

Asexual Morphological Differences in Male and Female Plants of *Commiphora wightii* (Arn.) Bhandari - An Endangered Medicinal Plant

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Abstract Studies were carried out on growth characteristics, stem branching pattern and leaf morphology to reveal the significant differences in male and female plants and development of reliable morphological markers to identify gender even in non-flowering phase. Female plants have spreading growth and weeping canopy whereas male plants have erected growth and divergent canopy. Female plants have significantly higher height ($p = 0.06$) and collar diameter ($p = 0.03$) than males. Similarly, female plants also have higher number of secondary and tertiary branches with wider branch angles leading to the differences in canopy shape and size between two genders. Other morphological differences observed in female and male plants are in leaf characters viz. leaf area, leaf margin and number. Male plants were only having higher stomatal density ($p = 0.03$) on both the sides of leaves as compared to females. Guggulsterone-Z was detected only in aerial branches of female plants (0.014-0.029%) and in males it was below detectable limits.

Keywords: apomixis, branch angle, Burseraceae, endangered, guggul, sexual dimorphism, stomatal density

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1. Introduction

Commiphora wightii (Arnott) is one of the important traditional and endangered medicinal plants of semi arid region of India [1]. It belongs to family Burseraceae. *C. wightii* is also known as 'guggal' or 'guggulu' in hindi and Indian bdellium tree in English ([2,3]). It is much-branched perennial shrub/small medium-sized tree, reaching to a height of 3-4 m [4]. It is distributed mainly in the arid regions of Rajasthan, Gujarat, Madhya Pradesh, Karnataka and Orissa states of India and also in the adjoining areas of Pakistan [5].

It is basically used for its oleo-gum resin commonly known as guggul gum which has been a key component in ancient Indian ayurvedic system of medicine [6]. This is a mixture of many important secondary metabolites mainly E and Z-guggulsterones, which are of potent medicinal uses mainly in arthritis, cold, bronchitis, laryngitis, obesity, heart disease, lipid disorders etc. [7,8]. Encroachment, unawareness of rural people, destructive harvesting methods, unsustainable overexploitation and lack of management cultivation practices led to decrease in its population [9,10,11], as a result it has become threatened species [12,13]. Thus the gap between supply and demand of guggul gum is increasing rapidly. According to the assessment reported by Ayurvedic Drug Manufacturing Association (ADMA), less than 10% of requirement of guggul gum is currently being met from indigenous

sources and remaining 90% of this material being obtained through imports [14].

It is a polymorphic flowering plant having male, female and andromonoecious type of plants [15]. Flowering occurs twice in a year i.e. in April/May and October/November, rest of the year the plant remains in vegetative phase. Male and andromonoecious plants are extremely rare in natural population and plantations [16,17]. In absence or scarcity of male plants, it evades the process of fertilization and adopted apomixis for its survival [16]. This situation may be leading the production of genetically identical plants and loss of genetic variation which may lead to genetic erosion of the species. Further the breeding programmes and production of pure line and homozygous plants would also get hampered in absence of male plants. Thus, there is a need to identify extremely rare male plants in natural habitats and conserve them for future breeding programmes. Male and female plants can be easily identified on the basis of flowers but difficult to identify them during non flowering season.

RAPD molecular markers have been identified to differentiate male and female plants [18] but it is very expensive, time consuming and not very useful to a field researcher. At the same time RAPD markers are also not highly reliable. Therefore, present studies were conducted with the aim to analyze mostly morphological, few microscopic and Z- guggulsterone differences between female and male plants. However, the main aim was to develop morphological markers and distinguish male and female plants during non flowering phase.

2. Materials and Methods

Germplasm bank of *C. wightii* was established from 25 different districts of Rajasthan in 2007. In this germplasm, two male plants were identified during flowering time. Some distinct morphological differences in male and female plants were also noticed in their form. Present studies were carried out on 6-year-old two male (M1 and M2) and three female (F1, F2 and F3) genotypes of *Commiphora wightii* growing in this germplasm. Initially these plants were irrigated for 1st & 2nd year and later these plants were kept under natural conditions at the site. Observations were taken in the mid of June 2013. Minimum average temperature was 29.8°C, maximum average temperature was 41.5°C, relative humidity I was 61% and relative humidity II was 32%. The annual rainfall for that year was 575 mm.

2.1. Morphological Parameters

Thirteen qualitative and quantitative morphological parameters under three categories i.e. growth characteristics, leaf morphology and branching pattern of the plant were studied. Growth habit, canopy, plant shape, stem bark color, leaf shape and margin were qualitative and height, crown, number of branches, branch angles, number of leaves, leaf length and leaf area were quantitative parameters.

(A) Growth characteristics: Plant shape, height, canopy, growth habit, and crown size were observed as growth characteristics. The overall shape, growth and canopy of the plant were evaluated visually on the basis of standard terminology used in taxonomy. Plant height was measured in cm from ground adjacent to the stem to the tip of leading branch of plant using graduated pole. Average crown spread was measured in cm using cross method of two longest spread at right angle and average of these two readings was considered as crown diameter [19].

(B) Branching pattern: Number of branches and branching angles were measured to study branching pattern. The numbers of branches of each genotype were counted at three different levels viz. primary, secondary and tertiary. The branching angles were evaluated using divider and compass at all the three different orders. First order (I) is the branching angle between primary and secondary branches, II order is the branching angle between secondary and tertiary branches and III order is the branching angle between tertiary and quaternary branches. Stem bark color was assessed visually of the same diameter stem, from almost similar location of every plant for the comparison.

(C) Leaf morphology- Data were recorded on shape, margin, size, number and area of leaf. To evaluate leaf shape and margins, ten fully expanded matured leaves from each genotype were taken and assessed visually to evaluate leaf shape. Leaf length of ten fully mature leaves from each genotype was measured using measurement scale. To count the number of leaves three apical twigs (15 cm long) were collected from each plant and leaves on each twig were counted excluding the small leaves at the top of the twig. Leaf area was also calculated using graph paper method [20].

2.2. Stomatal Studies

Microscopic studies were also conducted on stomatal density, stomatal index and stomata types. Fresh mature leaves of all the genotypes were collected, washed and dried before removing epidermal layers. To peel the epidermal layer method was adopted from TIEE [21]. Counting of stomata was performed on both adaxial and abaxial epidermal layer of each genotype in 3 different microscopic fields. An average no. of stomata and epidermal cells per microscopic field (400X magnification) were calculated and stomatal index was calculated using the equation of Salisbury [22].

$$\text{Stomatal index} = \frac{\text{no. of stomata} \times 100}{\text{no. of stomata} + \text{no. of epidermal cell}}$$

Stomatal density was also determined by calculating number of stomata per mm². The number of stomata present in a particular area of a grid was counted using motic image analyser and then it was divided by the grid area [23].

$$\text{Stomatal density} = \frac{\text{no. of stomata in a grid area}}{\text{grid area}}$$

2.3. Estimation of Z- Guggulsterone

Z- guggulsterone from aerial branches of two male and three female plant samples was estimated using a non- destructive procedure described by Agarwal *et al.* [24]. 50 gm powder of aerial branches from each sample were taken to perform High performance liquid chromatography using standard reference (M/s Natural Remedies, Bangalore, India) at 242 nm wavelength. The mobile phase used was 0.1% acetonitrile and formic acid/H₂O (60:40) and flow rate was adjusted at 1.0 ml/min. The column was used of RP-18 (250mmX4.6mm.5µm, Merck). The peak of standard Z- guggulsterone was observed and then readings of the samples were compared to estimate the Z guggulsterone concentration of samples. Z-guggulsterone was estimated in the laboratory of DMAPR, Anand.

3. Results

Significant differences were observed in different morphological parameters assessed in male and female plants, which are described category wise in following paragraphs.

3.1. Morphological Differences

(A) Growth parameters: In general, it was observed that male plants were having poor growth as compared to female. Male plants had erect growth habit with divergent type canopy (Figure 1B and Figure 2) i.e. branches were upwards and spreading little away from each other whereas in females had much more spreading branches leading to weeping type canopy (Figure 1A and Figure 2). Therefore, the male plants were having more symmetrical shape whereas female plants were less symmetrical. It was observed that the height and crown size were less in male

plants as compared to females (Table 1). Statistical test performed on various quantitative parameters indicate that crown size of the plant was significantly different between male and female plants whereas height of the plants was

found to be non significant at 5% level of chi square test (Table 1A). Quantitative measurements on height and crown diameter clearly indicate superiority of female plants over male plants.



Figure 1. Canopy of female and male *C. wightii* plants. A; Weeping type of canopy in female plant, B; Divergent type of canopy in male plant

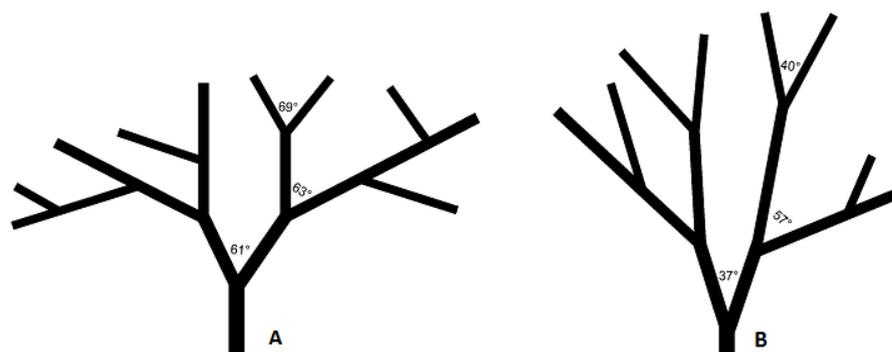


Figure 2. Pictorial representation of female (A) and male plant (B) on the basis of branch angles

(B) Branching pattern: The number of branches (3-8) at primary level was almost same in both type of plants but secondary and tertiary branches were significantly higher in females (unpaired student t-test $p = 0.041$ and $p = 0.043$, respectively) as compared to males. The branching angle between primary and secondary branches (I order; [Figure 3A](#) and [Figure 3B](#)) and between tertiary and quaternary branches (III order; [Figure 3E](#) and [Figure 3F](#)) was also more in females as compared to males (Table 1). However, there was insignificant difference in male and female plants for

branch angle between secondary and tertiary branches (II order; [Figure 3C](#) and [Figure 3D](#)) both type of plants were having knotty and crooked branches. Paired T test on branch angles in male and female plants revealed that the angle of branching at I order and III order varies significantly ($P = 0.00, 0.01$) whereas differences in II order branching angle was found to be insignificant (Table 1B). The stem bark of female was brown tinged with green colour and peels more frequently than that of male which is green tinged with brown colour ([Figure 4A](#)).

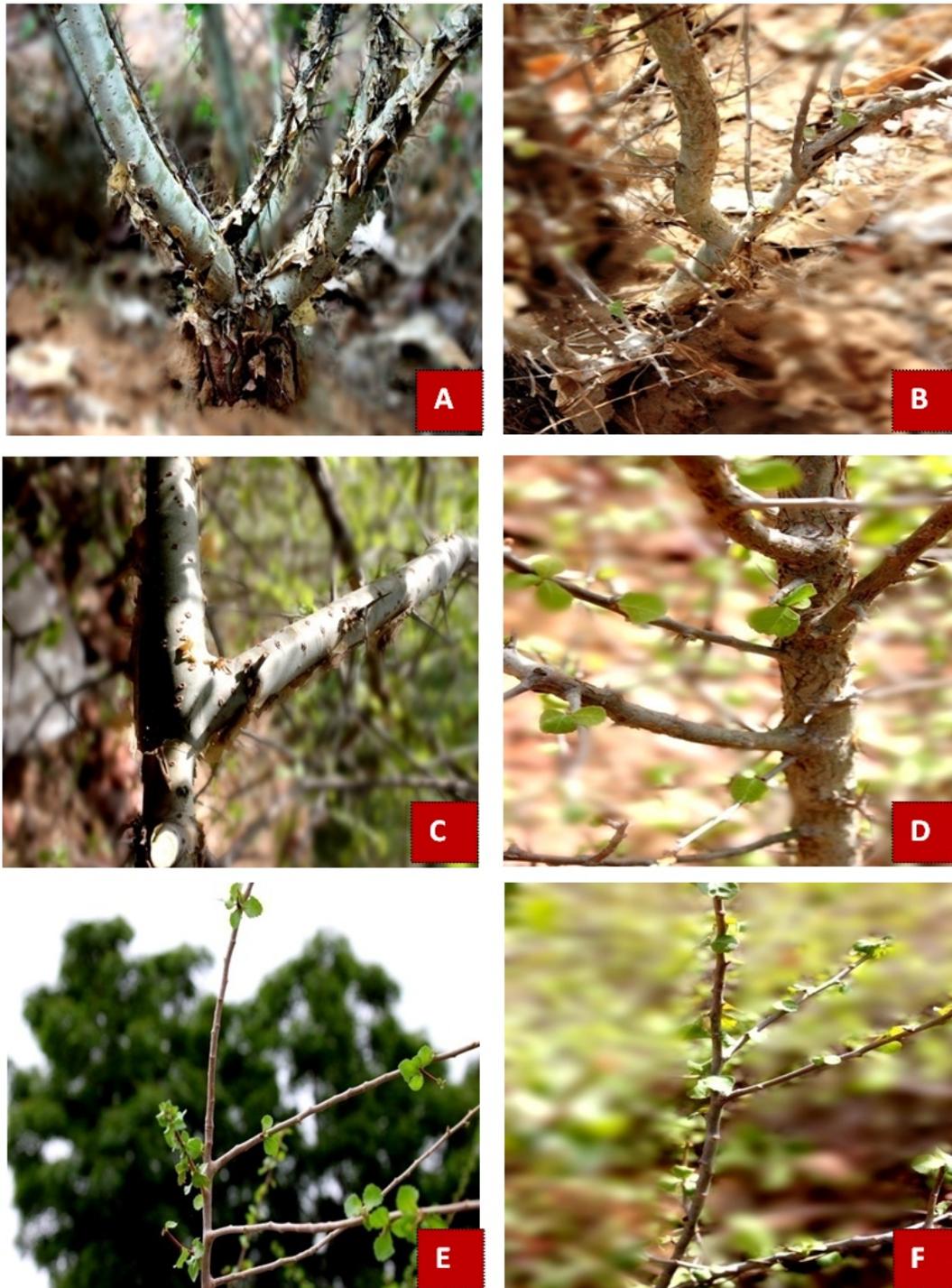


Figure 3. Branching angles of female and male *C. wightii* plants. A, C & E; I, II and III order branching angles of female plant, B, D & F; I, II and III order branching angles of male plant

(C) Leaf morphology: The leaf shape was obovate in males and ovate in female plants (Figure 4B). The margins of male leaf were of crenate type i.e. the edge of leaf had blunt, rounded teeth and dentate type in females i.e. the

edge of leaf had pointed teeth (Figure 4B). Both numbers and size of leaves were significantly greater ($p = 0.0$; Table 1B) in female than in male plants (Figure 4B and Figure 4C).

Table 1. Morphological parameters of male and female plants of *Commiphora wightii*

Parameters/Characters	MALE			FEMALE			
	(M1)	(M2)	(F1)	(F2)	(F3)		
Growth habit	Erect	Erect	Spreading	Spreading	Spreading		
Canopy	Divergent	Divergent	Weeping	Weeping	Weeping		
Plant shape	Wedge, symmetrical	Wedge, symmetrical	Wedge, round, less symmetrical	Wedge, round, less symmetrical	Wedge, round, non symmetrical		
Stem bark color	Brownish green	Brownish green	Greenish brown	Greenish brown	Greenish brown		
Leaf shape	Obovate	Obovate	Ovate	Ovate	Ovate		
Leaf margin	Crenate	Crenate	Dentate	Dentate	Dentate		
Plant height (cm)	175	162	211	185	203		
Crown size (cm)	115	100	170	140	160		
Quantitative parameters	(P)*	05	08	08	03	08	
	No. of branches (S)*	06	09	46	24	40	
	(T)*	150	180	1200	600	1000	
	Branching angle (Mean \pm SD)	I order	38.3 \pm 3.5	35.8 \pm 4.3	58.0 \pm 24	58.7 \pm 17	65.5 \pm 17
		II order	57 \pm 24.3	57.3 \pm 11.7	68 \pm 12.4	61.8 \pm 11.6	58 \pm 19.6
		III order	39.5 \pm 4.0	41.0 \pm 2.2	66.0 \pm 4.3	68.8 \pm 3.3	69.3 \pm 11.0
	No. of leaves (mean \pm SD)	14 \pm 2.0	15 \pm 1.5	26 \pm 4.0	21 \pm 1.5	24 \pm 2.0	
	Leaf length (cm)	2.4 \pm 0.0	2.4 \pm 0.2	3.9 \pm 0.2	3.6 \pm 0.4	3.6 \pm 0.5	
	Leaf area (cm ²) (mean \pm SD)	4.2 \pm 0.2	4.0 \pm 0.5	7.2 \pm 0.7	6.2 \pm 0.7	7.5 \pm 0.5	

Table 1A. Chi square test

S. No.	Parameters	T value	Df	P value
1.	Height	-2.82	3	0.06
2.	Crown	-3.87	3	0.03

Table 1B. T test

S.No.	Parameters	T value	df	P value	
1.	Number of Branches	-1.94	13	0.07	
	I order	-3.73	18	0.00	
2.	Branching angle	II order	-0.76	18	0.45
		III order	6.35	7	0.00
3.	Number of leaves	-5.41	13	0.00	
4.	Leaf size	-7.96	13	0.00	
5.	Leaf area	-7.71	13	0.00	

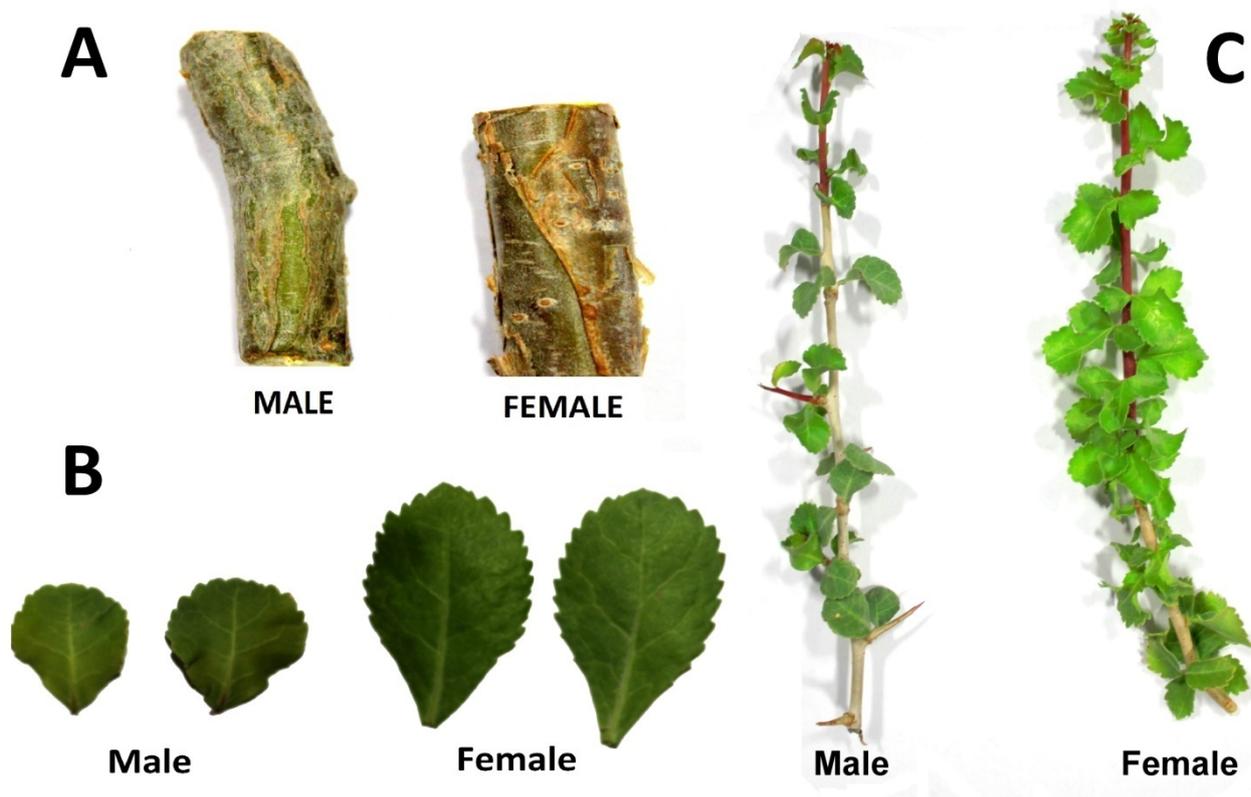


Figure 4. Morphological differences in male and female *C. wightii* plant. A: Stem bark colour; B: Leaf margin, shape and size; C: Number of leaves

3.2. Stomatal Studies

On the contrary of all quantitative characters studied stomatal density was significantly higher ($p = 0.03$; Table 1A) on both the sides of leaves in males (Figure 5A) as compared to female plants (Figure 5B). Whereas, there

was no significant difference ($p = 0.27$) in stomatal index between male and female plants (Table 2A). Stomata were of anomocytic type (irregularly arranged) and were hypoamphistomatic types (having more stomata on lower epidermis as compared to upper; Figure 5A and Figure 5B).

Table 2. Leaf stomatal index and stomatal density of male and female plants of *C. wightii*

S. No.	Parameters/ Characters	MALE			FEMALE		
		(M1)	(M2)	(F1)	(F2)	(F3)	
1.	Stomatal density (/mm)	(Ad)*	310 ± 11.6	308 ± 15.0	282 ± 15.0	275 ± 19.1	280 ± 24.2
		(Ab)*	338 ± 27.3	350 ± 19.3	300 ± 15	305 ± 36.8	288 ± 37.7
2.	Stomatal index	(Ad)*	12.4	11.7	11.3	12.0	11.1
		(Ab)*	13.0	12.6	11.8	12.5	10.0

* Ad= Adaxial, Ab= Abaxial

Table 2A. T test

S. No.	Parameters	T value	df	P value	
1.	Stomatal density	Ad*	3.59	13	0.03
		Ab*	3.34	13	0.05
2.	Stomatal index	Ad*	1.35	3	0.27
		Ab*	1.42	3	0.25

* Ad= Adaxial, Ab= Abaxial.

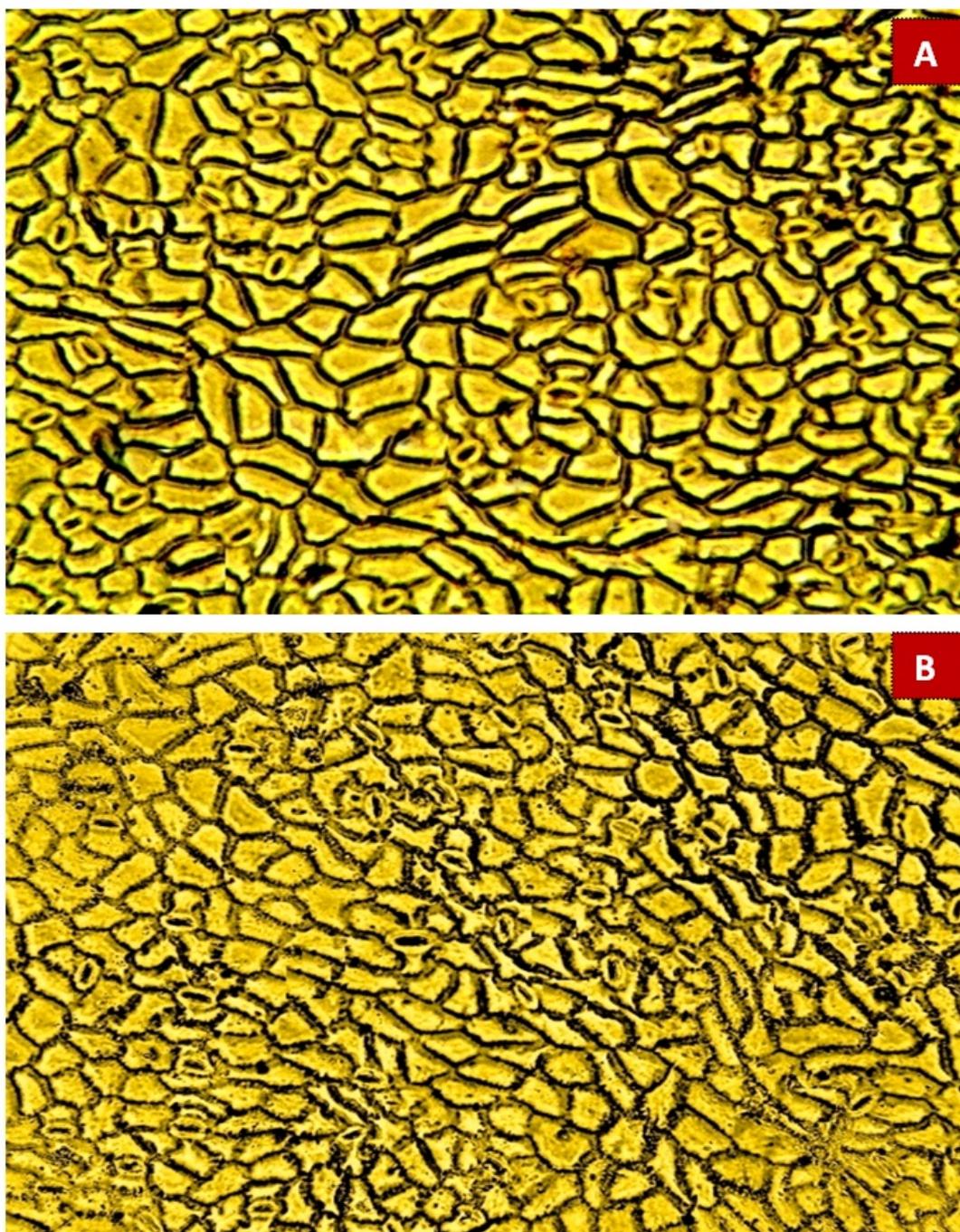


Figure 5. Stomatal density of male (A) and female *C. wightii* plant (B).

3.3. Z- guggulsterone

Z- guggulsterone was also estimated in male and female plants from aerial branches using non destructive method which revealed a difference among three female plants (140.96, 139.93 and 289.17 $\mu\text{g/g}$) whereas it was below detectable limits in male plants.

4. Discussion

Differences in morphology and growth characteristics have been documented between male and female plants of some species by many researchers [25,26,27]. In some plants like *Juniperus virginiana*, *Acer negundo* and *Lindera benzoin* females were smaller and slow growing

than male plants [28,29,30,31]. *Commiphora wightii* resembles later case of *Ailanthus excelsa* and *Mercurialis annua* where female plants have higher biomass and better growth characteristics as compared to males [32,33].

Differences in growth characteristics of *C. wightii* may be due to the reason that female plants may or may not be produced through sexual reproduction, but male plants can only be produced through sexual seeds. Hence, it is assumed that sexually produced male plants may be less adapted to environment as compared to apomictic female plants in *C. wightii*. The same is also reported in past by Bayer *et al.* [34] who demonstrated that sexual progenies have lower fitness as compared to apomictic. The mechanism of resources allocation is also different in male and female plants. Generally female plants allocate more nutrients for reproduction but apomixis mode of

reproduction requires less energy for seed production resulting in transfer of the supplements to increase the growth of the plants. Whereas male plants investing proportionately more in root growth, presumably to provide nitrogen for pollen production. Thus, they are not having superiority in above ground portion of plants. This hypothesis appears true in cases where male plants have poor growth like *C. wightii*.

Higher numbers of branches and leaves in female plants as compared to male can be interpreted as a sign of higher adaptability to the extreme arid condition of India [35]. The variations in leaf morphology, which may be due to differences in reproductive function of female plants for which they allocate more nutrients and carbohydrates for a longer period than males. Therefore, the optimal leaf size and longevity may be greater in females than males [36]. These variations in leaf size of male and female genotypes of *C. wightii* may be used for sex identification, as in litchi [37].

Stomatal density plays a key role in determining water use efficiency, Since, it controls leaf conductance, which is crucial for both photosynthesis and transpiration [38]. The present results are of great importance as the stomatal density of male plants is significantly greater than that of female plants which may lead to more evapo-transpiration in water deficiency which may be a possible reason for depletion of its male population. The stomatal index is fairly constant for leaves of a particular species [39] However, some researchers also reported that stomatal density was positively correlated with the stomatal index [40].

Previously Sinha *et al.* [35] also studied growth performance and morphological parameters in five female provenances of guggul. They revealed a significant amount of variation between provenances but not within provenances which is a clear identification of ecological adaptation of provenances. The present results include female plants from only one provenance so we did not observe much difference among female genotypes. Since, they observed a significant amount of differences among female genotypes for parameters like plant height, shape, growth habit and stem bark colour, these parameters were not considered as strong morphological parameters to differentiate male and female genotypes.

Z-guggulsterone screening also showed variation and it was very less (below detectable limit) in male plants as compared to females. The previous work done for the estimation of Z-guggulsterone from stem bark based on sex of *C. wightii* plant [41] also observed similar results. Secondary metabolites play a major role in the adaptation of plants to their environment in stress conditions [42,43]. In *C. wightii* male plants were less in number [16] and poor growth also indicated that probably the males were less adapted to stressful environment as compared to female plants.

Over all male plants were weaker in their growth as compared to females. Out of thirteen different qualitative and quantitative morphological characters, branch angles and tree form were the stronger markers for field level workers to identify extremely rare male plants even during vegetative phase also. If the rare male plants in the natural population of *C. wightii* can be identified using some simple descriptors provides, it can provide great

opportunities for *in situ* and *ex situ* conservation and integration in future breeding programmes. On the basis of these morphological characters we have identified ten new male plants at Deesa trials.

These types of studies will also help in understanding phenomenon of sex differentiation which is largely considered as an adaptation for out-breeding and also prove useful in judicious bio-resource utilization [44]. Thus, the gender-related bio-chemo diversity within a species is of considerable importance depending upon its uses.

Compting Interests (If Any)

The author(s) declare(s) that there is no conflict of interest

Authors' Contributions

This work was carried out in collaboration between all authors. Author UKT designed the study, performed the statistical analysis, wrote the protocol, and author AG wrote the first draft of the manuscript. Authors HS and AG managed the field study. All authors read and approved the final manuscript.

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