Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2011 Vol. 1 (1) Jan – Mar, 50-58/Negi et al.

Genetic Diversity and Variations in the Endangered Tree (*Tecomella undulata*) in Rajasthan

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ABSTRACT

Taxonomical and ecological study was carried out to find out diversity at intra-specific level in *Tecomella undulata* (Sm.) Seem throughout the state of Rajasthan. Three possible morphotypes with yellow, red and intermediate coloured (yellowish orange to dark reddish orange) petals were identified. This is accompanied by variations in colour of sepals and seeds. The abundance of the three morphotype varied with the orange coloured one showing a density of 15 plants per hectare and those of yellow and red ones hardly exceeding 1-3 plants per hectare. It was observed that orange and red flowered morphotype flower 15-20 days earlier than the yellow flowered ones. Analysis of total proteins from seeds when subjected to SDS PAGE revealed distinct and consistent variations in banding patterns among the three morphotypes. These distinct characters suggest existence of atleast two morphotypes in *Tecomella undulata* growing in Rajasthan –one with yellow flowers and the other with red flowers, the third morphotype with intermediate flower colour might be an intraspecific hybrid of the two morphotypes.

Key Words: *Tecomella undulata*, Biodiversity, Morpho-taxonomical, Proteins, Morphotypes.

INTRODUCTION

Diversity in any plant species enables it to adapt to future environmental changes that arise due to pollution, climatic change, disease and other forms of environmental adversities. Overexploitation of plants for agriculture and other commercial, scientific and educational purposes and the other developmental activities of mankind have resulted in serious threat to plant species that have evolved over the course of millions of years (Khan 1998).

Tecomella undulata (Sm.) Seem is a medicinally and economically important plant that originated in India, Arabia (Randhawa and Mukhopadhyay, 1986), appears in the list of endangered plants of Rajasthan (Bhau *et al.*, 2007). The plant grows under natural conditions in wild, unprotected and is highly exploited. It is very hardy and drought resistant plant and has potential application for afforestation and landscaping of dry tracts. The plant is used for its high quality timber. Its wood is soft, durable and takes a good polish and therefore is used in furniture, carving, and agricultural implements. The wood is prized equal to Teak and that's why it is also called as '*Marwar* Teak of Rajasthan'. The plant parts are used for the cure of syphilis and eczema. Bark posses' mild, relaxant, cardio tonic and chloretic activities. Hot water extract of the plant is used to treat enlarged spleen, gonorrhea, leucoderma and liver diseases (Anonymous 1986, Saxena 2000). Due to its economic importance vis-à-vis endangered status it was thought to carry out its extensive taxonomical (both morphological and biochemical) and ecological study in Rajasthan.

MATERIALS AND METHODS

Field survey was carried out throughout Rajasthan to study the distribution pattern of *Tecomella undulata*. The districts (and the sites) where *T. undulata* plants were located include – Jaipur (Rajasthan University campus, Amrita Devi Udyan, World arboretum, Jhalana forest, Queens road and Rajasthan University reserve forest), Jodhpur (Basni, Central Arid Zone Research Institute campus and Botanical Survey of India campus), Bikaner (Mukam, Nokha, Anajmandi), Jaisalmer (Gadisar), Barmer (Kawas), Sikar (Bawari, Bhopatpura), Nagaur (Tarnow), Jhunjhunu (Birmi), Churu (Natha forest, Salasar) and Ganganagar (Khalsa College campus) (Fig. 1).

Field study was carried out round the year continuously for three years (during 1999 to 2002). Plant density per hectare was observed. Various qualitative and quantitative characters like colour, shape, size of leaf and flower parts of the plant; plant height and number per hectare etc., were critically noted (Table 1, 2). Fifty readings of every morphological character from each population were taken from randomly selected three to four plants (where the plant number was more than two) and mean value was calculated. Herbarium sheets were prepared and deposited in the herbarium of Botany Department, University of Rajasthan, Jaipur and herbarium of NBPGR (National Bureau for Plant Genetic Resource), New Delhi. Three morphotypes of *Tecomella* were also compared using SDS-PAGE of total soluble protein extracted from seeds of all the three morphotypes growing in same environmental condition that

is desert habitat. Protein extraction was made following Mondal *et al.,* (1997). Protein samples were prestained with 0.1% coomassie brilliant blue and subjected to SDS – PAGE following Becker *et al.,* (1990). After electrophoresis gel was stained with coomassie brilliant blue for 24 hours and then destained with methanol: acetic acid: water (4:1:5) mixture.

Seeds collected during April to June were stored in normal room temperature. They were tested for germination in the month of September. Seeds were soaked in distilled water for 4 hours and then spreaded over the moist blotting papers in the petriplates for germination. Average percentage of seed germination was observed after two days of seed wetting.

RESULTS

Tecomella undulata is a medium sized tree with height ranging from 8m to 15m. In India it is mostly distributed in the desert of western Rajasthan. Taxo-morphological characters showed variations existing among and within the three morphotypes viz; yellow, orange and red flowered plants of *Tecomella* and are summarized in Table 1and 2 and well supported by the photographs (Figs. 2-4).

External Morphology and Habit

Three possible morphotypes were identified based on colour of the corolla viz., yellow, orange and red (Figs. 2A-E). In the Nagaur district yellow plants were also found bearing yellow corolla with a tinge or spot of reddish orange colour (Fig. 2F). Orange morphotype was most uniformly distributed followed by the yellow and red flowered morphotypes respectively. Maximum and minimum density (per hectare) in orange morphotype was found in Barmer (8.5) and Jaisalmer (1.0) districts respectively. Maximum number of yellow morphotype (2.5 plant/hectare) was found in Barmer district whereas minimum (1.0 plant /hectare) in Ganganagar, Jaipur and Sikar districts (Table 1, 2).

Fig. 1: Map of Rajasthan showing the districts visited to carry out the study

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Only one to two young plantlets were seldom found growing in the field. The mature plants were generally more than 10-20 years of age. However in laboratory upto 80% of seed germination without any hormonal or chemical treatment was noticed (Figs. 3A-C). About 10 plants of all the three morphotypes have been successfully propagated and are growing in the botanic garden of the University of Rajasthan, Jaipur since last 10 years.

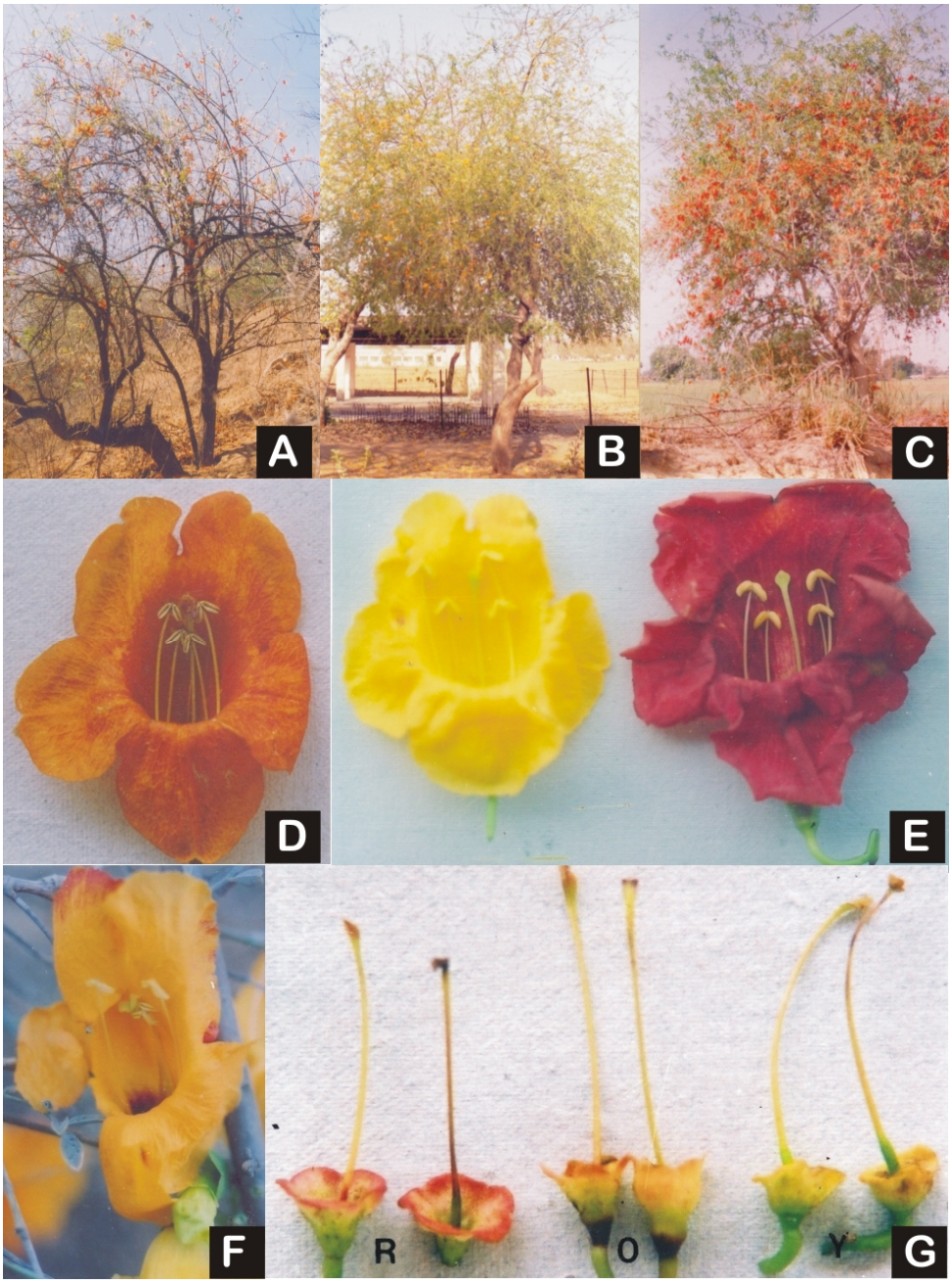

Fig. 2: Diversity in *Tecomella undulata* A – Orange morphotype showing profuse branching**; B** – Yellow morphotype; **C** – Red morphotype; **D** – Orange flower; **E** – Yellow and Red Flowers from respective morphotypes; **F** – Yellow flower with reddish orange spots on the corolla lobes; **G** – Flowers from different morphotypes (with corolla and androecium removed) to expose various coloured floral regions (***Abbrv.*** O: Orange morphotype, R: Red morphotype, Y: Yellow morphotype).

All the three morphotypes are successfully flowering since last three years. All the plants are bearing the respective colour which their maternal plants were bearing. The main trunk of the plant branches very early, at lower level of the stem, (Figs. 2A-C, Fig. 4) in comparision to other timber yielding plants such as *Acacia nilotica*, *A. tortalis* and *Dalbergia sissoo* commonly grown in the arid region of Rajasthan. This makes most of its trunk part unsuitable for timber production if the branches are left unpruned. Young plantlets growing in wild or unprotected areas are usually grazed upon by cattle, goats and camels which results in bushy habit of the plant.

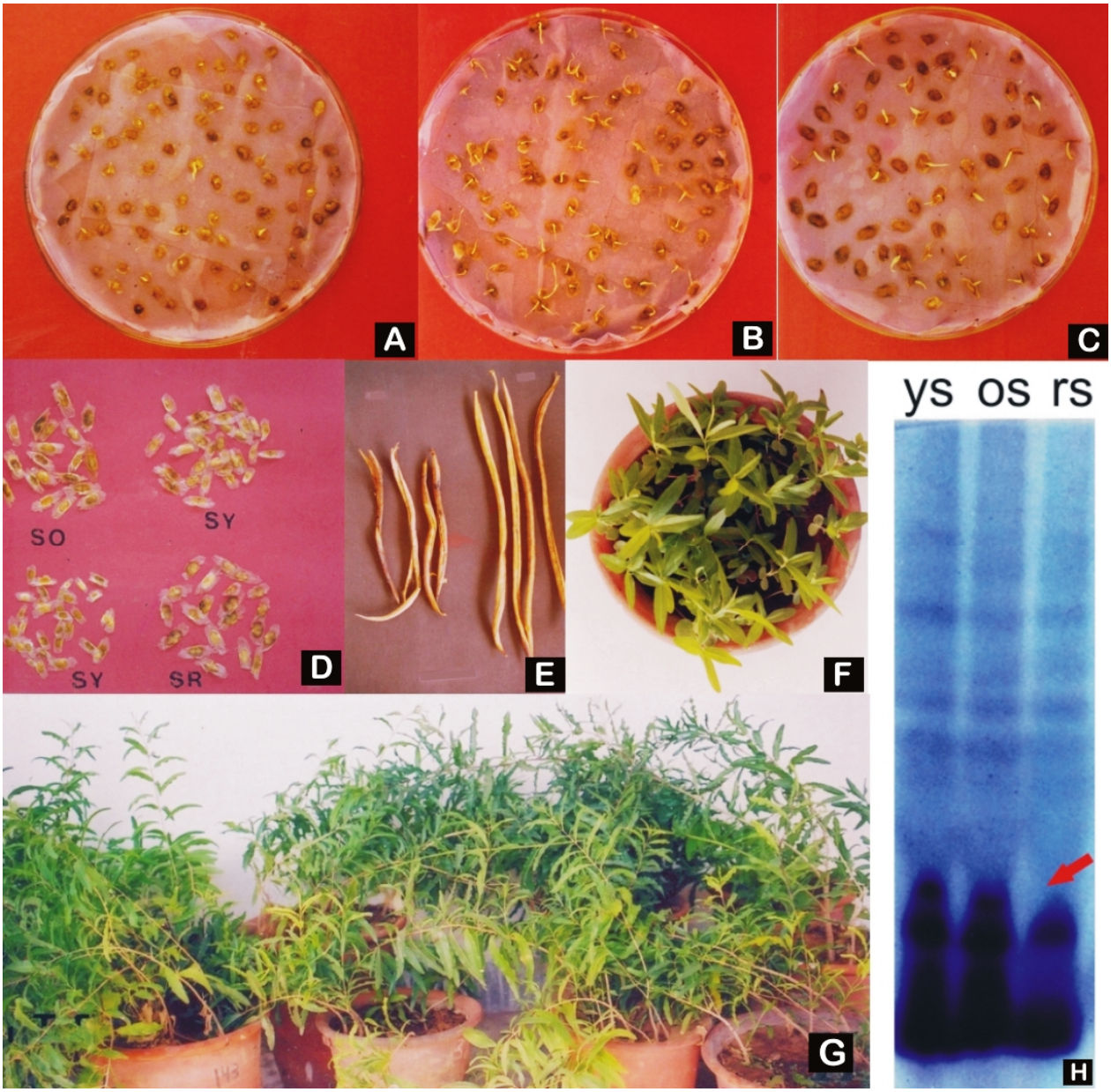

Fig. 3: Various study carried out on morphotypes of *Tecomella undulata* A – Seed germination study in yellow morphotype**; B** – Seed germination study in orange morphotype; **C** – Seed germination study in red morphotype; **D** – Seeds from the three morphotypes; **E** – Fruits from orange morphotype; **F** – Plantlets profusely growing in pot; **G** – 8 month old plantlets of *Tecomella* germinated from seeds; **H** – SDS-PAGE protein profile from the three morphotypes (***Abbrv.*** SO – Seed from orange morphotype, SR – Seed from red morphotype, SY – Seed from yellow morphotype; ys, os and rs –lanes with seed protein from yellow, orange and red morphotypes respectively; arrow indicating missing band).

Table 1: Comparative taxonomical characters of yellow, orange and red flowered morphotypes of *Tecomella undulata* (sm.) Seem. from different districts of Rajasthan

Parameters*	**Districts**							
	Jaipur			Sikar			Churu	Jhunjhunu
	Yellow	Orange	Red	Yellow	Orange	Red	Orange	Orange
Plant Height (m)	8.0	7.3	6.4	5	7.8	5.0	8.9	9.3
Plant density per hectare	1.0	2.67	1.0	1.0	7.4	1.0	14.31	16.07
Petiole (cm)	1.4	1.65	1.65	1.75	1.87	1.88	2.25	2.10
Leaf size (cm)	12.15x 2.75	12.50x3.01	12.64 x 3.00	11.33 x 2.19	11.6x 2.38	11.62 x 2.45	9.4 × 2.85	9.73 × 3.0
Number of flower per inflorescence branch	24	31.5	102.53	51.52	48.33	61.27	98.12	113.40
Calyx length (mm)	10.25	17.23	16.75	18.25	15.42	12.34	15.87	16.30
Calyx colour	Green with yellow lobes	Green with brownish pink lobes	Green with red lobes	Green with reddish yellow lobes	Green with pink lobes	Green with pink lobes	Green with pink lobes	Green with pink lobes
Corolla length (mm)	65.5	63	74.5	66.5	62.5	59.5	55.90	57.50
Corolla colour	Yellow, some flower with orange patch on the lobe	Orange, with red corolla tube & yellow patch on the lobes	Red	Yellow with dark brown corolla tube, red patches on the lobes	Orange with dark brown corolla tube	Red with long yellow patches on the corolla tube	Orange with long yellow patches on the corolla tube	Orange with long yellow patch in corolla tube
Length of Anther filament (mm)								
Anterior	50.25	44.72	51.23	48.63	47.10	47.00	37.22	41.50
Posterior	42.36	41.32	43.64	43.75	40.5	41.25	33.80	36.42
Style length (mm)	44.5	51.75	55.25	51.25	42.75	39.25	42.21	42.83
Ovary length (mm)	5.0	7	6.8	6.7	5.0	4.9	5.0	5.2
Fruits per plant	15	144.30	13.50	73.0	387.50	43.0	360.32	473.4
Fruits per inflorescence	2-5	2	1.80	2.3	2.39	1.8	2.52	2.70
Fruit length (cm)	38.6	35.5	35.75	41.29	42.5	45.25	29.63	35.25
Seeds per fruit	180.1	189.5	191.25	191.25	194.25	216.4	169.5	190.0
Seed size, along with wing (mm)	22.71×9.36	24.5x9.25	28.25x10.40	24.75x9.70	34.24x10.25	27.5x9.38	26.75 × 9.75	27.5 × 7.75
Seed colour	Yellowish brown	Brownish yellow	Brownish yellow	Yellowish brown	Yellow to brown	Yellowish brown	Yellow brown to brown	Yellow to brown with pinkish wing

*Quantitative characters are shown as mean values of 50 readings except few parameters where no. of plants were limited, such as plant height, plant density per hectare, fruits per plant

Table 2: Comparative taxonomical characters of yellow, orange and red flowered morphotypes of *Tecomella undulata* (sm.) Seem. from different districts of Rajasthan

Parameters*	**Districts**							
	Nagaur		Ganganagar	Barmer		Bikaner	Jodhpur	Jaisalmer
	Yellow	Orange	Yellow	Yellow	Orange	Orange	Orange	Orange
Plant Height (m)	8.6	9.4	6.0	5.3	5.8	8.9	7.7	4.8
Plant density per hectare	1.5	3.5	1	2.5	8.5	2.5	2.5	1
Petiole (cm)	1.8	1.8	1.4	1.90	1.11	1.35	1.95	1.48
Leaf size (cm)	15.10x2.7	10.12x2.77	9.74x2.29	11.03x2.33	6.22x2.00	10.32x 2.13	10.46x1.92	9.37 × 2.18
Number of flower per inflorescence branch	28.54	32.74	24.34	129.0	128.56	97.31	42.51	63.46
Calyx length (mm)	16.70	17.46	17.31	16.5	14.5	14.03	13.5	14.81
Calyx colour	Green with yellow lobes	Basal purple with green middle region and pink lobes	Green with yellow lobes	Green with yellow lobes	Green with pink lobes	Green with yellowish green lobes	Green with pink lobes	Green with pink lobes
Corolla length (mm)	58.5	51.5	53.73	58.5	56.75	53.5	72.5	58.37
Corolla colour	Yellow with dark brown corolla tube	Orange with dark purple corolla tube, sometimes red corolla lobes	Yellow with brown corolla tube	Yellow with orange patches in the lobes	Orange with red corolla tubes	Orange with red corolla tubes	Orange with red corolla tubes	Orange with red corolla tubes
Length of Anther filament (mm)								
Anterior	43.41	42.57	45.93	36.5	35.75	37.20	49.24	44.26
Posterior	34.00	36.50	42.03	33.5	32.20	31.25	44.75	41.91
Style length (mm)	47.50	44.0	46.5	40.5	41.6	42.50	49.50	43.14
Ovary length (mm)	5.3	5.1	5.0	4.7	4.7	4.9	6.8	5.1
Fruits per plant	444	422.40	87.0	217.70	362.35	212.3	174.5	43
Fruits per inflorescence	2.2	2.54	2.03	2.3	2.57	2.4	2.52	1.77
Fruit length (cm)	35.75	33.75	36.4	36.03	37.3	34.8	37.24	30.78
Seed per fruit	186.50	185	191.31	185.8	190.5	180.71	176.6	172.3
Seed size, along with wing (mm)	19.35x8.25	27.25x9.5	26.86×9.48	27.39×9.61	27.68×8.95	25.32×9.66	27.9×9.97	25.98 × 9.8
Seed colour	Yellowish brown	Yellowish brown to brown	Yellow to yellowish brown	Yellow to yellowish brown	Yellow to yellowish brown	Yellowish brown to brown	Yellowish brown	Yellowish brown

*Quantitative characters are shown as mean values of 50 readings except few parameters where no. of plants were limited, such as plant height, plant density per hectare, fruits per plant

Distinct and consistent variations in colour of the plant parts among the three morphotypes were observed. Three morphotypes were recognized based on petal colour. These are yellow, orange and red morphotypes, bearing yellow, orange and red coloured corollas respectively (Fig 2A-E). In the yellow morphotype the calyx was green with yellow coloured lobes, whereas in orange and red morphotypes the calyx was green with pink lobes and green with red lobes respectively (Fig. 2G). Ventral side of the corolla tube was dark purplish red in all the three morphotypes. Ovary was green in yellow morphotype and red in red and orange morphotypes. Seedcoat colour also varied among different morphotypes with yellowish brown in yellow morphotype and yellow and brownish yellow to brown in orange and red morphotypes respectively (Fig. 3A-D) (Table 1, 2).

Protein Profile

To find out the variation at protein level among the three morphotypes, SDS-PAGE of the protein extracted from seed samples from all the three morphotypes was performed. Variations in number of protein bands were observed (Fig. 3H). A total of 9 protein bands were observed. Eight band were common between all the three morphotypes. Ninth band, just heavier than the bottom two lighter bands was absent (shown with arrow) in the red morphotype and was found present in orange and red morphotypes.

Flowering Season

Variations in the onset of the flowering seasons among the three morphotypes were observed. Orange and red morphotypes show flowering about 15-20 days earlier than the yellow morphotype. This variation was always observed during flowering season wherever yellow, orange and red flowered plants were found together in a locality (Jaipur, Sikar and Nagaur districts) or separately in other districts under observation.

Seed Germination

Highest (78.72) and lowest (70.30) percentage of seed germination was observed in orange flowered and yellow flowered morphotypes respectively whereas red flowered morphotype showed 73.74 percent of seed germination (Figs. 3A-C). Seeds sown in soil at a depth of 1-2cm also sprout out within two to three days (Figs. 3A-C, F, G).

DISCUSSION

Bhau *et al.,* (2007) studied genetic diversity using 8 AFLP primers in *Tecomella undulata* populations collected from various parts of Western Rajasthan. Though they reported high level of genetic diversity and attributed it to its out-crossing nature, yet the study would have been interesting if had they categorized the samples based on flower colour. We have observed consistent variations in morpho-taxonomical characters such as colour of the seed coat and flower and onset of flowering time among the three morphotypes. These suggest that there occur two morphotypes of *Tecomella undulata* in the state of Rajasthan, one with yellow and the other with red flowers. The orange flowered morphotype seems to be the hybrid between these two morphotypes and is well manifested by its hybrid vigour like – highest plant density, plant height, fruit per plant and percentage of seed germination etc.

Traditionally taxonomy is generally based on visible morphological characters. However powerful analytical techniques in biochemistry and molecular biology are helping refining natural systems of classification and establishing phylogenetic relationships among the morphologically similar or disputed taxa. Singh and Gupta (1978) studied esterase isoenzyme variation in 24 genotypes of *Brassica* species using SDS gel electrophoresis. They suggested to use the esterase isoenzymes as genetic markers for screening *Brassica* population and for studying both intra and inter specific variations using polyacrylamide gel electrophoresis. Similarly Mondal and Mondal (2002) compared three species of *Chara* using SDS-PAGE of their total soluble proteins. In *Tecomella* SDS-PAGE of the soluble protein from seed revealed less similarity between yellow and red morphotypes whereas highest affinity between yellow and orange morphotypes in seed protein. Not a single protein band was observed which was present in yellow and red morphotypes but absent in orange morphotype. This further supports the hybrid nature of the orange morphotype. Permanent gap in time of the onset of flowering period between yellow and orange or red morphotypes at different places further supports lesser affinity between yellow and red morphotypes.

It seems that orange morphotype is well adapted to the stressful environmental conditions of the desert region. It is exclusively distributed in the western and north-western part of Rajasthan which include Jaisalmer, Barmer, Bikaner, Jodhpur, Nagaur, Churu and Jhunjhunu districts. The density of the plant, except at very few sites in Churu, Jhunjhunu and Nagaur districts, was very low. Hardly one to two young plantlets per hectare were observed growing in the field under natural conditions. Local people are unaware of good seed germination practices associated with this species. Consistent grazing by animal makes the plant bushy if kept unprotected in the younger age. That's why farmers are showing lesser interest in growing this plant. During our survey we hardily saw any young (one to two year old) plant growing in the fields. At the same time the older individuals usually show cracks in their bark and hole in the bole. This hollow trunk makes the tree useless for timber production and also leads to death of the plant. Earlier Investigations have revealed the causes of cracking of bark and hollowness of the main bole in *Tecomella undulata* trees. The malady was assigned to infestation of wood by borers and wood decaying fungi. Two species of imperfect fungi (*Phoma sp.* and *Botryodiplodia theobromae*) were found primarily

responsible for canker-rots, which subsequently attract borers and heart rot pathogen (*Fomes* sp.). No new plants and vulnerable older plants is a dangerous situation posing a great threat to the existence of this species. Although research has shown that mature trees if treated with paste containing linseed oil, copper carbonate and red lead in 2:1:1 ratio exhibit reduced cankered area and healing of canker due to formation of callus (Amal Kar *et al.*, 2009) even it require fresh initiatives by the villagers to propagate this plant. All the above reasons are responsible for diminishing density of such an important desert species. Due to this an alarming situation has arrived and the plant should be considered as seriously endangered. Particularly the distribution and the densities of the yellow and red ecotypes are very low. The steps for conservation of this diverse and important species should urgently be taken. The best way of growing the plant is to prepare a nursery. The plant should get regular water and manure for one year and then only should be transferred to the field, well protected from the grazing animal, which is hardly practised by local people. In our case we have observed that few plants which we grew in our campus portion devoid of regular watering and manuring were just 2-3 meters tall and didn't show flowering untill they were nine years old. Contrastingly, those plants which were grown in well fed soil with continuous watering are now 8-10 meters tall showing regular flowering after seven years of age (Fig. 4). Multiple branching in the trunk at a very low level also makes most of the boles lesser attractive for timber production (Figs. 2A-C, Fig. 4).

Fig. 4. A ten years old, red morphotype (progeny of a red morphotype shown in Fig. 2C) growing in the garden of the Department of Botany, University of Rajasthan, Jaipur.

We further recommend growing of plants in protected areas with regular pruning off of the lateral branches emerging out, in order to get straight boles. Comparative studies of various economical aspects like wood or timber quality, leaf forage quality and medicinal quality among all the three morphotypes is needed to be done. We also recommend carrying out its phylogenetic study, at molecular level, but in different manner. Samples must be grouped based on the colour of the flower, so that phylogenetic relationship among the three morphotypes could be found out.

ACKNOWLEDGEMENT: Authors are thankful to the Department of Biotechnology, New Delhi for providing financial assistance to undertake the study

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