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Original article

FLORAL AND MOLECULAR CHARACTERIZATIONS OF TWO MORPHOTYPES OF PRIDE OF BARBADOS (*CAESALPINIA PULCHERRIMA* L.)

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ABSTRACT

Floral and molecular characterizations of two morphotypes of *Caesalpinia pulcherrima* were undertaken in this study. The sampled plants were growing in three different locations as ornamentals within Ilorin metropolis, Kwara State. Leaf and floral samples were obtained and transported to the laboratory of Plant Science and Biotechnology, Federal University, Oye Ekiti where the floral characteristics (numbers of the floral leaves, their lengths and colours; the fertility of the pollens, their diameter and their production per anther and also per flower) were evaluated. Molecular analyses were also done at the Bioscience Center, Institute of Tropical Agriculture, Ibadan, following standard procedures. The variegated red type has red coloured petals with yellow fringes while its sepal is tinged red. Its pedicels and ovule are green coloured while its style, anther and the filaments were all red coloured. The yellow type had yellowish petals and sepals, its pedicels and ovules are also green while the style, anther and the filaments were all yellow coloured. The petals and the sepals in the two morphotypes were five each; the pedicel, ovule and style for the flower were one each while the number of anthers and filaments were 10 for each. The lengths of pedicels (variegated red, 2.98cm; yellow, 1.86cm), styles (variegated red, 4.18cm; yellow, 2.94cm) and the filaments (variegated red, 3.98cm; yellow, 2.90cm) were significantly different in the two morphotypes. Their pollen diameters showed no significant differences while the pollens produced per anther (red, 3765.0; yellow, 3860.25) and pollen produced per flower (red, 37650; yellow, 38602.5) showed significant differences between the two types. Their pollen fertilities (red, 77.5%; yellow, 75.9%) were also statistically different. Molecular assays using bar code method also revealed marked differences in their phylogenetic relationships. This study helps to understand the importance of the floral features in plant diversity studies, in plant adaptation, identification and classification.

Keywords: Floral characterization, Molecular, Pollens, Identification, Classification

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INTRODUCTION

The Pride of Barbados (*Caesalpinia pulcherrima* L.) belongs to the family Fabaceae. It is a fast-growing ornamental plant with clusters of brightly coloured, showy flowers. It is a native to central and South America. It produces yellow, white, red and variegated flower forms which is peculiar for each plant variety, hence they are identified by the colour of the flower they produce. The showy flowers attract insect pollinators which leads to the production of dark brown seeds in a pod at maturity (1, 2). The Pride of Barbados, also called the peacock flower, often produce green, bi-pinnate leaves on highly branched and spiny stems, growing as shrubs. *C. pulcherrima* is a hardy plant with great resilience to drought. It grows, tolerating a wide range of soil types and even conditions, although it prefers full sun and moderate to low water (1).

Caesalpinia pulcherrima is very popular for ornamental purpose, however it has proved to be of immense medicinal values in the traditional treatment of ailments such as fever, skin infections, inflammations and many more (3, 4). *Caesalpinia crista* seeds showed high potency as antioxidant, anti- inflammatory and analgesic activity on mice, thus, very useful therapeutic potentials (5). Almost all parts of *C. pulcherrima* (leaves, pods, flowers, seeds and the bark) have been variously reported to be of immense therapeutic uses, treating rheumatism, ulcers, fevers, infections, wounds, eye irritations, bronchitis, asthma and tumors, as abortifacient and anticonvulsant (6 - 13). These showed the level of importance of this plant for human wellbeing. Saline extract of *C. pulcherrima* leaves showed significant antioxidant properties and inhibition of Candida strains (4, 14). Also, there was no cytotoxicity against mice splenocytes thus promoting the proliferation of the cells. These results, according to the authors, may be very important when using *C. pulcherrima* leaf extracts in saline conditions as agents in formulating herbal remedies to treat inflammation of hair follicles and boosting of the immune systems. Cytotoxicity of the leaf extract of *C. pulcherrima* was also reported (15). Dyes for fabrics and leather products are also obtained from the leaves and stems of *C. pulcherrima*. The leaves, pods, and seeds are used as fodder for livestock, providing excellent source of protein, minerals, and vitamins, making it a valuable feed for animals (1).

Variations in DNA sequences and the occurrence of specific alleles have been detected among the different varieties of *Caesalpinia pulcherrima*, indicating genetic differentiation among different colour varieties. This genetic diversity is crucial for the adaptation and survival of the species in different environments (16).

The techniques of molecular characterization have played immense roles in species identification and classification in recent years. DNA sequences of plants have been conserved and their variability used in delineating taxa boundaries (17). Closely related species that are difficult to distinguish based on morphological or anatomical characters have been delineated by high level of accuracy and sensitivity. Sequences of plants data bases have made global references very easy, thus facilitating reclassification of world flora and reducing ensuing confusions that may arise due to the emergence of new plant forms by natural hybridization or biotechnology.
This study is aimed as a preliminary y investigation into the reproductive botany

of *Caesalpinia pulcherrima* variegated red and yellow morphotypes.

MATERIALS AND METHODS

Samples were collected from *Caesalpinia pulcherrima* red and yellow morphotypes growing in three different locations as ornamentals within Ilorin metropolis, Kwara State. Open flowers and those at the final bud stages were collected in plastic sample bottles and transported in ice to the Laboratory of the Plant science and Biotechnology, Oye Ekiti within three hours, for study on the same day of collection.

Leaf and Stem Features

The leaves and stems of the plants from which flower samples were obtained were observed visually for the leaf shape, the distribution of spines on the leaves and stems.

Floral Morphology

Freshly opened flowers were obtained and the following parameters were studied:
The numbers of the sepals, petals, stamens and pistil were visually counted; the heights of the sepals and petals were measured with a meter rule, so also the lengths of the stamens and the pistil. Measurements were made using five flowers each. The colours of the sepals, petals, stamen and pistil were all taken by visual observations.

Pollen Fertility

Pollen grains from freshly opened anthers were dusted on clean glass slides, two drops of Potassium Iodide solution were placed on it and a cover slip was used to cover it. Under the X10 objective lens of the microscope, 70 pollens were randomly counted out of which the homogenously dark brown stained pollens were visually counted as fertile. This process was repeated using two other flowers, to make three replicates.
Pollen fertility (%) was determined as:

$$\frac{Number\ of\ fertile\ pollens}{Total\ number\ of\ pollens\ counted} \times 100$$

Pollen Diameter

Pollen grains were dusted on a clean glass slide, clean cover slip was placed on it. Eye piece graticule was used to measure the diameter (at X10 objective) of 70 pollens at random, this was done for all the samples under study. The measurements made with the eye piece graticule were converted into millimeters after calibration with the stage graticule.

Pollen Production

Following the method of (18), the quantity of pollen grains produced in a flower per sample under study were evaluated. Flower buds that are already matured, ten (10) in number and which were about to open (and the anthers still intact) were collected from ten different flowers which are hermaphrodites. The buds were preserved in 70% ethanol in labelled vials. Five anthers from different flower buds per variety (each flower contains ten anthers) were placed in a 2 mL vial with distilled water (1 mL) and were gently crushed using a glass rod to release the pollen into the suspension. A drop of each thoroughly stirred suspension was put on the counting area of a Haemocytometer slide (with depth of 0.10 mm) and covered with its cover slip. The pollen grains were counted in four counting areas, under a binocular microscope at 10 × magnification.

Pollens per anther (P/A) = $\frac{Pollen\ counts\ \times 1000mm^3}{0.1\ mm^3\ \times 5\ Anthers}$

An estimate of the pollens grains produced by a flower was computed by the number of pollens in an anther multiplied by the number of anthers in a flower.
Pollens per Flower (P/F) =

$$(P/F) = P/A\ \times 10\ anthers$$

The mean $\pm$ SE were computed.

Molecular analyses

Young freshly harvested leaves of the samples, packaged in labelled paper envelopes taken in ice blocks were to the Bioscience laboratory of the IITA (International Institute of Tropical Agriculture), Ibadan for DNA barcoding molecular assays. DNA isolation was done by the CTAB method while the Sanger sequencing method was used for amplicon sequencing. The sequences obtained were verified by blasting on the NCBI website. The molecular analyses were done using MEGA X program (DNA alignment with the CodonCode Aligner and the construction of the Phylogenetic Neighbor Joint tree).
The molecular analyses were done using MEGA X program (DNA alignment with the CodonCode Aligner and the construction of the Phylogenetic Neighbor Joint tree).

RESULTS

Leaf and Stem Features

The leaf form in both the variegated red variety and the variety are bi-pinnate compound with short hard spines at the base of the petioles and the base of the midribs. The short hard spines are also present on the stems of both varieties (Table 1).

Table 1: Leaf and stem features in variegated red and the yellow varieties of *Caesalpinia pulcherrima*

PARAMETERS	VARIEGATED RED	YELLOW
Leaf Form	Bi-pinnate compound	Bi-pinnate compound
Leaf Spine	Present on Petiole and Midrib	Present on Petiole and Midrib
Stem Spine	Present	Present

Floral Morphology

The variegated red variety has red coloured petals with yellow fringes while its sepal is tinged red (Plate 1). Its pedicels and ovule are green coloured while its style, anther and the filaments were all red coloured (Plate 2, Table 2). The yellow variety on the other hand had yellowish petals and sepals. The pedicels and ovules are also green while the style, anther and the filaments were all yellow coloured (Table 2).
The two varieties had five petals and five sepals each, one pedicel for the flower, one ovule and one style each while the number of anthers and filaments were 10 for each (Table 3). However, there are slight variations in the lengths of their floral parts. The lengths of pedicels, styles and the filaments are significantly different in the two varieties (Table 3).

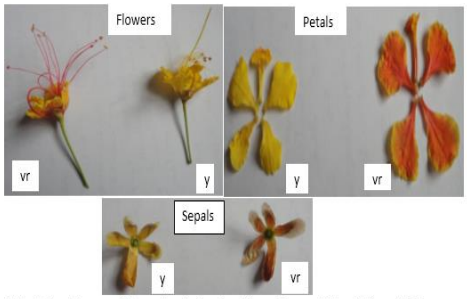

Plate 1: Floral leaves of the variegated red and the yellow varieties of *Caesalpinia pulcherrima* [Vr is variegated red; y is yellow]

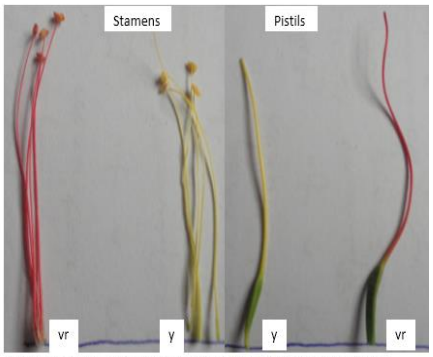

Plate 2: The stamens and pistils of the variegated red and the yellow varieties of *Caesalpinia pulcherrima* [Vr is variegated red; y is yellow]

Table 2: Colours of the floral parts in variegated red and the yellow varieties of *Caesalpinia pulcherrima*

STRUCTURE	VARIEGATED RED	YELLOW
Petal	Red with yellow fringes	Yellow
Sepal	Tinged red	Yellow
Pedicel	Green	Green
Ovule	Green	Green
Style	Reddish	Yellow
Anther	Reddish	Yellow
Filament	Reddish	Yellow

Table 3: Numbers and Lengths of floral parts in variegated red and the yellow varieties of *Caesalpinia pulcherrima*

	VARIEGATED RED		YELLOW	
Structure	Number	Length (cm)	Number	Length (cm)
Petal	5	1.3 ± 0.00a	5	1.2 ± 0.00a
Sepal	5	0.89 ± 0.20a	5	0.9 ± 0.00a
Pedicel	1	2.98 ±.0.05a	1	1.86 ± 0.06b
Ovule	1	0.80 ± 0.00a	1	0.82 ± 0.05b
Style	1	4.18 ± 0.05a	1	2.94 ± 0.06b
Anther	10	0.10 ± 0.00a	10	0.10 ± 0.00a
Filament	10	3.98 ± 0.05a	10	2.90 ± 0.00b

*Values with different letters in the same row are significantly different (P=.05). Each mean is a number of five replicates.

Pollen Diameter, Fertility and Production

The pollen diameter in the two varieties showed no significant differences while the pollen produced per anther and pollen produced per flower showed significant differences between the two varieties (Table 4). Their pollen fertility are also statistically different (Table 4).

Table 4: Pollen diameter, production and fertility in in variegated red and the yellow varieties of *Caesalpinia pulcherrima*

	Pollen Diameter (mm)	Pollen/ Anther	Pollen/ Flower	Pollen fertility (%)
Variegated Red	0.068 ± 0.025a	3765.0 ± 294.50b	37650 ± 2945.00b	77.5 ± 4.70a
Yellow	0.064 ± 0.21a	3860.25 ± 413.08a	38602.5 ± 4130.80a	75.9 ± 2.32b

*Values with different letters in the same column are significantly different (P=.05).

Molecular analyses

The molecular analyses revealed that *Caesalpinia pulcherrima* has 90.69% similarity with *Caesalpinia sappan* chloroplast on the NCBI data base. The sequence alignment, using forward and reverse primers H1 and 72A are shown in figures 1-4 while a phylogenetic tree drawn, using the Neighbor Joining and the nucleotide distance measure showed clear differences in the genomes of both variegated red and the yellow varieties of *Caesalpinia pulcherrima* (Figure 5).

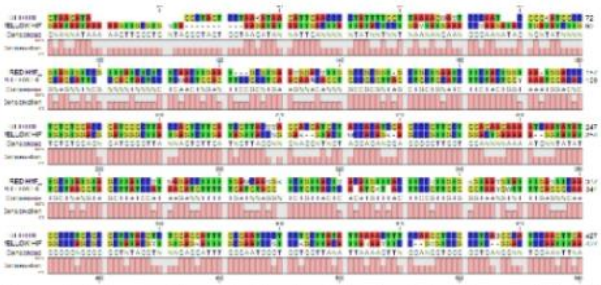

Figure 1: Sequence alignment of variegated red and the yellow varieties of *C. pulcherimma* in HI primers on CLC sequence viewer 8.0

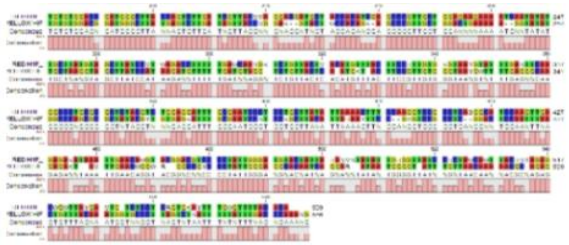

Figure 2: Sequence alignment of variegated red and the yellow varieties of *C. pulcherimma* in HI primers on CLC sequence viewer 8.0

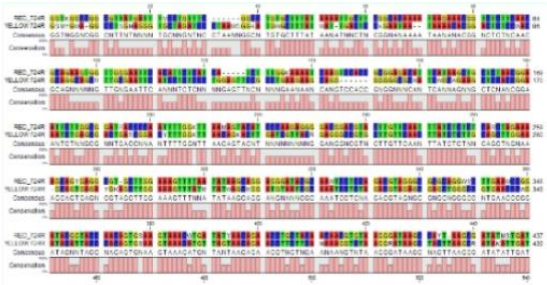

Figure 3: Sequence alignment of variegated red and the yellow varieties of *C. pulcherimma* in 72A primer on CLC sequence viewer 8.0

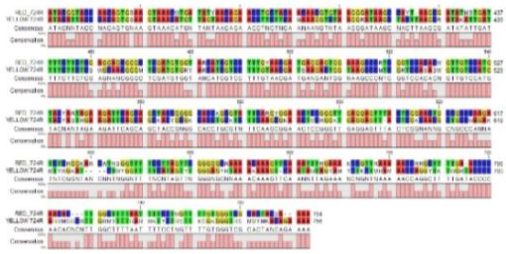

Figure 4: Sequence alignment of variegated red and the yellow varieties of *C. pulcherimma* in 72A primers on CLC sequence viewer 8.0

Figure 5: Dendogram of phylogenetic relationships between the red and yellow varieties of *C. pulcherimma* on CLC sequence viewer 8.0

DISCUSSION

Caealpinia pulcherrima L. is a perennial shrub commonly grown as ornamental plant in many parts of the world. It is however a very important plant whose use can be harnessed for human benefits if more studies are done to unravel its potentials. *C. pulcherrima* flowers are very showy, occurring in various colours, however, the commonest types in Nigeria are the variegated red and the yellow flowered forms. Despite their similarities, very distinctive features abound that could be useful in delineating the taxon, thus helping to resolve the existing mix-ups in the names of the various forms in the species.

The results from this study showed that there are very distinct differences in the morphologies of both variegated red and the yellow flowered morphotypes of *C. pulcherrima*. These differences, as observed in the colours and lengths of many of the floral leaves, pollen production and fertility, and also in the molecular evaluations are very important to understand the floral biology of this plant. This knowledge is key to understanding their evolution and diversities (19, 20). *C. pulcherrima* morphotypes under this study produced very high amount of pollen grain, this is a reproductive strategy by plants

(21). **The floral display size, that is, the number of opened flowers on a plant at a particular time, can influence attraction of pollinators, thus the plant production (22). The observed differences (both in floral and molecular characteristics) between the variegated red and the yellow morphotypes in this study corroborate the earlier findings** (16) where variations were observed in the DNA sequences and the detection of specific alleles in colour varieties of *Caesalpinia pulcherrima.* This according to them is very important for the adaptation and survival of the plant in several environments. This study is very important in understanding the diversity in the taxon *Caesalpinia* and also in their identification and classification.

Declarations

Authors' contributions: OAO conceptualized and designed the study, AAJ and OOM participated in fieldwork and data collection ATO performed the data analyses. OAO and ATO prepared the manuscript. All authors contributed to the development of the manuscript and also approved its submission.

Conflict of Interest: None

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