdam, The Netherlands.

Shaw RK, Acharya L, Mukherjee AK. 2009 Assessment of genetic diversity in a highly valuable medicinal plant *Catharanthus roseus* using molecular markers. Crop breeding and applied biotechnology **9,** 52-59**.**

Tharachand C, Immanuel Selvaraj C, Mythili MN. 2012. Molecular markers in characterization of medicinal plants: An overview. Research in Plant Biology **2,** 01-12**.**

Vega-Avila E, Cano-Velasco JL, Alarcón FJ, Aguilar MDC, Fajardo Ortíz, Almanza Pérez JC, Román-Ramos R. 2012. Hypoglycemic activity of aqueous extracts from *Catharanthus roseus*, Evidence-Based Complementary and Alternative Medicine 1- 7.

Van der Fits L, Deakin EA, Hoge JH. Memelink J. 2000. The ternary transformation system: constitutive VirG on a compatible plasmid dramatically increases Agrobacterium-mediated plant transformation. Plant Molecular Biology **43,** 495–502.

Vardhan S, Yadav AK, Suria RK, Nag BBSP. 2012. Genetic relatedness and In vitro antimicrobial activities of alkaloids isolated from Indian varieties of *Catharanthus roseus* (L.). Current Botany **3,** 43-48.

Wahua C, Okoli BE, Sam SM. 2013. Comparative Morphological, Anatomical, Cytological, and Phytochemical Studies on Capsicum frutescens Linn. and Capsicum annum Linn. (SOLANACEAE); International Journal of Scientific and Engineering Research **4.**

Watson L, Dallwitz MJ. 1992. The Families of Flowering Plants. Descriptions illustrations, identification and information Retrieval version alpha 35.

Zietkiewicz ER, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics **20,** 176-183.