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Anatomical Response of Regenerated Bark in *Terminalia arjuna* (Roxb.) Wight & Arn.

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Abstract: *Terminalia arjuna* is a large tropical tree whose both original and regenerated bark is commercially exploited for medicinal purpose mainly to cure cardiac diseases. For sustainable supply of Arjuna bark, the regenerated bark of tree is usually harvested within 18-24 months with controlled wounding at different girth classes. During course of investigation, it was found that the biomass and rigidity of regenerated bark was higher than original bark in each girth-class of tree. To explore the reason for higher biomass and rigidity in the regenerated bark of Arjuna, a comparative anatomical investigation was carried out between original and regenerated bark from randomly selected trees of middle girth-class (50-75cm). Results showed that there was significantly higher proportion of phloem fibres and ray cells and lower proportion of sieve tubes in regenerated bark in comparison to original bark. Most of the fibre dimensions and ray parameters varied between original and regenerated bark and also between first and second harvest of the regenerated bark. The wounded tree gives first preference to the protection of the injured bark from surroundings rather than food conduction through sieve tubes.

Keywords: *Terminalia arjuna*, Regenerated bark, Wound, Harvesting, Bark anatomy

Terminalia arjuna (Roxb.) Wright & Arnot ('Arjuna') is a large tropical medicinal tree species of family Combretaceae. It's bark is commercially exploited for Ayurvedic medicines, primarily to cure cardiac diseases (Dhingra et al 2013, Chander and Chauhan 2014). Most bark collectors girdle the trees or sometimes harvest entire bark by means of unscientific way, which may lead to death of trees. Considering these issues, sustainable bark harvesting techniques have been developed (Pandey and Mandal 2012, Pandey 2015, Gunaga et al 2017, Anonymous 2020). During sustainable harvesting practices, outer and middle bark is generally removed longitudinally from the stem and branches by making incision of specific strip size leaving inner bark for regeneration. However, regeneration largely depend upon the age and girth of trees, depth of incision, strip size (width and length), moisture of exposed surface, method, season of harvesting and also largely by tree-to-tree variation (Pandey and Mandal 2012). Bark regrowth usually completes within 18-24 months after each harvesting in *Terminalia arjuna* (Pandey 2015).

Trees respond to bark injury with a series of chemical, anatomical and physical changes adjacent to wound surface. When tree is injured for bark collection, the injured tissue is not repaired or heals from inside out like animals (Shigo 1986). The replacement of removed bark tissues initiates with callus formation from newly formed wound cambium in the surrounding area of injury (Dickison 2000). The callus mostly grows from the peripheral parts of the wound and suberinized. Thus, trees respond to injury by compartmentalizing or creating a wall around the wounded tissue with gradual growth of new tissue (Shigo 1986, Sinha et al 2010). The harvesting of regenerated bark is important for sustainable supply of bark in *Terminalia arjuna,* since the regenerated bark is also exploited commercially for medicinal purpose. During preliminary stages of the present investigation, it was reported that bark regeneration after first harvest completed within 8-9 months in *Terminalia arjuna* trees of middle (50-75 cm) to higher girth classes (>100cm) in comparison to smaller girth-class (25-50 cm); however, bark regeneration completed within 9-12 months after second harvest (Figure 1a-f). Interestingly, the bark biomass was found to be maximum in regenerated bark as compared to original bark in each girth-class. Furthermore, during microtomy, the regenerated bark sections were found to be harder to cut than original bark sections. Studies on changes in anatomical properties of original and regenerated bark in forest tree species are scanty. The current study aimed to explore the cause of higher biomass and rigidity in the regenerated bark of *Terminalia arjuna* by comparing the changes in anatomical properties of original and regenerated bark.

MATERIAL AND METHODS

The experiment was conducted among arjuna trees of different girth classes (25-50 cm to 100-150 cm) to study the sustainable bark harvesting from block and road side plantations established at Navsari Agricultural University, Navsari, Gujarat (20.95°N latitude, 72.90°E longitude), India. For anatomical investigations, three trees were randomly selected from the middle girth-class (50-75cm) and used for the study. A longitudinal bark strip of 10 cm (length) and 5cm (width) was removed in month of March from each selected tree at the breast height of 1.37m from the ground level (Fig. 1a-b). The renewed bark was harvested twice after completing its regeneration at the interval of nine months and brought to the laboratory in order to compare the anatomy of original bark with regenerated bark from same trees.

Anatomical measurements: In the laboratory, both original and regenerated bark (after first and second harvest) were converted into rectangular blocks and then transverse and tangential sections were cut with a sliding microtome, and anatomical observations were made at 4x, 10x and 40x objectives under a Leica trinocular microscope. Ray parameters (Ray width, ray height and ray frequency) were measured from the slides of tangential sections at 10x objective. However, tissue proportions (Phloem fibre, ray and sieve tube proportions) were determined by point sampling method under an eyepiece scale (11-point micrometer scale) attached with a Leica stereo-zoom microscope (Rao et al 1997). The slides of transverse section were moved randomly at 10 places using a 10x objective to identify and record the different tissue proportions. For the measurement of fibre dimensions such as length, width, lumen width and cell-wall thickness, the maceration of bark samples was carried out by Schult'z method (Jane 1956). Minimum 25 observations were taken for the measurement of fibre dimensions and tissue proportion as per IAWA guidelines (Wheeler et al 1989).

Statistics: The anatomical features of original and regenerated barks were compared using t-test to confirm the significant differences between treatments using the online statistical software package (Sheoran et al 1998).

RESULTS AND DISCUSSION

Anatomical variation between original and regenerated bark: The results of anatomical properties of regenerated bark after first and second years of harvesting was compared with original bark in *Terminalia arjuna* trees of 50-75 cm girth-class (Table 1). Results showed that there was significantly higher proportion of phloem fibres (about 52%), ray cells (about 32%) and lower proportion of sieve tubes in regenerated bark (about 17%) as compared to original bark (about 40%). Among the fibre dimensions, lumen width (about 15 μm) was significantly higher in regenerated bark than original bark; while, fibre width (26.25 μm) was higher in regenerated bark of second harvest. In fact, fibre wall thickness (6.94 μm) was found to be higher in original bark. The similar trend was also observed for the ray parameters, where ray height and ray frequency were significantly higher in original bark than regenerated bark. Interestingly, aggregate rays were observed along with uniseriate rays in the original bark (Fig. 2c); however, aggregate rays were absent in the regenerated bark (Fig. 2d).

The present comparative anatomical studies of original and regenerated bark showed that the higher proportion of

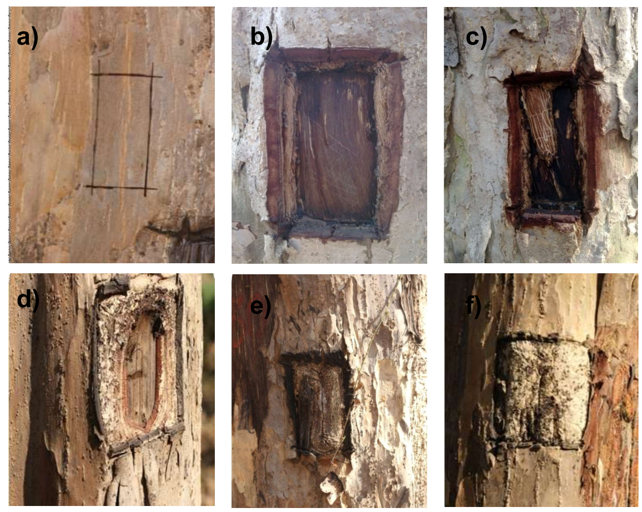

Fig. 1a-f. Regeneration process of *Terminalia arjuna* bark after harvesting

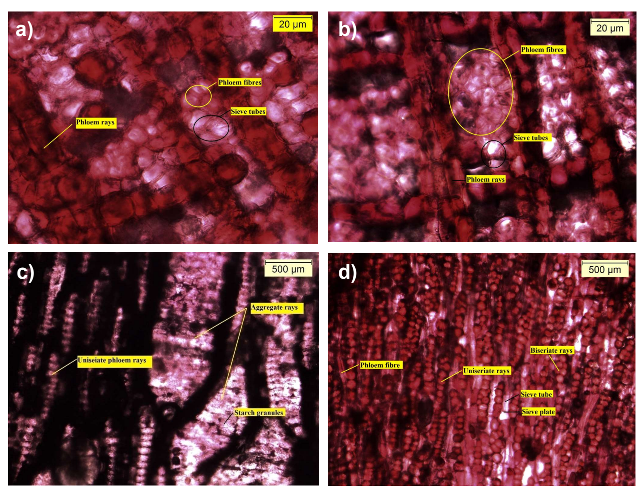

Fig. 2. Bark anatomy of *Terminalia arjuna* in Transverse Section (TS) at 40x and Tangential Longitudinal Section (TLS) at 10x objective (a) TS of original bark showing lower proportion of phloem fibres (b) TS of regenerated bark showing higher proportion of phloem fibres (c) TLS of original bark showing uniseriate and aggregate rays (d) TLS of regenerated bark showing uniseriate rays only

Table 1. Comparison of anatomical properties of original bark and regenerated bark of *Terminalia arjuna* in girth class of 50-75 cm after first and second year of harvesting

Anatomical properties	Original bark (O)		Regenerated bark at first harvest (R_1)		Regenerated bark at second harvest (R_2)		't' value		
	Range	Mean	Range	Mean	Range	Mean	(OxR_1)	(OxR_2)	(R_1xR_2)
Fibre length (mm)	0.78-1.89	1.18±0.27	0.79-1.36	1.10±0.14	0.86-1.47	1.19±0.15	1.545 ns	-0.179 ns	-2.587 *
Fibre width (µm)	16.59-27.01	22.18±3.72	18.97-28.62	22.88±2.53	20.66-37.42	26.25±4.42	-0.665 ns	-2.440 *	2.749 **
Fibre lumen width (µm)	5.40-13.03	8.29±3.21	12.92-18.33	15.77±1.72	9.97-20.27	13.34±3.39	-7.819 **	-3.720 **	-2.645 *
Fibre wall thickness (µm)	5.56-8.96	6.94±1.12	2.64-5.15	3.56±0.73	4.77-9.39	6.46±1.35	9.172 **	0.837 ns	7.818 **
Ray width (mm)	0.03-0.06	0.05±0.01	0.03-0.04	0.04±0.01	0.02-0.07	0.05±0.01	1.456 ns	-0.267 ns	-2.350 *
Ray height (mm)	0.21-0.78	0.44±0.19	0.24-0.44	0.32±0.06	0.24-0.58	0.35±0.09	2.216 *	1.927 ns	-0.756 ns
Ray frequency (Nos./mm)	6-10	9±1.17	5-9	7±1.42	5-10	8±0.77	2.984 **	0.636 ns	-3.138 **
Fibre (%)	27.27-54.54	36.36±8.57	36.36-72.73	53.64±10.01	18.18-72.72	50.00±16.18	-4.146 **	-2.356 *	0.604 ns
Ray (%)	9.09-36.36	23.64±9.77	18.18-36.36	30.91±7.48	18.18-45.45	33.64±8.62	-1.635 ns	-2.426 *	-1.007 ns
Sieve tube (%)	27.27-54.54	39.99±9.78	9.09-27.27	17.3±8.35	9.09-36.36	16.14±11.17	5.813 **	5.035 **	0.002 ns

*,** Significant at 5 and 1% level

phloem fibres and lower proportion of sieve tubes in regenerated bark may have occurred for quick healing of wounded bark and protection from the surroundings (Fig. 2b). It seems that tree gives first preference to the protection of the wounded bark than food conduction, since proportion of sieve tube is already higher in the remaining uncut bark. Hence, the protection of the wounded bark of tree from surroundings becomes the first priority to save itself from injury. Similar type of result was also reported in regenerated bark of *Hevea brasiliensis*, where, a large number of sclereids was observed in the regenerated bark (Thomas *et al.* 1995). The higher proportion of ray cells in regenerated bark was observed and it may be due to the reason that rays play a significant role in the process of wound healing by formation of wound phellogen at the site of injury (Fig. 2d). As a result of bark injury, there is an increased ethylene production in the parenchyma cells (either ray or axial parenchyma) below the cut surface that increases the growth either by cell enlargement and/or by cell division (Lev-Yadun and Aloni 1992). The increase in total width and lumen width of fibre of regenerated bark may have occurred due to dilatation of growth of cells during compartmentalization process. However, the thicker cell-wall of fibre and increase in ray height and frequency in original bark may be caused by the normal growth of the original bark.

Anatomical variation between regenerated bark of first and second harvest: Considering the regenerated bark of first and second harvests, the fibre dimensions such as fibre length, fibre width, lumen width and cell-wall thickness and ray parameters such as ray width and ray frequency varied significantly between the barks of two harvests. It was found that most of the fibre and ray parameters were higher in the regenerated bark of second harvest. This anatomical variation between regenerated barks of two harvests may be caused due to drastic change in the physiological and biochemical activities of wounded plant after repeated bark harvesting. The regrowth of injured bark is usually influenced by both vascular cambium and the polar patterns of periderm formation which is regulated by the intensity of ethylene and auxin production in wounded tree at physiological levels (Lev-Yadun and Aloni 1990, Thomas et al 1995). The comparative anatomical studies showed that sustainable harvesting in terms of strips of recommended size is feasible in Arjuna bark, since the change in the anatomical properties of regenerated bark has given priority for its protection from the surroundings by quick healing.

CONCLUSION

The wounded tree of *Terminalia arjuna* produces higher proportion of phloem fibres/ray cells and lower proportion of sieve tubes in regenerated bark as compared to original bark for fast wound healing/protection from the surroundings. The wounded tree gives first priority to the protection of the injured bark rather than food conduction through sieve tubes. Information provided in the study supports the sustainable harvesting of arjun tree bark.

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