

Biometrical, palynological and anatomical features of *Chrozophora rottleri* (Geiseler) Juss. ex Spreng.

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Abstract: *Chrozophora* belongs to the family Euphorbiaceae, the spurge family that includes 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. The family is distinguished by the presence of milky sap, unisexual flowers, superior and usually trilobular ovary, axile placentation and the collateral, pendulous ovules with carunculate micropyle. In the present investigation the biometrical, palynological and anatomical features of *Chrozophora rottleri* was studied. The results revealed that the length of pollen grains of *Chrozophora rottleri* varied slightly, ranging from 19µm to 25 µm. The pollen aperture was in the range of 0.5µm to 0.7µm. P/E ratio was 0.65 to 0.78. The pollen grains were elliptical to rounded. Pollen grains were tricolpate with pentoporate and hexoporate in some specimens and spinose exine. The viability of pollen grains was maximum 72 % to 87%.

Staminate flowers 4-6 mm in diameter, yellow; calyx white, united c. 1 mm high, lobes 3.2-4 by c. 1.2 mm, sepals lanceolate, stellate-pubescent; androphore 3.3-3.8 mm long; anthers 0.9-1.3 by c. 0.7 mm, yellow. Pistillate flowers 3.2-3.3 mm in diameter, greenish to yellow; pedicel 1.4-2 mm long, elongating in fruit to up to 1.1 cm; calyx lobes only basally united, 1.5-2.2 by 0.5-0.7 mm; petals 1.3-2 by 0.4-0.6 mm; ovary ovoid, 2.7-3 by 2.2-3 mm wide; style 0.5-0.8 mm long, bifid, red, stigmas erect, up to 2.3 mm long, apically split for up to 1.8 mm, red.

Anatomy of root, stem and foliar parts of *Chrozophora rottleri* revealed the presence of thick in stem and foliar parts; single row of epidermal cells (parenchyma) in leaves; mesophyll tissues differentiated into palisade and spongy parenchyma; vascular bundles in the mid-vein, lateral veins in the mesophyll tissues and in the laminar surfaces; the latex, laticiferous tissues, latex vessels, and resinous ducts. These features suggested polyphyletic origin and evolution of taxa of Euphorbiaceae.

Keywords: Biometric, Palynology, Anatomy, *Chrozophora rottleri*, Euphorbiaceae

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I. Introduction

Chrozophora belongs to the family Euphorbiaceae, the spurge family [1, 2, 3] (Webster, 1967; Webster, 2007; Hyam and Pankhurst, 1995) that encompasses 7,500 species; 422 species are described from India. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. The family is distinguished by the presence of milky sap, unisexual flowers, superior and usually trilobular ovary, axile placentation and the collateral, pendulous ovules with carunculate micropyle. The species of spurge family widely occur in warmer climate, also they extend into the temperature regions of Northern and Southern hemisphere but are not found in the arctic region [4] (Lawrence, 1951). This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America. A large variety occurs in tropical Africa, but they are not as abundant or varied as in these two other tropical regions [5] (Gibbs, 1974). However, Euphorbia also has many species in non-tropical areas such as the Mediterranean Basin, the Middle East, South Africa, and Southern USA. The leaves are alternate, seldom opposite, with stipules. They are mainly simple, but where compound, are always palmate, never pinnate. Stipules may be reduced to hairs, glands, or spines, or in succulent species [6, 7, 8] (Paul *et al.*, 2014; Paul *et al.*, 2014; Betancur- Galvis *et al.*, 2002) are sometimes absent.

Chrozophora is the sole genus in the subtribe *chrozophorinae* of Euphorbiaceae. It comprises 11 species, which are mostly monoecious herbs and under shrubs. This genus is distributed in Pakistan, India, West Africa and Mediterranean regions [9, 10] (Tene Vicente *et al.*, 2007; Caius, 1938). Five species of *Chrozophora* are known to occur in India. The plant occurs naturally in tropical African, Asia and India [10] (Caius, 1938).

Botanical Description: Annual herbs, prostrate or ascending; main stem up to 50 cm long, stellate-pubescent or at times scabrid. Leaves alternate, 2-5 x 1-4 cm, rounded or obtuse at apex, rounded or subtruncate at base, entire or shallowly crenate-sinuate, 3-5-veined from base, somewhat bullate above when young, becoming less so with age, pubescent above, densely so beneath; petiole 1-4 cm long, densely stellate-pubescent; stipules 2 mm long, linear. Inflorescence 1-5 cm long, leaf-opposed. Male flowers: pedicels 1 mm long; sepals c. 3 mm long, lanceolate, stellate-pubescent; petals pink, 3 mm long, elliptic-oblong, lepidote without; stamens 15, united into 4 mm tall column; anthers 1 mm long. Female flowers: pedicels c. 5 mm long, extending up to 1.5 cm or more in fruit; sepals 1.5-2 mm long, linear-lanceolate, stellate-pubescent; petals minute or absent. Ovary 2 mm diameter, densely stellate-pubescent; styles 1-1.5 mm long, bifid almost from base, stellate-pubescent without, densely papillose within. Fruit 4 x 7 mm, rounded, 3-lobed, stellate-pubescent; seeds 3-3.5 x 2-2.5 mm, globose-ovoid, grey.



Floral twigs of *Chrozophora rotleri*

Scientific Classification:

Kingdom: Plantae; Clade: Angiosperms; Clade: Eudicots; Clade: Rosids; Order: Malpighiales;
Family: Euphorbiaceae; Subfamily: Acalyphoideae; Tribe: Chrozophoreae; Subtribe: Chrozophorinae;
Genus: *Chrozophora* Neck. Ex A. Juss. (1824), Pax and K. Hoffm. (1919); Species: *Chrozophora tintoria*,
Chrozophora rotleri

The leaves of *C. rotleri* are very much beneficial in treatment of skin diseases [11] (Khare, 2007) and are also used as depurative agent. From this plant, aqueous extract of this leaves has a significant anti-helmintic property against *Pheritima posthuma* [12] (Priyanka *et al.*, 2010) (Indian Earth worm) and possess phytotoxic activity on rice, wheat and mustard. Suparna and Tapaswi (1999) [13] reported that, the leaf extracts of *C. rotleri* exhibited higher inhibition of shoot, root and radial elongation than the stem and root Juice of the fruit is given in cases of cough and colds [11] (Khare, 2007) in countries like Nepal and leaf is used as purifying agent and seed is used as laxative [14] (Singh *et al.*, 2010), having bioactive components [15] (Mander, 1998). The seeds are used as cathartic [16] (Sasinath, 2007) and have with purgative properties [17] (Srivastava and Agarwal, 1953). *Chrozophora* genus has several interesting medicinal uses, the plant ash of *Chrozophora brocchiana*, is applied to sore and the crushed leaves were rubbed on the affected sites to treat stitch in the side. The aerial parts are taken in decoction to strengthen lactating mothers and their children, and to treat fever and dysentery. While powdered dried leaves in water are taken to treat diarrhea. Root sap in water is used as ear drops to treat otitis [18] (Yushau, 2011). Analysis of the chemical content shows no particular reason for a beneficial action as a wound-dressing; however, there is unusually high silica content. While *Chrozophora senegalensis* plant has been reported is an astringent for treatment diarrhea mainly caused by *Salmonella* specie, and in Senegal a root decoction is given to suckling babies to treat diarrhea [19] (Etkin, 1997). It is boiled with cereal foods and the pregnant women used a decoction of it as a body wash, also used as a remedy for syphilis; and treatment of intestinal pain, typhoid and boils [20, 2] (Usman *et al.*, 2007; Benoit- Vical *et al.*, 2008). The fruit juice is used as eye drops to treat more severe cases, a maceration of leaves and roots is drunk to treat loss of hair and diabetes, and a water extract of aerial parts caused an in-vivo hypoglycemic response in rats [22] (Delazar *et al.*, 2005). It has been reported that leaves and stems extracts of *Chrozophora senegalensis* showed a high anti-plasmodial activity against two chloroquine-resistant *Plasmodium falciparum* strains, without toxicity in vitro and no toxicity in vivo by oral way in mice. While the leaf extracts alone showed antimicrobial activity

against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*; with highly active on *Salmonella typhi*. In Sudan, *C. oblongifolia* stem and leaf extracts are used to treat gonorrhoea and the chloroform and methanol extracts showed considerable antidiabetic activities. Ugulu *et al.*, (2009) [23] reported that *Chrozophora tinctoria*, has a high solubility in water, and produced dark red color, but it did not show reaction with wool fiber. The plant is used traditionally to treat warts, also has been used as an emetic, cathartic, and for the treatment of fever elsewhere [24] (Gamble, 1967).

Chrozophora plicata has an emetic, drastic and corrosive property. Its seeds are used as cathartic [25] (Manandhar *et al.*, 2000). The leaf extracts exhibited strong fungi toxicity against *P. aphanidermatum*, the plant poisoning causes salivation, dyspnea, bloat, dullness, diarrhea, paresis of the hind limbs, recumbence and lateral deviation of the head and neck. While *Chrozophora rottleri* is traditionally used for the treatment of various diseases. In Sudan people use stems or whole plant as powdered and applied it to wounds to improve healing. The plant also used in Saudi Arabia and India to treat Jaundice and purifying blood. An infusion of seeds and leaves is taken as a laxative in Ethiopia and in Senegal, the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhea, whereas in Kenya, camels graze it. The fruits yield a purplish blue dye, which is used to dye mats in East Africa. The fruit juice is given in cases of cough and cold in Nepal [11] (Khare, 2007). The leaves of *Chrozophora rottleri* are used as a depurative agent and they are very much beneficial in treatment of skin diseases [12] (Priyanka *et al.*, 2010). The seeds are used as cathartic like Ghodtapde and credited with purgative properties. Priyanka *et al.*, (2010) [12] reported that, the aqueous extract of the leaves of this plant has a significant anti-helminthic property against *Pheritima posthuma* (Indian Earth worm). The aqueous extract of *Chrozophora rottleri* possessed phytotoxic activity on rice, wheat and mustard. In an experimental study Suparna and Tapaswi (1999) [13] reported that, the leaf extracts of *Chrozophora rottleri* exhibited higher inhibition of shoot, root and radial elongation than the stem and root.

The major phytochemicals of *C. rottleri* include Alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins, quercetin 3-o-rutinoside (rutin), acacetin 7-orutinoside, and apigenin 7-o-b-d-[6-(3,4-dihydroxybenzoyl)]-glucopyranoside (chrozophorin).

The oil from the seed of *Chrozophora rottleri* was reported to be rich in linoleate, while the leaves and root contain xanthone glycosides and chromone glycoside. The tannin was found in the whole plant [26] (Madane *et al.*, 2013). Another study revealed the presence of alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins in the chloroform extract of *C. rottleri* (Maharaj *et al.*, 2013) [27]. Maharaj and Prabhakaran (2013) [27] and Mothana *et al.*, (2011) [28] reported that the weed *C.rottleri* had adverse allelopathic effects on the germination and growth of rice seedlings.

II. Materials and Methods

The specimens of *C. rottleri* were collected from five different localities of Begusarai district of Bihar viz. Begusarai, Brauni, Bacchwara, teghra and Matihani. A total of twenty five samples (five from each site) was collected for their biometric measurements. The mean and standard deviation values of the measurements were calculated according to Rummel (1970). The samples (roots, stems, leaves, petioles) of each site were fixed in 70% alcohol, and then anatomical sections of root, stem, petiole and leaf were taken. The pollen grains of the *Chrozophora rottleri* were collected from five different areas of Begusarai. Palynological characters such as pollen type, aperture morphotype, exine ornamentation, spine type and length, grain size and shape, pollen fertility like features were observed.

Mature pollen grains from mature anthers were dusted on a clean slide and stained with one percent acetocarmine. The acetolysed pollen grains were mounted in glycerine jelly and the slides were sealed with paraffin wax. Acetocarmine was found to be the most suitable stain for pollen grain studies in Boraginaceae. The size of the pollen grains was measured by using ocular micrometer. Twenty readings were taken in each case. The pollen grains were micro photographed to study the shape of pollen grains and the wall ornamentation. Photomicrographs were taken for all plant materials. Jetner – Biolux research microscope was used for micro photographing. Pollen size was calculated by taking measurements of polar axis and the maximum breadth in the equatorial view of the grain and applying the formula $P/E \times 100$. The results obtained have been presented in Table-1 and 2; Figure1-4.

Table-1: Morphological features of Pollen grains of *Chrozophora rottleri* collected from five different areas of Begusarai District

Pollen features	Localities				
	Begusarai	Barauni	Bacchbara	Teghra	Matihani
Length of pollen in μm	21	24	22	19	25
Width of pollen in μm	19	17	18	17	20
Pollen aperture in μm	0.6	0.7	0.5	0.7	0.7
P/E ratio	0.66	0.68	0.67	0.68	0.65
Shape of pollen	Elliptical	Elliptical	Rounded	Rounded	Elliptical
Pollen type	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate
Aperture morphology	Pentoporate	Pentoporate	Pentoporate	Hexoporate	Pentoporate
Exine ornamentation	Spinose	Spinose	Spinose	Spinose	Spinose
Spine type	Pointed	Pointed	Pointed	Pointed	Pointed
Spine length in μm	7.4	7.6	7.8	7.5	7.5
Grain size in μm	75	75	70	85	85
Pollen fertility (%)	72	71	76	87	87

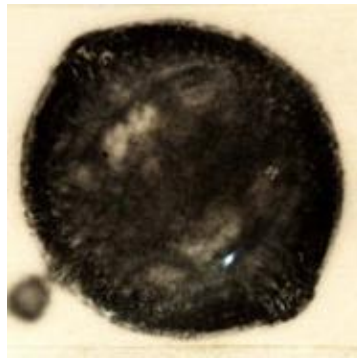


Fig-1: Pollen grain of *C. rottleri* collected from Begusarai

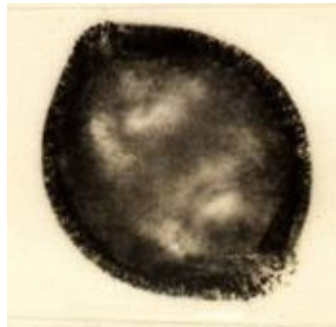


Fig-2: Pollen grain of *C. rottleri* collected from Barauni

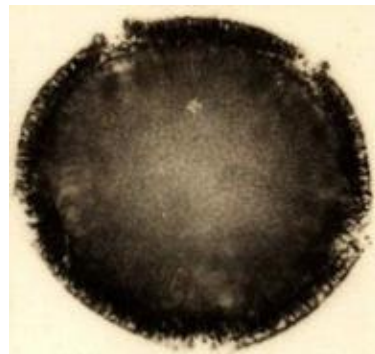


Fig-3: Pollen grain of *C. rottleri* collected from Bacchwara

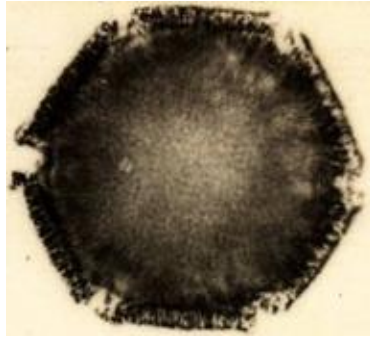


Fig-4: Poolen grains of *C. rottleri* collected from Teghra

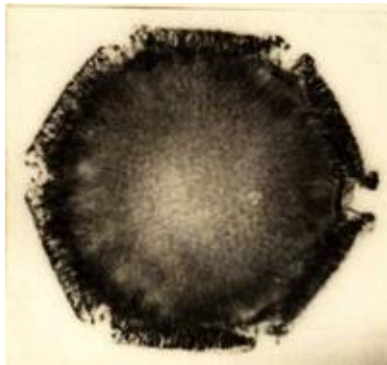


Fig-5: Pollen grains of *C. rottleri* collected from Teghra

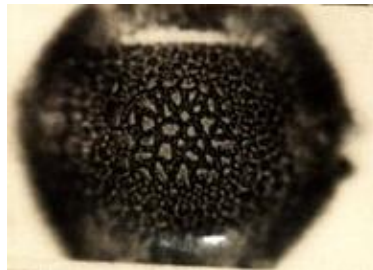


Fig-6: Pollen grain of *C. rottleri* collected from Matihani

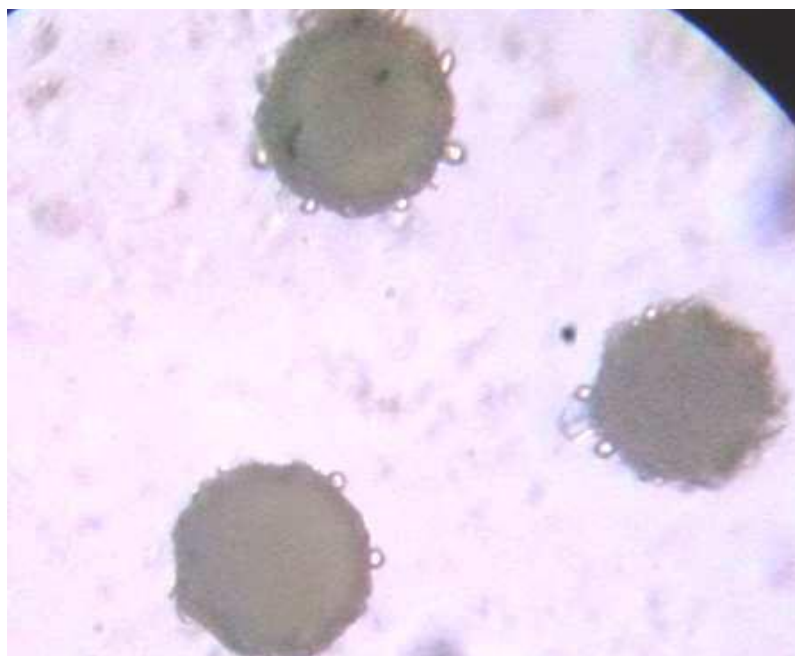


Fig-7: Pollen grains of *Chrozophora rottleri*

Table-2: Biometric measurement of *Chrozophora rottleri*

Plant parts	Number of measurements	Width Minimum	Length Maximum	Mean \pm SD	Minimum	Maximum	Mean \pm SD
Plant	25	9.0 cm	63.50 cm	38.55 \pm 12.07	27.00 cm	54.60 cm	47.75 \pm 8.25
Root	25	3.55 mm	10.7 mm	5.17 \pm 1.83	7.25 cm	33.65 cm	17.35 \pm 5.75
Stem	25	2.55 mm	14.50 mm	6.15 \pm 2.65	2.70 cm	7.50 cm	5.65 \pm 1.54
Leaf	25	1.75 cm	6.65 cm	3.75 \pm 1.65	1.82 cm	7.50 cm	4.75 \pm 1.51
Petiole	25	-	-	-	0.76 cm	11.00 cm	5.21 \pm 2.17
Pedicel	25	-	-	-	3.5 cm	31.25 cm	15.65 \pm 6.88
Calyx (female)	25	0.35 mm	1.37 mm	0.97 \pm 0.23	2.55 mm	4.35 mm	3.15 \pm 0.45
Calyx (Male)	25	0.55 mm	1.57 mm	0.99 \pm 0.25	2.56 mm	4.40 mm	3.15 \pm 0.63
Corolla (female)	25	1.25 mm	1.60 mm	1.20 \pm 0.25	2.77 mm	5.35 mm	3.71 \pm 0.71
Corolla (male)	25	1.25 mm	1.65 mm	1.26 \pm 0.23	3.15 mm	5.15 mm	3.67 \pm 0.52
Stigma	25	-	-	-	0.77 mm	2.50 mm	1.35 \pm 0.37
style	25	-	-	-	0.76 mm	2.0 mm	1.08 \pm 0.21
Ovary	25	-	-	-	2.5 mm	3.85 mm	3.14 \pm 0.35
Anther	25	0.55 mm	1.25 mm	0.75 \pm 0.19	1.25 mm	1.85 mm	1.25 \pm 0.21
Filament	25	-	-	-	0.50 mm	1.35 mm	0.99 \pm 0.14
Seed	25	3.25 mm	4.50 mm	3.75 \pm 0.42	3.15 mm	6.25 mm	4.61 \pm 0.62
Fruit	25	0.42 cm	1.60 cm	0.57 \pm 0.21	0.40 cm	0.82 cm	0.57 \pm 0.12

III. Results

The pollen grains of higher plants constitute the most vital unit of the flower with regard to their form and function. These one-celled microscopic haploid units represent the essential genetic bridge between generations. Description of the fundamental features of pollen morphology by early Botanists has demonstrated the potential value of palynology in phylogeny and plant taxonomy. In the present investigation the morphological features of pollen grains of *Chrozophora rottleri* in five different localities have been studied (Table- 1 and Figure 1-7). From the result it is evident that the length of pollen grains of *Chrozophora rottleri* varied slightly, ranging from 19 μ m to 25 μ m. The pollen grains of *Chrozophora rottleri* was larger in size from specimen collected from Matihani (25 μ m) followed by Barauni (24 μ m), Bacchwara (22 μ m), Begusarai (21 μ m) and Teghra (19 μ m) (Table-1). Similarly, the width of pollen grains collected from specimens of Matihani, Begusarai, Bacchwara, and Barauni and Teghra were 20 μ m, 19 μ m, 18 μ m and 17 μ m respectively (Table-1). In all specimens of *Chrozophora rottleri* the pollen aperture did not show great variation and was in the range of 0.5 μ m to 0.7 μ m. P/E ratio was 0.65 to 0.78. The pollen grains of *Chrozophora rottleri* were elliptical in shape in specimens of Begusarai, Barauni and Matihani, whereas rounded in specimens of Bacchwara and Teghra (Fig-1 to 6). In all the specimens of *Chrozophora rottleri* the pollen grains were tricolpate with pentoporate aperture, hexoporate in specimens of Teghra (Fig-4 and 5) and spinose exine. The spine was pointed in all specimens. The length of spines was more or less similar in all specimens ranging from 7.4 to 7.8 μ m. The size of pollen grains was larger in specimens of teghra and Matihani (85 μ m), followed by 75 μ m in specimens of Begusarai and Barauni and relatively small in specimens of Bacchwara (70 μ m). In specimens collected from teghra and Matihani the viability of pollen grains was maximum (87%), followed by Specimens of Bacchwara (76%) and low in specimens from Barauni (71%) and Begusarai (72%).

IV. Discussion

The family Euphorbiaceae is distinctly eurypalynous. The pollen grains are either colpate, colporate, porate or inaperturate. Sexine pattern also varies considerably. It is either tectate, semitectate, reticulate or microreticulate, and provided with spinules or a crotonoid-pattern. This diversity in pollen morphology clearly illustrates the heterogeneity of this family. The present investigation gains support from the work of Saad *et al.*, (1988) [29] who also studied the pollen morphology of some species of Euphorbiaceae. Aachal Tiwari *et al.*, (2014) [30] have studied the biology of pollen grains in some species of Euphorbiaceae and found a more or less similar pattern.

Chrozophora rottleri is monoecious and produces flowers on a raceme inflorescence which arises in the leaf axils of the terminal branches. The flower visitors are day-active and composed of flies, wasps, bees, and beetles. The beetles consistently stayed on the plant by moving to the undersurface of the leaves when not in feeding. While in their foraging activity, they moved between male and female flowers and walking over the receptive stigmas invariably to reach and male flowers and vice-versa. This resulted in the pollination of female flowers.

The male and female flowers of *Chrozophora rottleri* are spatially at inflorescence level but both anthesed about the same time during morning hours. The female flowers are compatible to xeno- and geitonogamous pollen. All these structural features of this plant favour beetle pollination. Beetle pollination or cantharophily is a feature of tropical environments [31] (Faegri and van der Pijl, 1979). This type of pollination suggests the characteristics of early angiosperms [32] (Gottberg, 1988).

Chrozophora rottleri, which belongs to the family Euphorbiaceae, is an annual herb. The phenological observations of this plant showed that the flowering season is from June to September. In the morphological studies, the measurements of the flower, seed and fruit, as well as the position of the leaves and other parts of the plant were noted. The average of these measurements and the standard deviations were calculated (Table- 2).

Chrozophora rottleri is an annual herb or under shrub, monoecious, prostrate or ascending, up to 60 cm in height. Indumentum consisting of very dense, sessile and peduncled stellate or lepidote hairs, next to simple hairs. Leaves alternate, 2-5 X 1-4 cm, rounded or obtuse at apex, rounded or subtruncate at base, entire or shallowly crenate-sinuate, 3-5-veined from base, somewhat bullate above when young, becoming less so with age, pubescent above, densely so beneath; petiole 1-5.5 cm long, densely stellate-pubescent; stipules 2 mm long, linear; lower surface of leaf bears two glands near the base. Inflorescences 1-5 cm long, terminal racemes (in fruit pseudo-lateral and opposite to leaf due to extension of axillary buds), solitary (or two together), not branching, basal flowers pistillate, apical ones staminate. Bracts very inconspicuous to conspicuous 1.6 by 0.3 mm, narrowly triangular, only hairy outside. Flowers actinomorphic, staminate flowers usually two per node. Staminate flowers 4-6 mm in diameter, yellow; calyx white, united c. 1 mm high, lobes 3.2-4 by c. 1.2 mm, sepals lanceolate, stellate-pubescent; androphore 3.3-3.8 mm long; anthers 0.9-1.3 by c. 0.7 mm, yellow. Pistillate flowers 3.2-3.3 mm in diameter, greenish to yellow; pedicel 1.4-2 mm long, elongating in fruit to up to 1.1 cm; calyx lobes only basally united, 1.5-2.2 by 0.5-0.7 mm; petals 1.3-2 by 0.4-0.6 mm; ovary ovoid, 2.7-3 by 2.2-3 mm wide; style 0.5-0.8 mm long, bifid, red, stigmas erect, up to 2.3 mm long, apically split for up to 1.8 mm, red. Ovary 2 mm diameter, densely stellate-pubescent; styles 1-1.5 mm long, bifid almost from base, placentation free central; Fruit 4 x 7 mm, rounded, 3-lobed, stellate-pubescent; seeds 3-3.5 x 2-2.5 mm, globose-ovoid, grey. Fruit slightly lobed capsules, triangular in transverse section, dehiscing usually septically and partly loculicidally into 3 bivalved parts, outside densely stellate, inside glabrous, thin walled; column slender, with frayed remnants of the septa, apically triangular, septa single veined. Seeds 3 per fruit, obovate, angular, covered by a thin, incomplete sarcotesta; the latter carunculate apically. Embryo flat; endosperm copious. The male flowers of *Chrozophora rottleri* contain 15 stamens arranged in two tiers, the lower tier with 10 and the upper one with 5. The pollen production per anther of the upper tier averaged 395 while that of lower tier averaged 505, and together, the total pollen production per flower averaged 7235 [33] (Subba Reddy *et al.*, 1998). The pollen grains are yellow, 57-68 μ m in size, and have reticulate exine with eight pores. The pollen-ovule ratio approximated to 2400:1. The present observations are in full conformity with Davis (1988) [34] and Suleyman Baslar (2000) [35] who have also measured a similar biometric values in *Chrozophora tinctoria*.

Anatomical observation: In the root cross-section of *Chrozophora rottleri*, it was observed that the epidermis had been destroyed by the excessive development of secondary tissues. The cortex consisted of sclerenchyma and parenchyma. Sclerenchyma cells were dispersed in the cortex. The next structures after the cortex were the phloem and xylem. The xylem covered a wide area. The endodermis and pericycle could not be seen clearly (Fig- 8). There was no pith in the centre of the root due to the excessive development of the xylem. These findings are in agreement with those of Metcalfe and Chalk (1957) [36], except that these authors did not report the sclerenchyma in the cortex.

The transverse section of stem anatomy of *Chrozophora rottleri* showed one layer of epidermis. Under the epidermis, there was a cortex consisting of collenchymas and parenchyma cells. It was observed that the collenchyma was interspersed with chlorenchyma at some points. The sclerenchyma groups which were among the cortex parenchyma cells surrounded the vascular system from the outside and they were not clearly separated from the central cylinder. It was observed that the area up to the vascular system showed the character of the primary stem structure. After this structure came the phloem. It was observed that a secondary structure had

developed in the xylem. There was a parenchymatous pith in the centre of the stem (Fig-9). These findings are in agreement with those of Metcalfe and Chalk (1957) [36]. However, these authors did not report chlorenchyma in the cortex.

The leaf anatomy of *C. rottleri* showed the cuticle and epidermis covered with stellate hairs; in addition, there was a single layer of palisade with small intercellular spaces which was rich in chlorophyll, and a narrow region of spongy parenchyma with wide intercellular spaces and well developed vascular bundle (Fig-10 and 13). These results are in agreement with those of Metcalfe and Chalk (1957) [36]. The leaves were bifacial and amphistomatous. Their stomata were of the amaryllis and parasitic type (Fig- 11 and 12).

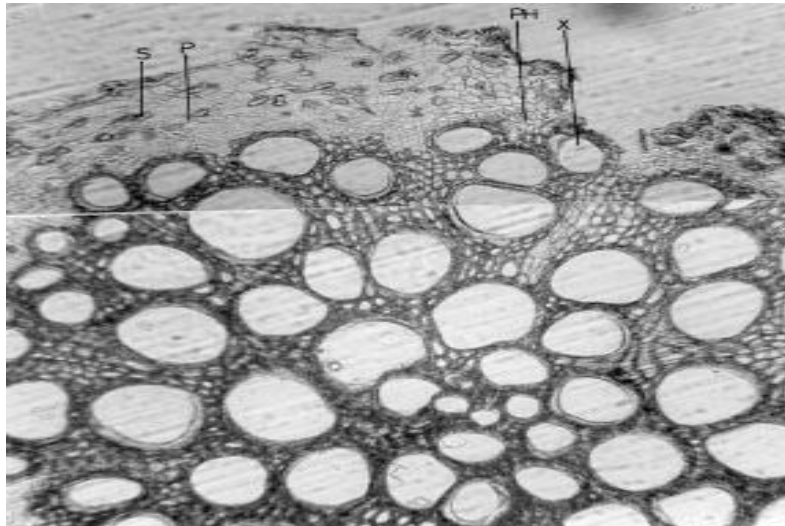


Figure- 8. The cross section of the root of *C. rotleri* (10x6.3). P, Parenchyma; S, sclerenchyma, PH, Phloem, X-Xylem.

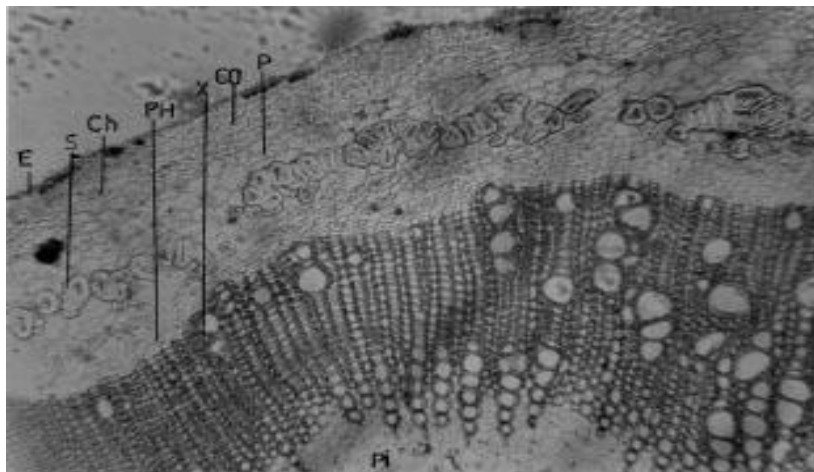


Figure-9: The cross section of the stem of *C. rotleri* (10x6.3) E, Epidermis; PH, Phloem; X,Xylem; CO, Collenchyma; S, Sclerenchyma; Ch, Chlorenchyma; P, Parenchyma; Pi, Parenchymatic Pith.

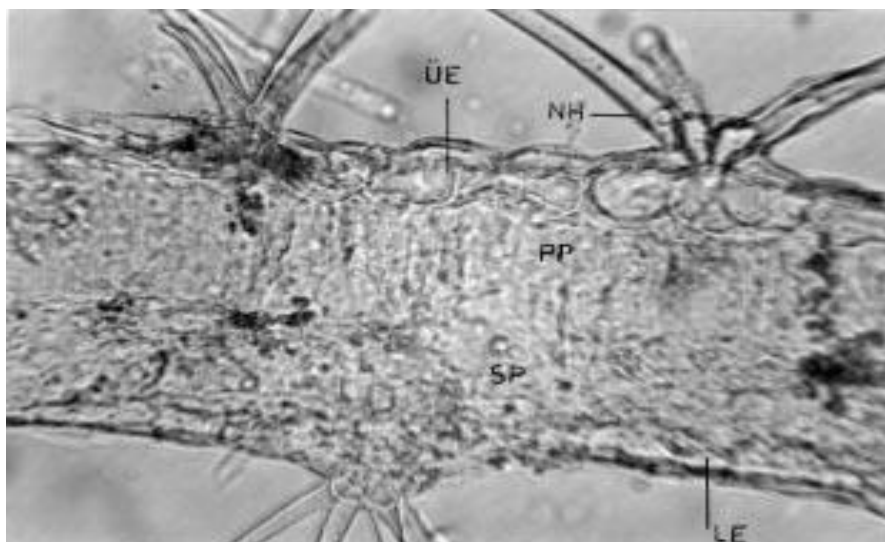


Figure-10: The cross section of the leaf of *C. rotleri* (40x6.3): NH-Nonglandular hair; Pp-Palisade parenchyma ; Sp, Spongy parenchyma ; EU-Upper Epidermis ; LE-Lower Epidermis.

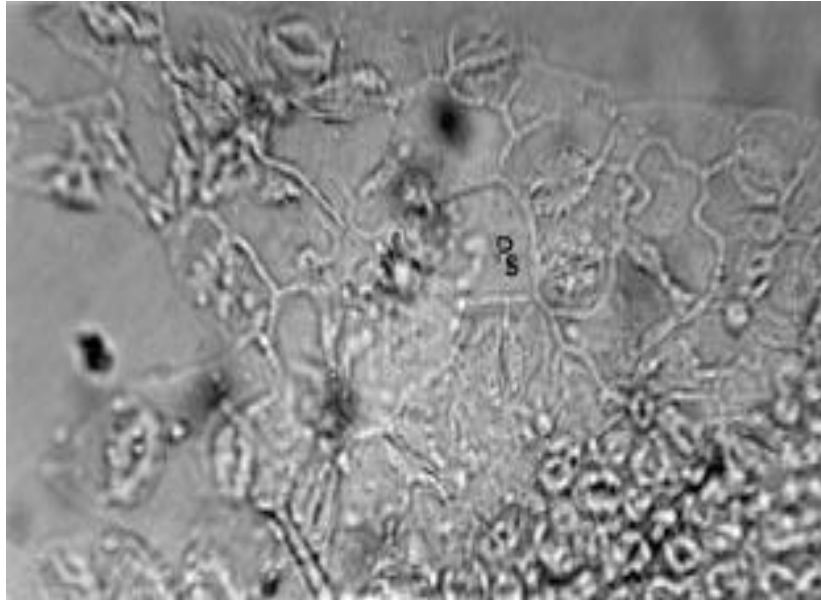


Figure-11: Upper epidermis with stomata in the transverse section of the leaf of *C. tinctoria* (40x6.3). Ps- Paracytic stomata.

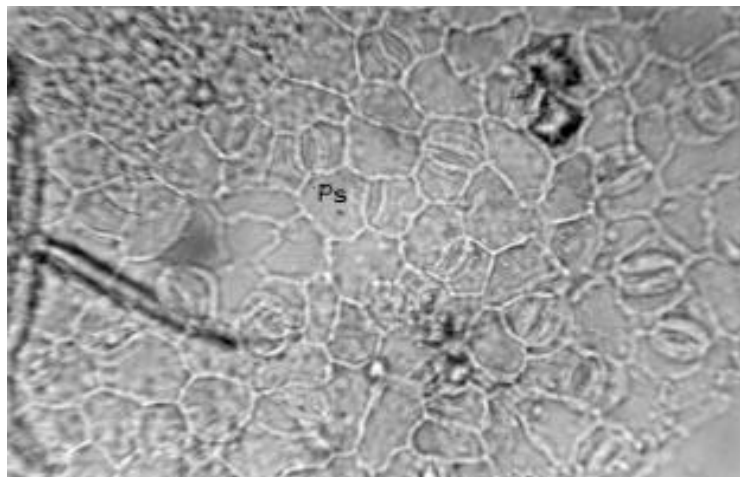


Figure-12: Lower epidermis with stomata in the transverse section of the leaf of *C. rotleri*(40x6.3). Ps- Paracytic stomata.

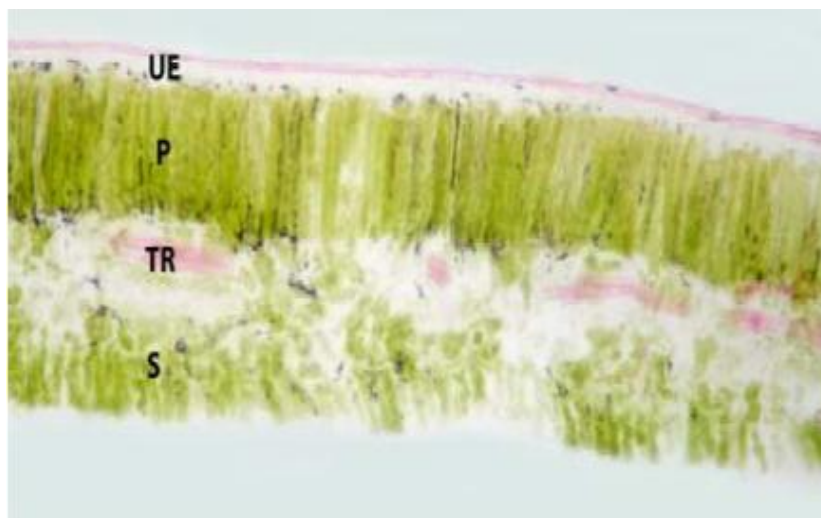


Figure-13: Cross section of leaf of *Chrozophora rotleri*, UE, Upper epidermis; P, Palisade parenchyma; TR, Transfusion tissue; S, Spongy parenchyma

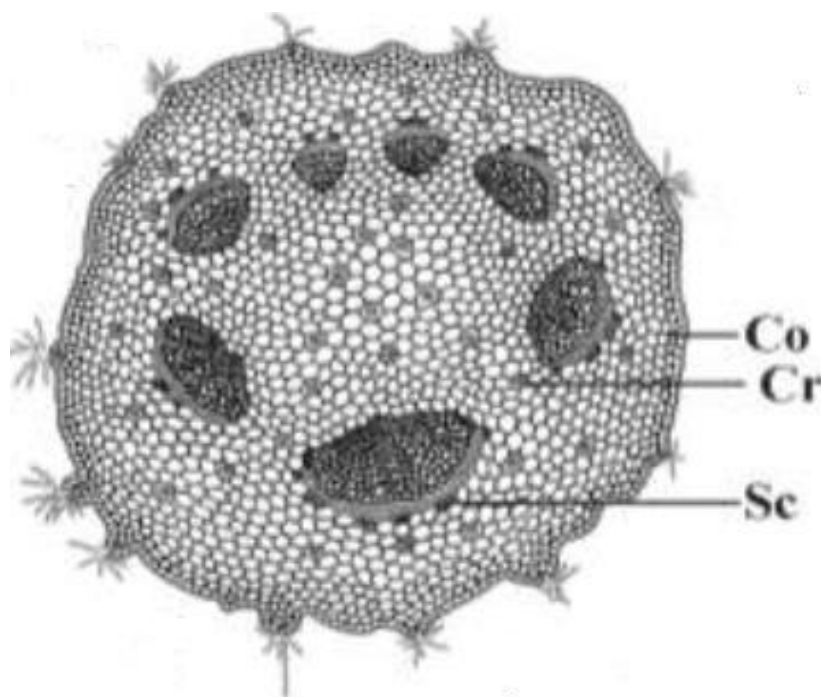


Figure- 14: T. S. of Petiole of *Chrozophora rottleri*. Co – Collenchyma, Cr – Crystals, Sc – Sclerenchyma

Transverse section of petiole of *Chrozophora rottleri* showed a few layers of collenchymas in the hypodermal region as continuous ring. The individual vascular bundles have phloic sclerenchymatous patches (Fig-14). The crystals, both solitary and/or clustered occurred.

All the plant samples (25) collected from five different localities of Begusarai district have simple leaves. The shape of the petiole/rachis as observed in transverse section is generally circular. The epidermis of rachis, petiole and petiolule has small cells with thick or thin cuticle layer. Epidermal cells are generally barrel shaped or rounded but in some cases they are squarish. Trichomes are variable. The present study exhibits an interesting variation in the organization of mechanical tissues in the petiole and rachis. A similarity in the internal structure of the petiole/petiolule and rachis is noted. The mechanical tissue is sclerenchymatous and collenchymatous. The xylem elements are additionally mechanical in function. The distribution of collenchyma is of much significance. In the petiole, few layers of collenchyma occurs in the hypodermal region as continuous ring. The petiole are adopted to support the weight of leaf lamina and therefore are inextensible and naturally possess the mechanical tissue at the central region.

The considerable variability in distribution of sclerenchyma is noticed in petiole/ rachis of studied taxa. Phloic sclerenchymatous patches on individual vascular bundles are observed in most of the plants studied. Apart from these, varying quantum of vascular tissue - xylem acts together with the sclerenchyma as a sort of central skeleton for the petiole/rachis. Comparatively higher amounts of vascular variability are noticed in the petiole. The vascular tissue is in the form of a ring of discrete bundles in the petiole.

Lignier (1887) [37] is of the opinion that the vascular arc increases in size within the petiole to develop folds, which separate towards inner and outer side to result in the medullary or cortical bundles. The present study reveals comparatively higher amount of vascular variability than that of other tissues. It is presumed that the structure of the rachis/petiole of different taxa is related to the mechanical requirement of the leaf/leaflets.

In the comprehensive survey of petiole anatomy, De Candolle (1879) [38] recognized a principle and accessory system, the latter composed of the cortical and or the medullary bundles. The principle system has open and closed types. According to Petit (1886, 1887) [39, 40] an open system is one in which vascular bundles are distinct or separated and closed system has fused bundles. Petit holds that herbaceous plants show distinct bundles and shrubby or woody taxa exhibit fused (closed) bundles to form an arc or ring. Further, he noted that perivascular or pericyclic sclerenchyma is lacking in herbaceous plants and occurs in woody taxa.

The pattern of vascular system along with the distribution of sclerenchyma, occurrence of collenchyma in some species and the shapes in the transverse section of the rachis, petiole and petiolule can be used as adjuncts for the delineation of the taxa studied. Howards (1962) [41] upheld the importance of petiolar vasculature as an aid to horticultural taxonomists. The study on petiole structure emphasizes its utility in Meliaceae [42] (Bhadane, 2006). The present observations are also in agreement with the work of Sarala and Bhadane (2014) [43] and Elumalai *et al.*, (2014) [44] who have studied the taxonomic significance of rachis

petiole and petiolule anatomy in some plants of Euphorbiaceae and found a more or less similar pattern in case of foliar anatomy of *Chrozophora rotleri*.

V. Conclusions

In the present investigation anatomy of root, stem and foliar parts of about twenty plants of *Chrozophora rotleri* was studied. The results revealed the following characteristics that suggested polyphyletic origin and evolution of taxa of Euphorbiaceae.

1. Presence of thick cuticle was observed in all the specimens of stem and foliar parts of *Chrozophora rotleri*.
2. In upper epidermis of leaf, single row of epidermal cells (parenchyma) occurred in all the specimens.
3. The mesophyll tissues were differentiated into palisade and spongy parenchyma in all specimens.
4. The occurrence of palisade parenchyma on both sides of lamina surfaces (upper and lower side) and spongy parenchyma and vascular bundles occur in between them. Hence, *Chrozophora rotleri* is considered as unique and specific species with regard to the distribution of mesophyll tissue.
5. The vascular bundles in the mid-vein, lateral veins in the mesophyll tissues and in the laminar surfaces were uniform throughout the specimens under study.
6. The latex, laticiferous tissues, latex vessels, and resinous ducts were observed in all the specimens.

Thus the present study is an attempt to investigate the anatomical behavior of *Chrozophora rotleri* which forms an attainment towards advanced knowledge.

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