Indian Journal of Ecology (2023) 50(2): 417-421
DOI: https://doi.org/10.55362/IJE/2023/3913

Manuscript Number: 3913
NAAS Rating: 5.79

Variation in Seed Germination of *Dysoxylum binectariferum:* An Endangered Medicinal Tree Species, from Different Indian Seed Sources

Suraj R. Hosur, A. Krishna, M.R. Jagadish and R. Vasudeva

College of Forestry Sirsi, University of Agricultural Sciences, Dharwad-581401, India
E-mail: srj1419@gmail.com

Abstract: *Dysoxylum binectariferum* is an endangered medicinal tree species of India that needs urgent conservation attention. In the present study, fruits and seeds from seven different sources covering four states namely, Arunachal Pradesh, Assam, Maharashtra, and Karnataka were collected to study the variation in germination attributes. A significant variation was observed for all germination traits viz. germination per cent, germination rate, speed of germination, mean daily germination, peak value of germination, and germination value. Seed germination varied significantly (28.89-98.33%) among seven seed sources. Seeds collected from the Jog (Karnataka) location were superior in all germination attributes. Seed germination traits with altitude showed a significant positive relationship and a significant negative relationship with latitude and longitude. The information generated in the study shows that there is potential scope for further tree improvement and conservation aspects of *Dysoxylum binectariferum* across India.

Keywords: *Dysoxylum binectariferum*, Recalcitrant, Provenance, Germination, Seed traits

Dysoxylum binectariferum (family: *Meliaceae*) is an endangered small to a medium-sized tree found in the Western Ghats of India (Masur et al 2014, Sumangala et al 2016). They are widely distributed in subtropical and tropical regions. These species occur in a variety of habitats, from rainforests, and mangrove swamps to semi-deserts (Julian et al 2012). Bark is pale brown or ashy in colour, smooth or rough surface with an outer corky layer. Twigs are smooth but sturdy, leaves are alternate and imparipinnate. Flowers are 7.5 to 10.0 cm in diameter. The fruit is pear-shaped having a diameter of 4.5–5.0 cm, four-valved, yellow-orange in colour and contains a few brownish-black recalcitrant seeds. Due to the existence of dysobinin and rohitukine, *D. binectariferum* has gained global scale significance. Dysobinin, a tetranortriterpene isolated from fruits, is a central nervous system (CNS) depressant with mild anti-inflammatory activity (Singh et al 1976). The pharmacologically significant chemical rohitukine is a key natural product of this speciesand it inspired the innovation of three anti-cancer clinical candidates, flavopiridol, P-27600, and IIM-290. Among all known plant sources of rohitukine, *D. binectariferum* was produce the highest amount of rohitukine in stem bark nearly 7 percent of its dry weight. Further, the bark of the tree is be used for the treatment of foul ulcers and leprosy (Overharvesting for bark and habitat degradation have made this species endangered in India's western and north-eastern regions (Nath et al 2005, Sumangala et al 2016). In *D. binectariferum,* distribution is mainly affected by recalcitrant behaviour of seeds, rodent predation on seeds, seeds are infected by the fungus on surface litter and adverse climatic conditions such as lack of moisture for germination of fallen seeds just after the winter are major causes that hinder seed germination. One of the most important steps in tree domestication is mapping provenance variation and examining for germination traits across species distribution. However, it is difficult if the species occur in isolated patches of the interior forest has different reproductive stage timings in different regions, and is recalcitrant; losing viability in a short time frame (Gunaga et al 2020). As a result, the current study was conducted to better understand the provenance variation in seed germination traits for the endangered and recalcitrant *D. binectariferum* across its distribution in India.

MATERIAL AND METHODS

The mapping of *D. binectariferum* populations was undertaken across India. Total seven populations covering four states were earmarked for the study (Table 1, Fig. 1). In each population, five superior trees were marked and from each tree 75 fruits were collected. Trees were coded with source and tree number ex. JT1 means First numbered tree from Jog source. Since there is a variation in fruit maturity between Western Ghat and North-Eastern region, matured fruits were collected at different time i.e., 2nd fortnight of January 2022 from Western Ghat and 1st fortnight of March

2022 from North-East India . After collection, fruits were opened, black pulpy seed coat covered seeds were separated. Then these seeds were washed thoroughly in water to remove the pulpy seed coat and to get green seeds. Germination trials were laid out in the sand bed of nursery of College of Forestry, Sirsi. For each population, 120 seeds (40 seeds of 3 replications) were sown following a completely randomised design (Plate 1, 2). Number of seeds germinated was monitored everyday up to 60 days after sowing. Emergence of plumule above the soil followed by opening of cotyledon leaves was considered as germination. Based on daily germination count, Germination per cent was calculated by using International Seed Testing Association guidelines (Anonymous 2010) as proportion of seeds germinated from the total number of seeds sown and expressed as per cent. Germination speed (GS) was estimated using following formula given by Czabator (1962). GS = n1/d1 + n2/d2 + n3/d3 + ……; where, n = number of germinated seeds, d = number of days. Mean daily germination (MDG) was estimated as total number of germinated seeds divided by total number of days taken for germination. Peak value (PV) was estimated as highest number of seeds germinated in a given day divided by the number of days at this peak value. Germination value (GV) was estimated (Czabator 1962). GV=PV X MDG, where PV is peak value and MDG is mean daily germination. Data were subjected to statistical analysis using Operational Statistic software performed for all the studied parameters.

RESULTS AND DISCUSSION

Dysoxylum binectariferum is a recalcitrant seed-bearing tree species found in the Western Ghats and North-Eastern parts of India. Fruit maturity occurs at different times in

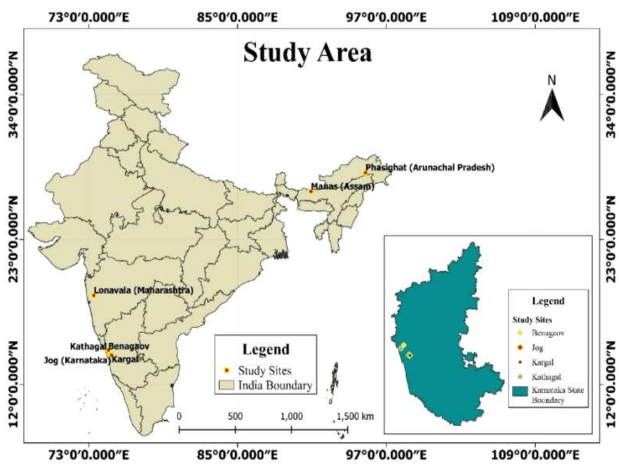

Fig. 1. Location of the study sites

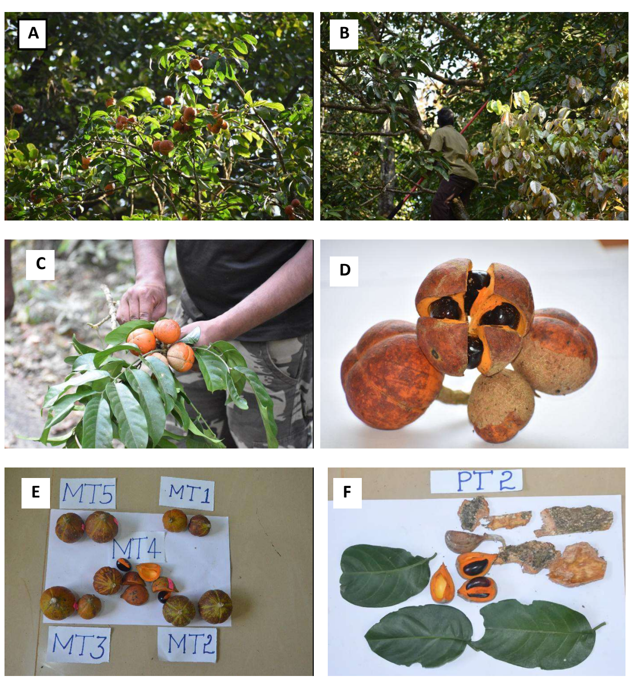

Plate 1. A: Fruits bearing branches, B: Collection of fruit using tree pruner, C, D: Pictorial representation of Fruits of *D. binectariferum,* E: Fruit samples from Manas, F: Fruit, Seed, Bark and Leaf samples of Phasighat tree number two

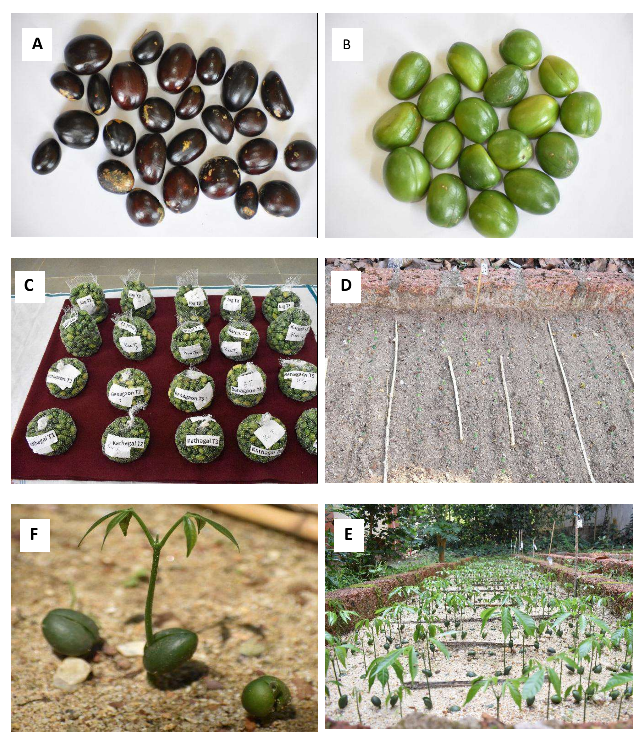

Plate 2. A: Seeds with fatty black seed coat, B: Mature green seeds after removing the seed coat, C: Seeds ready for sowing. D: Sowing of seeds in sand bed, E & F: Pictorial representation of seedlings

different regions, for instance, in the Western Ghats, in the second fortnight of January and in North-East India in the first fortnight of March. The current study found a significant variation in seed germination among seven *D. binectariferum* provenances from four states. Seed germination showed a wide range of variation, ranging from 98.33 to 28.89 percent (Table 2). The seed lot collected from the Western Ghat region (except Lonavala) resulted in significantly higher germination (>74%) than the seed lot collected from the North-East region, namely Manas (72.44%) and Phasighat (57.78%). Among the populations, Jog was superior in germination percent (97.17%), speed of germination (0.76), peak value germination (1.50), germination value (2.13). The highest mean daily germination (1.21), germination rate (19.53) were recorded for Kargal and the least germination traits were recorded by the Lonavala population. The result showed that germination per cent was positively related with altitude (R^2= 0.36), negatively with latitude (R^2 = 0.62) and longitude (R^2 = 0.59). Hence, higher altitudinal and lower longitudinal, latitudinal seed sources performed better germination traits than others. Racial variation among populations of various origins was related to locality factors such as latitude, longitude, and altitude (Jagadish et al 2014). Variations in seed germination traits among natural populations can help with tree selection for domestication. Higher seed weight populations have resulted in higher germination, as confirmed by using families derived from across distribution. As a result, heavier seeds should be preferred to produce higher quality seedlings (Manjunath 2003). The variations could be attributed to the genetic potential of the seed sources and mother trees (Hanumanth 2020). The study suggested that differences in germination traits could be due to wide variations in microclimate and local environmental conditions across the species' range of distribution (Mahajan et al 2015). The genetic constitution of the species for specific traits must have changed as a result of a specific set of local environmental conditions, resulting in geographically distinct clines (Kumara et al 2014). The species' occurrence across a broad geographical range encompassing a wide range of edapo-climatic conditions in its habitat is expected to reflect in the genetic makeup of its diverse populations (Jagadish et al 2014). Similar variations in tropical tree species such as *Dysoxylum malabaricum* (Vasudeva et al 2005) and *Garcinia gummigutta* have also been observed (Bhagyavanth et al 2010).

The germination traits had shown larger variations among the trees of different provenances. JT1 tree from Jog showed maximum germination percent (98.33%) followed by JT2 and JT5 with 97.50%. The maximum germination rate was 21.04 (KaT3) followed by 20.26 (KaT4) and least 2.02 (LT3). KaT5 recorded 0.79 speed of germination. The variation of the peak value of germination ranged from 1.53 (JT1) to 0.20 (LT1) while mean daily germination starts from 1.62 (KaT4) to 0.29 (LT5) and germination value varied from 2.20 (JT5) to 0.05 (LT3) (Table 2). Gunaga et al (2015) observed tree-to-tree variation in seed germination of *Dysoxylum binectariferum*, with seeds collected from ten distant individuals of a single population revealing an overall mean germination per cent (77.4%). The germination percentage of the DB-41 tree was highest (93.7%), while the germination percentage of the DB-02 tree was lowest (64.00%). This variation could be attributed to environmental factors that shaped genetic variation in reproductive traits. The germination study on *Dysoxylum malabaricum* revealed average germination after 12 days, with overall germination ranging from 98 to 100% (Manjunath 2003).

CONCLUSION

The current research reveals that in *Dysoxylum binectariferum*, different regions, provenances, and individual trees within each provenance influenced seed germination. The extent of variation was unearthed to be greater between provenances than between trees. The significant variation could be attributed to the seed source's altitude, longitude, and latitude, which appear to have a direct influence on seed germination. Higher tree to tree variation

Table 1. Passport data of *D. binectariferum* seed sources considered for the investigation

Bioclimatic region	State	Location/Place	Altitude (msl)	Latitude (°)	Longitude (°)
North-East India	Arunachal Pradesh	Phasighat	194	28.06407	95.31755
	Assam	Manas	110	26.64237	90.91727
Western Ghat	Maharashtra	Lonavala	619	18.75083	73.41416
	Karnataka	Jog	583	14.2291	74.8181
		Kargal	491	14.2214	74.8422
		Benagaov	525	14.6021	74.5925
		Kathagal	67	14.4832	74.4807

Table 2. Effect of seed source variation on germination of *Dysoxylum binectariferum*

Source	Trees	Germination (%)	Germination rate	Speed of germination	Mean daily germination	Peak value of germination	Germination value
Joga	JT1	98.33 (84.26)	19.67	0.78	1.22	1.53	2.16
	JT2	97.50 (83.25)	18.35	0.77	0.86	1.51	2.17
	JT3	96.67 (82.82)	18.08	0.77	1.31	1.50	2.05
	JT4	95.83 (78.78)	14.32	0.73	0.87	1.48	2.08
	JT5	97.50 (83.25)	15.44	0.72	0.79	1.50	2.20
	Mean	97.17^{a} (82.47)	17.17	0.76	1.01	1.50	2.13
Kargal	KaT1	90.00 (70.84)	18.50	0.73	0.83	1.43	1.96
	KaT2	90.00 (70.84)	18.23	0.72	1.53	1.43	2.01
	KaT3	93.33 (73.73)	21.04	0.75	1.26	1.46	2.10
	KaT4	95.83 (78.78)	19.63	0.78	1.62	1.47	2.00
	KaT5	95.00 (77.08)	20.26	0.79	0.82	1.46	2.14
	Mean	92.83^{b} (74.25)	19.53	0.75	1.21	1.45	2.04
Benagav	BT1	82.50 (64.67)	16.90	0.63	0.36	1.27	1.99
	BT2	80.00 (64.06)	16.40	0.64	0.87	1.23	2.04
	BT3	81.67 (65.84)	16.58	0.64	0.80	1.23	1.94
	BT4	82.50 (64.67)	14.06	0.63	0.92	1.28	2.25
	BT5	83.33 (65.53)	14.25	0.59	1.02	1.32	1.98
	Mean	82.00^{c} (64.96)	15.64	0.62	0.79	1.26	2.04
Kathagal	KtT1	74.17 (61.00)	12.60	0.65	0.77	1.20	1.94
	KtT2	76.67 (61.62)	11.31	0.61	1.13	1.27	1.91
	KtT3	80.00 (64.06)	10.78	0.61	0.74	1.27	2.01
	KtT4	72.50 (59.57)	15.30	0.61	0.72	1.24	1.73
	KtT5	70.83 (57.53)	11.81	0.62	0.82	1.17	0.49
	Mean	74.83^{d} (60.75)	12.36	0.62	0.84	1.23	1.62
Lonavala	LT1	35.56 (36.57)	3.70	0.10	0.31	0.20	0.06
	LT2	48.89 (44.79)	6.85	0.18	0.36	0.33	0.06
	LT3	28.89 (29.55)	2.02	0.08	0.36	0.22	0.05
	LT4	55.56 (51.25)	7.80	0.24	0.51	0.37	0.09
	LT5	35.56 (36.57)	6.13	0.08	0.29	0.24	0.06
	Mean	40.89^{f} (39.75)	5.30	0.14	0.37	0.27	0.07
Manas	MT1	97.78 (88.34)	17.68	0.48	0.69	0.92	0.29
	MT2	75.56 (61.35)	9.96	0.32	0.53	0.60	0.40
	MT3	64.44 (55.72)	4.44	0.25	0.46	0.45	0.24
	MT4	68.89 (58.58)	5.70	0.30	0.49	0.55	0.19
	MT5	55.56 (51.25)	6.73	0.25	0.41	0.44	0.20
	Mean	72.44^{d} (63.05)	8.90	0.32	0.52	0.59	0.26
Phasighat	PT1	53.33 (49.54)	6.90	0.28	0.51	0.42	0.17
	PT2	53.33 (49.54)	8.90	0.24	0.41	0.44	0.13
	PT3	62.22 (52.67)	7.51	0.28	0.44	0.49	0.18
	PT4	55.56 (51.25)	7.51	0.27	0.41	0.55	0.19
	PT5	64.44 (55.72)	8.52	0.28	0.46	0.51	0.23
	Mean	57.78^{e} (51.74)	7.87	0.27	0.44	0.48	0.18
			CD (p=0.05)	CD (p=0.05)	CD (p=0.05)	CD (p=0.05)	CD (p=0.05)
Sources			4.16	0.12	0.12	0.04	0.07
Trees			3.52	0.10	0.10	0.03	0.06
Sources×Trees			9.30	0.27	0.27	0.09	0.17

* Values in parenthesis are arcsine transferred value

observed for seed germination traits could be due to genetic dissimilarity among the trees, as recalcitrant seeds of the species may be dispersed within a narrow range due to a lack of dormancy. Jog source was superior with respect to germination traits. Therefore, this source may be used for domestication by using superior trees and large-scale plantation programme. Information generated here would be helpful for nursery entrepreneurs and researchers for further tree improvement programme.

REFERENCES

Anonymous 2010. *ISTA International rules for seed testing* Secretariat, Zuerich, Switzerland.

Bhagyavanth NM, Gunaga RP and Vasudeva R 2010. Development of fruit and seed descriptors in *Garcinia gummigutta* (L.) In: *National symposium on Garcinia Genetic resources: linking diversity, livelihood and management* (ed. R. Vasudeva, B.S. Janagoudar, B. M. C. Reddy, Bhuwon Sthapit and H. P. Singh), pp.1-210, Sirsi. 103-111. College of Forestry, India.

Czabator FJ 1962. Germination value: an index combining speed and completeness of pine seed germination. *Forest Science* **8:** 386-396.

Gunaga RP, Manjunath AV, Gunaga SV and Vasudeva R 2015. Tree to tree variation in seed traits and germination in *Dysoxylum binectariferum* Hook. F. *Indian Forester* **141**(5): 578-580.

Gunaga RP, Mayur MM, Wanage SS, Mirgal AB and Rane AD 2020. Morphological variation in seed traits, germination and seedling growth in endangered medicinal tree species, *Saraca asoca* (Roxb.) de Wilde., from different seed sources of Konkan region, Maharashtra. *Journal of Pharmacognosy and Phytochemistry* **9**(4): 157-162.

Hanumantha M 2020. Assembling, genetic evaluation and selection of superior types in *Cinnomomum zeylaniucum* Blume. Ph.D. Thesis. University of Agricultural Sciences, Dharwad, India.

Houghton PJ 2000. Chemistry and biological activity of natural and semi-synthetic chromone alkaloids.*Studies in Natural Products Chemistry* **21**: 123-155.

Jagadish MR, Venkatareddy YB, Venaktanaidu MJ, Vachana HC, Devakumar AS and Patil CSP 2014. Seed source variation in germination behaviour of *Vetaria indica* Linn. *International Journal of Science and Nature* **5**(2): 231-234.

Jain SK and Defilipps RA 1991. *Medicinal Plants of India*. Reference Publications Inc., Michigan, USA vol. I.

Received 12 November, 2022; Accepted 26 February, 2023

Jain SK, Meena S, Qazi AK, Hussain A, Bhola SK, Kshirsagar R, Pari K, Khajuria A, Hamid A and Shaanker RU 2013. Isolation and biological evaluation of chromone alkaloid dysoline, a new regioisomer of rohitukine from *Dysoxylum binectariferum*. *Tetrahedron Letter* **54**(52): 7140-7143.

Julian PF, Sanford DE and Luko HQ 2012. Alkaloids, limonoids and phenols from Meliaceae species decrease survival and performance of *Hypsipyla grandella* larvae. *American Journal of Plant Sciences* **11**(3): 13-18

Kumara PM, Soujanya KN, Ravikanth G, Vasudeva R, Ganeshaiah KN and Shaanker RU 2014. Rohitukine, a chromone alkaloid and a precursor of flavopiridol, is produced by endophytic fungi isolated from *Dysoxylum binectariferum* Hook. f and Amoora rohituka (Roxb). Wight & Arn. *Phytomedicine* **21**(4): 541-546.

Mahajan V, Sharma N, Kumar S, Bhardwaj V, Ali A, Khajuria RK, Bedi YS, Vishwakarma RA and Gandhi SG 2015. Production of rohitukine in leaves and seeds of Dysoxylum binectariferum: an alternate renewable resource. *Pharmaceutical Biology* **53**(3): 446-450.

Manjunath L 2003. *Reproductive biology and half-sib family performance of Dysoxylum malabaricum Bedd.: An important threatened timber species.* M.Sc. Thesis, University of Agricultural Sciences, Dharwad, India

Masur U, Kumar H and Kumar A 2014. Anti-larval effects of leaf and callus extract of *Dysoxylum binectariferum* against urban malaria vector, *Anopheles stephensi*. *Journal of Natural Products* **7**: 147-154.

Mohanakumara P, Sreejayan N, Priti V, Ramesha BT, Ravikanth G, Ganeshaiah KN, Vasudeva R, Mohan J, Santhoshkumar TR and Mishra PD 2010. *Dysoxylum binectariferum* Hook. f (Meliaceae), a rich source of rohitukine. *Fitoterapia* **81**(2): 145-148.

Nath PC, Arunachalam A, Khan ML, Arunachalam K and Barbhuiya AR 2005. Vegetation analysis tree population structure of tropical wet evergreen forests in around Namdapha National Park, northeast India. *Biodiversity Conservation* **14**: 2109-2135.

Singh S, Garg HS and Khanna NM 1976. Dysobinin, A New tetranortriterpene from *Dysoxylum binectariferum*. *Phytochemisitry* **15**: 2001-2002.

Sumangala RC, Kumara PM, Shaanker R, Vasudeva R and Ravikanth G 2016. Development and characterization of microsatellite markers for Dysoxylum binectariferum: A medicinally important tree species in Western Ghats, India. *Journal of Genetics* **93**(2): 85-88.

Vasudeva R, Manjunath L, Gunaga RP, Hombegowda HC, Umashaanker R and Ganeshaiah KN 2005. Reproductive biology and half-sib family performance for early vigour in White Cedar, *Dysoxylum malabaricum* Bedd. Multiplication trees in the *Tropics: Management and Improvement Strategies*, pp. 705-711.