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Genetic characterisation and assessment of Chironji (*Buchanania lanzan* Spreng.) seed source in Uttara Kannada district

Sumed RG, Uma A, Shahbaz Noori, Honnesh SH, Surendra P and Shivanna H

Sumed RG
Department of Forest Products and Utilisation, College of Forestry, Sirsi, UAS Dharwad, Karnataka, India

Uma A
Department of Forest Products and Utilisation, Karnataka, India

Shahbaz Noori
Department of Silviculture and Agroforestry, Karnataka, India

Honnesh SH
Department of Silviculture and Agroforestry, Karnataka, India

Surendra P
Department of Forest Biology and Tree Improvement, Karnataka, India

Shivanna H
Department of Forest Biology and Tree Improvement, Karnataka, India

Corresponding Author:
Sumed RG
Department of Forest Products and Utilisation, College of Forestry, Sirsi, UAS Dharwad, Karnataka, India

Abstract
Buchanania Lanzan Spreng., commonly known as Chironji, is an important medicinal tree in the family of Anacardiaceae. The present investigation was carried in College of Forestry, Sirsi to estimate the genetic variation for fruit, seed and seedling growth of *Buchanania lanzan* Spreng., in Uttara Kannada district. Fruits were collected from five seed sources *viz.*, Mundgod, Sirsi, Siddapur, Kumta, and Honnavar in Uttara Kannada during the fruiting season. Fruit, seed, kernel, germination, seedling and genetic traits were analysed. The highest fruit thickness, kernel length, and seed coat thickness were recorded in Mundgod seed source (61.16%) followed by Sirsi seed source (9.96 mm), Siddapur seed source (6.31 mm) and least was recorded in Kumta seed source (8.70 mm). The GCV was highest in leaf biomass (40.15%) and stem biomass (32.49%). The PCV and GCV of collar diameter showed highest in 30 days old seedlings (45.56 and 26.31%) and least in diameter at 180 DAG (11.02 and 5.22%) respectively. The maximum magnitude of broad sense heritability and genetic advance as percent of mean were highest in collar diameter at 360 DAG (0.76 and 0.54) and minimum was found in seedling height at 30 DAG (1.06 and 0.05). The maximum PCV, GCV, H2 and GA as per percent of mean was recorded for total biomass that is 31.01 percent, 28.83 percent and 4.33 respectively. The Mundgod seed source proved to be promising with respect to seed and seedling traits.

Keywords: *Buchanania lanzan*, Chironji, seed source, heritability, genetic variation

Introduction
India, renowned for its rich biodiversity, boasts a vast repository of natural flora and fauna, including a multitude of plant species. Among these, more than 45,000 plant species hold the potential for medicinal applications within Indian trade. Officially, India recognizes over three thousand plant species for their medicinal value, with an estimated 6,000 plants in active use in traditional, folk, and herbal medicine. Notably, the World Health Organization's 1993 survey revealed that traditional medical practitioners in India treat around 85% of patients, underscoring the crucial connection between local tribal communities and medicinal plants.

Buchanania lanzan Spreng., commonly known as Chironji, belongs to the Phylum Tracheophyta, Class Magnoliopsida, Order Sapindales, and Family Anacardiaceae. This evergreen tree is indigenous to India and can be found in various states, including Madhya Pradesh, Bihar, Orissa, Andhra Pradesh, Jharkhand, Gujarat, Rajasthan, Maharashtra, and Uttar Pradesh. Additionally, it is prevalent in the Western Ghats of Karnataka, where it is referred to as "Nurukalu" in Kannada. Chironji holds immense significance as a life-sustaining and medicinally valuable tropical tree species, serving as a vital source of livelihood for local tribal populations. It primarily thrives in dry and moist deciduous forests.

The fruit of the Chironji tree contains a hard nut, which, when dehusked, yields a kernel with approximately 52% oil content. These seeds lose their flavour when exposed to open conditions and may become bitter (Kumar *et al.*, 2012) [7]. The International Union for Conservation of Nature and Natural Resources (IUCN) included Chironji in its Red Data Book in 1996, designating it as a vulnerable medicinal plant (Anon., 2014) [2]. Chironji plays a crucial role in both Ayurvedic and Unani medicine, known for its tonic, cardiotoxic, and astringent properties. Ayurveda employs Chironji fruits for treating conditions such as leprosy, diuretic issues, haemorrhage, cardiac diseases, asthma, and fever.

The seed fat is rich in lipids (59.0%), protein (19.0–21.6%), carbohydrates (12.1%), fibre (3.8%), and essential minerals like Calcium (279.0 mg), Phosphorus (528.0 mg), and Iron (8.5 mg). Furthermore, it contains vitamins such as Thiamine (0.69 mg), Ascorbic acid/Vitamin C (5.0 mg), Riboflavin (0.53 mg), and Niacin (1.50 mg). The seed oil, comprising 34-47% fatty oil, is a valuable substitute for Olive and Almond oils. The seed oil's fatty acid composition includes Myristic (0.6%), Palmitic (33.4%), Stearic (6.3%), oleic (53.7%), and Linoleic (6.0%) acids. The kernel contains neutral lipids (90.4%), glycolipids (3.4%), and phospholipids (6.2%), with neutral lipids primarily composed of triacylglycerols (82.2%) and free fatty acids (7.8%). Chironji also contains flavonoids, tannins, glycosides, phenols, steroids, saponin, and myricetin 3'-rhamnoside-3-galactoside (Mehta *et al.*, 2010) [9]. The root exhibits anticancer, antihypertensive, larvicidal, and anti-diabetic properties. Various parts of the tree are used for treating ailments, including seed oil for reducing granular swelling, kernels for alleviating itching and prickly heat, bark gum for diarrhoea and intercostal pain, and leaves for anti-diarrheal, anti-rheumatic, wound healing, and anti-ophidian purposes (Mehta *et al.*, 2010) [9].

Despite its ecological and medicinal importance, Chironji genetic diversity faces a serious threat due to the large-scale removal of trees from natural populations. Local communities often refrain from cultivating this species in their fields or gardens due to its limited commercial value, opting instead to exploit the wild populations for various commercial purposes. As a result, the four remaining natural populations of Chironji in forested and marginal lands face the looming threat of extinction (Malik *et al.*, 2012) [8]. It is imperative to emphasize conservation strategies for Chironji, focusing on preserving existing populations while also exploring its potential for cultivation in different farming systems. Hence, this research aims to assess the extent of variation in fruit and seed traits and evaluate the genetic diversity of seedling growth among different seed sources of *Buchanania lanzan* Spreng.

Materials and Methods

The study was conducted at the research plot of the University of Agricultural Sciences, Dharwad during the year 2021-22. The study encompassed a comprehensive survey of five distinct geographical locations (seed source) of the Uttara Kannada District, namely Mundgod, Sirsi, Siddapur, Kumta, and Honnavar and was carried out during the fruiting season, involving the collection of mature fruits from approximately 10 trees of similar age of *Buchanania lanzan* within each seed source. A minimum distance of 100 meters separated the selected trees to prevent the narrowing of genetic diversity. Notably, trees grown along roadsides were excluded from the selection process to minimize the influence of environmental factors. To document relevant data, the team utilized a Ravi-altimeter and wooden calliper to measure the characteristics of the mother trees, including tree height and Girth at Breast Height (GBH). Fruits collected from individual trees were carefully stored in separate gunny bags, each appropriately labelled with essential details such as the date of collection, locality, tree height (in meters), and GBH (in centimetres).

The variations for fruit, seed and kernel characteristics during the nursery stage as follows: A) Fruit Characteristics: Mature fruits were collected and transported to the laboratory, where they were subjected to sun-drying. A sample of about 200 fruits was reserved for further analysis. Manual seed extraction from the fruits was performed, involving soaking the fruits in water for one day and subsequently rubbing the seeds using a gunny bag to remove the pulp. The key fruit attributes for fruit were meticulously measured such as 1) Fruit Length (mm): Measured using a digital calliper, the length of the fruit was assessed from the base to the tip, with measurements accurate to 0.001 cm; 2) Fruit Width (mm): Employing a digital calliper, the width of the fruit was determined by measuring the horizontal dimension intersecting the vertical line of the fruit, with measurements precise to 0.001 cm; 3) Fruit Thickness (mm): The thickness of the fruit was gauged using a digital calliper and recorded in millimetres; 4) Fruit Weight (g): utilizing an electronic balance, the weight of the fruit pod was measured, with precision up to 0.1 grams.

The attributes measured for seed characteristics adhering to guidelines by International Seed Testing Association (ISTA) wherein uniform, healthy seeds were selected at random from the seed lot, with four replications of 100 seeds each. The parameter recorded were 1) Seed Length (mm): Measured from the tip to the bottom of the seed and expressed in millimetres; Seed Width (mm): The diameter was taken at the middle of the seed using a calliper, with measurements in millimetres; 2) Seed Thickness (mm): Measured using a digital calliper and recorded in millimetres; 3) Seed Test Weight (g): Approximately 100 stones from each seed source were weighed using an electrical balance and expressed in grams and 4) Seed Coat Thickness (mm): Stones were broken open, and the thickness of the stone or seed coat was measured using a calliper and expressed in millimetres.

The key attributes measured for kernel characteristics were 1) Kernel Length (mm): Measured from the tip of the seed to the bottom and expressed in millimetres; 2) Kernel Width (mm): The diameter, measured at the midpoint of the kernel using a calliper, was recorded in millimetres; 3) Kernel Thickness (mm): Measured with a calliper and expressed in millimetres; 4) Estimation of Seed Setting Percentage (%): Randomly, 100 seeds were sampled from each seed source in four replications. Seed setting percentage was estimated based on the presence or absence of kernels within the seeds; 5) Germination Analysis: The germination test was conducted following the procedure outlined by the International Seed Testing Association (ISTA). The number of normal seedlings was counted on the 30th day (final count) of germination, with the average of four replications expressed as germination percentage; 6) Peak Value of Germination (PVG): PVG, representing the maximum mean daily germination reached at any stage during the germination period, was calculated using a specific formula; 7) Germination Value (GV): GV, a composite value that combines germination speed and total germination, was calculated based on the cumulative percentage of seed germination at the end of the test, following the method prescribed by Czabator in 1962.

The seedling parameters were recorded after the germination was complete. Forty seedlings per seed source, with 10 seedlings in each replication, were considered for various growth traits. Observations were recorded at 180-day intervals throughout the first year of seedling development such as 1) Number of Fully Opened Leaves: Counted per seedling, and the average number of leaves per seedling was calculated; 2) Fresh Weight (g): Five seedlings per seed source per replication were uprooted, washed, and weighed after removing adhering soil particles. The fresh weight was recorded; 3) Dry Weight (g): Samples were oven-dried at 70 °C for 72 hours to assess dry weight using a digital electronic balance and to estimate genetic parameters, data were subjected to analysis of variance (ANOVA) to partition the variability into genetic and environmental components. A specific model was adopted for this purpose.

Results and Discussion

Considerable variations in seed and fruit traits were observed among different seed sources of *Buchanania lanzan*. Fruits collected from the Sirsi seed source exhibited the highest fruit length (12.90 mm), fruit width (12.91 mm), and fruit weight (1.51 g), whereas the lowest values for these attributes were recorded in the Honnavar seed source (10.84 mm, 10.37 mm, and 8.70 mm, respectively), with the Kumta seed source having the lowest fruit weight (0.85 g) (Table 1). This variation could be attributed to the species natural adaptation to a wide range of environmental conditions, including variations in rainfall, temperature, and climatic factors, along with potential influences from the mother tree's size.

The Mundgod seed source displayed the maximum seed length, width, thickness, and weight (10.89 mm, 10.0 mm, 7.25 mm, and 0.79 g, respectively), while the Kumta and Honnavar seed sources exhibited the lowest values for these attributes (Table 2). This variability can be attributed to the adaptability of the Mundgod seed source to the dry deciduous and moist deciduous conditions of its habitat, providing the ideal conditions for the expression of these traits.

The seed setting percentage per fruit was highest in the Mundgod seed source (61.16%) and lowest in the Honnavar seed source (35.87%). Seed setting percentage also exhibited significant differences between the Upghat and Coastal seed sources, potentially due to environmental conditions and forest types (Table 3). This distinction highlights the clear variations between Upghat and Coastal seed source for various seed parameters.

Mundgod seed source exhibited excellent germination (76.2%) compared to all other sources (Table 3), while the lowest germination rate was observed in the Honnavar seed source (43.2%). This variation is likely due to the larger seed size, seed weight, and kernel size of the Mundgod source, as larger seeds provide more nutrients for germination, and thinner seed coats lead to reduced seed dormancy. Heavier seeds generally germinate more quickly and completely than smaller ones due to a richer endosperm nutrient pool.

Peak germination values were highest in the Mundgod seed source, followed by the Sirsi seed source, while the lowest peak germination values were recorded in the Siddapur seed source. The Mundgod seed source also displayed the highest mean daily germination values, with the lowest values recorded in the Siddapur seed source (Table 3). These variations in germination attributes can be attributed to genetic differences among seed sources (Renuka, 2013) [10].

Mundgod seed source exhibited better growth and Vigor parameters compared to all other sources. This source produced seedlings with higher biomass accumulation in leaves, roots, stems, and overall seedlings (Table 4). The Upghat sources, located in drier areas, performed better in terms of seedling development. The vigor index was higher in sources with larger seed size and weight. The data revealed significant differences in shoot vigor index among the seed sources at different intervals after germination. Root vigor index also exhibited significant variations among seed sources (Table 5). These differences can be attributed to the fast growth and establishment of seedlings with larger seeds, which generally result in taller and more vigorous seedlings.

Variation among different seed sources can be attributed to the wide range of environmental conditions in which the species grows. The variability in seed sources is primarily due to genotypic differences and genotype-environment interactions. The genetic population's variability is reflected in the relative values of PCV and GCV. The study indicated that GCV was generally less than PCV for many traits, suggesting the role of the environment in the expression of germination traits. In the present study (Table 6), heritability estimates were moderate to high, with germination demonstrating the highest heritability (0.88) and genetic advance as a percentage of the mean being highest for germination percentage (32.97). High heritability coupled with high genetic gain is essential for effective selection (Gairola *et al*., 2011) [4].

The PCV ranged from 11.02 to 45.56 percent and GCV was ranges from 5.22 to 40.15 percent. The diameter at 360 days showed minimum PCV value (11.02%) and diameter at 30 days showed maximum PCV value (45.56%). The GCV was highest for leaf biomass (40.15%) followed by stem biomass (32.49%) and the total biomass showed maximum magnitude of broad sense heritability (0.86) at 360 DAG. The genetic advance as percent of mean was highest in seedling height at 360 DAG (8.06) and least in diameter at 30 DAG (0.05). The study indicates that there is a significant genetic variation among different seed sources of *Buchanania lanzan* (Table 6). These variations can be attributed to genetic differences, environmental factors, and adaptability to specific ecological conditions.

Table 1: Variation in fruit and kernel traits of *Buchanania lanzan* Spreng. Among five seed sources

Sl. No.	Seed source	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Fruit weight (g)	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)
1	Mundgod	11.78	10.82	10.34	1.41	6.65	4.81	3.45
2	Sirsi	12.90	12.91	09.96	1.51	6.27	4.85	3.33
3	Siddapur	11.65	10.91	10.42	1.40	6.21	5.15	3.40
4	Kumta	11.11	10.44	09.10	0.85	6.19	5.08	3.50
5	Honnavar	10.84	10.37	08.70	0.98	6.31	4.82	3.24
Mean		11.66	11.09	09.70	1.15	6.32	4.94	3.38
C.V. (%)		2.65	01.94	04.23	25.38	7.61	10.45	6.35
S.Em ±		0.219	0.152	00.29	0.206	0.34	0.365	0.152
C.D.(0.05)		0.477	0.331	0.632	0.449	N.S.	N.S.	N.S.

Table 2: Variation of seed traits of *Buchanania lanzan* Spreng. among different seed sources

Sl. No	Seed source	Seed Length (mm)	Seed Width (mm)	Seed Thickness (mm)	Seed weight (g)	Seed coat thickness (mm)
1	Mundgod	10.89	10.00	7.25	0.79	0.80
2	Sirsi	10.41	9.63	6.93	0.64	1.30
3	Siddapur	10.42	9.28	6.53	0.33	1.14
4	Kumta	9.70	9.93	6.98	0.51	0.80
5	Honnavar	9.97	8.88	6.48	0.48	1.86
Mean		10.28	9.55	6.84	0.55	1.19
C.V. (%)		7.4	4.39	4.87	16.55	5.34
S.Em ±		0.538	0.296	0.236	0.064	0.045
C.D.(0.05)		N.S.	0.645	0.514	0.139	0.098

Table 3: Variation studies on seed setting, germination, mean daily germination, peak germination value in *Buchanania lanzan* across different seed source.

Sl. No.	Seed source	Seed setting (%)	Germination %	Germination rate	MDG	PGV	Germination value
1	Mundgod	61.16	76.2	4.91	3.27	2.62	12.86
2	Sirsi	39.38	53.0	2.51	2.02	1.58	3.63
3	Siddapur	44.30	43.4	2.28	01.5	1.21	2.68
4	Kumta	37.96	45.2	1.96	2.28	1.52	2.99
5	Honnavar	35.87	43.2	1.68	2.27	1.29	2.20
Mean		43.74	40.25	2.67	2.27	1.65	4.88
C.V. (%)		05.02	16.15	14.55	10.7	15.88	37.08
S.Em ±		01.55	4.597	0.275	0.172	0.185	1.279
C.D.(0.01)		4.744	14.042	0.84	0.525	0.565	3.907

Table 4: Variation in biomass production at 360 days after germination in *Buchanania lanzan* across different seed sources

Sl. No.	Seed source	Root biomass (g)	Stem biomass (g)	Leaf biomass (g)	Total biomass (g)
1	Mundgod	5.40	1.70	3.30	10.46
2	Sirsi	4.80	1.10	2.20	8.09
3	Siddapur	5.50	1.20	1.80	8.51
4	Kumta	2.20	0.60	1.30	4.11
5	Honnavar	3.40	1.30	2.60	7.30
Mean		4.27	1.18	2.43	7.69
C.V. (%)		17.37	14.12	16.30	11.43
S.Em ±		0.52	0.11	0.28	0.63
C.D. (0.01)		1.60	0.36	0.85	1.94

Table 5: Variation in SVI and RVI at 30 days, 180 days and 360 days after germination across different seed sources

Sl. No.	Seed source	SVI	RVI	SVI	RVI	SVI	RVI
		30 DAG		180 DAG		360 DAG	
1	Mundgod	466.2	410.40	995.58	1668.60	2421.00	2809.80
2	Sirsi	271.33	212.92	541.91	860.35	1001.99	1358.96
3	Siddapur	152.00	154.27	392.64	623.37	725.87	984.44
4	Kumta	161.12	151.60	367.58	654.84	619.12	931.86
5	Honnavar	153.56	136.00	325.03	585.73	736.73	932.34
Mean		240.85	213.04	524.55	878.58	1100.94	1403.48
C.V. (%)		12.15	5.51	8.24	9.73	12.37	8.09
S.Em ±		20.695	8.308	30.547	60.439	96.301	80.255
C.D.(0.01)		63.214	25.377	93.307	184.613	294.155	245.142

Table 6: Estimates of variance components, heritability and genetic advance for seed germination and seedling growth parameters in *Buchanania lanzan*

Sl. No.	Genetic parameters → ↓ Traits	PCV	GCV	H2	GA as percent of mean
1	Germination percent	45.67	42.72	0.88	32.97
2	Height (cm) 30 DAG	13.00	10.36	0.63	0.95
3	Height (cm) 180 DAG	12.11	10.41	0.73	2.21
4	Height (cm) 360 DAG	26.69	20.63	0.59	8.06
5	Collar diameter (mm) 30 DAG	45.56	26.31	0.33	0.05
6	Collar diameter (mm) 180 DAG	19.63	17.14	0.76	0.54
7	Collar diameter (mm) 360 DAG	11.02	5.22	0.22	0.22
8	Root Mean wt (g)	36.58	32.20	0.77	2.48
9	Stem Mean wt (g)	35.45	32.49	0.84	0.72
10	Leaf Mean wt(g)	43.34	40.15	0.85	1.85
11	Total biomass	31.01	28.83	0.86	4.33

Conclusions

This study has elucidated clear distinctions between the Upghat and Plains regions regarding fruit traits, seed traits, germination, and seedling growth parameters. The findings highlight Mundgod as the most favourable seed source for *Buchanania lanzan* in the sampled regions of Uttara Kannada, based on a comprehensive assessment of fruit, seed, kernel, germination, and seedling traits. These results underscore the significance of assessing seed sources, not only for the evaluation of natural variation for afforestation but also for the enhancement of breeding efforts and the development of improved planting stocks within a specific seed source.

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