Variation in seedling vigour and camptothecin content of Pyrenacantha volubilis Wight: insights for domestication

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RESEARCH ARTICLE

Variation in seedling vigour and camptothecin content of *Pyrenacantha volubilis* Wight: insights for domestication

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Abstract As a pioneering work in the field of domestication of *Pyrenacantha volubilis* Wight, an anti-cancer drug (camptothecin) yielding plant, this study identifies superior seed sources of southern peninsular India for various parameters related to its growth and yield. The experiment was set up following the common garden experiment protocol in Completely Randomised Design. Laboratory extraction of camptothecin from whole seeds was carried out using High Performance Liquid Chromatography. Whereas seeds from Kizhoor population were the heaviest (test weight of 171.5 g), seedlings obtained by sowing the seeds from Villiampakkam population performed better in terms of total yield per plant (5.25 g dry weight) as well as total camptothecin yield per hectare (223.125 g). Almost three times variation was observed in the experiment between the poorest and the best performing populations. We attempt to discuss the potential for domestication, large-scale planting and sustainable harvesting of this promising and versatile new cash crop.

Keywords Agroforestry · Anti-cancer · High performance liquid chromatography · Icacinaceae · Seed source · Sustainable harvesting

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10722-020-01048-6) contains supplementary material, which is available to authorized users.

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Introduction

With a global turnover of over two billion US dollars, camptothecin (CPT) has become the third most important anti-cancer chemotherapeutic of this century (Lorence and Nessler 2004; Shaanker et al. 2008). CPT was first isolated from *Camptotheca acuminata* Decne. (Icacinaceae), a tree native to China and later cultivated in the Nearctic regions. China has dominated the production and trade of CPT for several years (Wani and Wall 1969). There has been a drastic reduction in the Indian substitute source for CPT, i.e.,

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wild populations of *Nothapodytes nimmoniana* J. Graham (Icacinaceae), due to the indiscriminate and unsustainable harvesting since the last two decades (Shaanker et al. 2008; Ved et al. 2016). A few potential alternate plant sources of CPT viz., *Apodytes dimidiata* E. Mey. Ex Arn., *Codiocarpus andamanicus* (Kurz) R. A. Howard, *Gomphandra comosa* King, *G. coriacea* Wight, *G. polymorpha* (Wall.) Sleumer, *G. tetrandra* (Wall.) Sleumer, *Iodes cirrhosa* Turcz., *I. hookeriana* Baill., *Miquelia dentata* Bedd., *M. kleinii* Meisn., *Natsiatum herpeticum* Buch.-Ham. ex Arn., *Pyrenacantha volubilis* Wight and *Sarcostigma kleinii* Wight & Arn., (all belonging to family Icacinaceae) have also been recently identified from India (Ramesha et al. 2013). Among them, *P. volubilis*—a climbing shrub native to south and south eastern parts of India, is the richest source of CPT possessing an appreciable amount of 1.35% in its seed cotyledons (Padmanabha et al. 2006; Suma et al. 2014).

In view of the high demand for this plant-based anti-cancer drug, coupled with the short supply from natural habitats due to ban on green harvesting from the wild in India, there is an urgent need for domestication of high-yielding plant sources of high-value chemotherapeutics such as CPT (Suma et al. 2014). Despite several other wild sources for CPT being available (Ramesha et al. 2013), not many of them have been domesticated and deployed into farmers' field or for industrial level captive CPT production systems. *P. volubilis* may be considered for multi-locational species suitability trials around the globe for large-scale commercialized cultivation, in the shaded niches of agroforests where *Camptotheca acuminata* and *Nothapodytes nimmoniana*, the original source of CPT cannot thrive (Suma et al. 2014). As a pioneering step in this direction, a rapid domestication process was initiated for *P. volubilis* (Pownitha 2017; Ramachandran 2017; Shaanker et al. 2018), the present work being a part of the same project, "Biotechnological interventions for conservation and utilization of forest resources" funded by Department of Biotechnology, Government of India. Since this is an initial attempt to domesticate *P. volubilis* much attention has been given to the identification of seed source variations for growth and CPT yield traits.

Seed source studies are fundamental to any tree improvement programme and for domestication of a species (Zobel and Talbert 1984). It provides an idea about the range of variations, kind and nature of genetic control of traits in the species. It facilitates identifying superior genotypes to be deployed for domestication. It leads to the development of local land races that suit a specific geographic condition to which planting out is proposed (Zobel and Talbert 1984). Several reports have clearly suggested that seed sources differ significantly in their metabolite content and are vital for genetic improvement (Liu and Adams 1998; Liu et al. 2002; Koul 2011). Indeed, identification of a good seed source/provenance is the quickest, cheapest and most economical way of achieving genetic improvement (Zobel and Talbert 1984). In this study, based on seed source testing in a common garden experiment, potential and possibility of promoting the non-destructive harvesting of parts such as seeds (as opposed to bark harvesting in *N. nimmoniana*) to isolate CPT and cultivation of the high CPT-producing species i.e. *P. volubilis* as a cash crop is analysed. Additionally, based on the findings of the present study and our initial studies, an optimal scheme for the harvesting of seeds of *P. volubilis* for CPT extraction is discussed.

Materials and methods

Natural populations of *P. volubilis* were identified (Table 1, Fig. 1) through literature survey of published flora including Fischer (1917), Pate (1917), Fischer (1921), Bhuvaneswari (2003), Reddy and Parthasarathy (2006) and Amirthalingam (2008). An ecological exploration was undertaken in these sites to locate the scanty natural populations and repeated field visits were done to collect the fruit samples. Matured and healthy fruits were handpicked from the labeled lianas from different locations and when the lianas were beyond reach, the fruits were collected by climbing the supporting tree. Fallen and damaged fruits were excluded from the collection. The collected fruits were immediately transferred into zip lock covers. A bulk seed collection was also made from each population. The collected fruits were soaked in tap water for few hours and rubbed against a wet cotton cloth to remove any traces of pulp from the fruit. After de-pulping, the seeds were obtained and were treated with 0.1% Carbendazim (Bavistin—Carbendazim 50% WP) to prevent possible fungal attack. Maintaining the identity, the seeds were then air dried for 2 days. The seed handling procedure was

Table 1 Latitude, longitude, altitude, rainfall and temperature details of various seed sources of *Pyrenacantha volubilis* considered in the study (Source of climatic data: https://en.climate-data.org/location/)

Sl. No.	Seed source	Latitude	Longitude	Altitude (m)	Average annual temperature (°C)	Average annual precipitation (mm)
1	Walajabad, Tamil Nadu (TN)	12°46′37.2″N	79°51′29.1″E	107	28.3	1083
2	Pazhaiyasivaram, TN	12°46′19.4”N	79°51′59.7″E	64	28.3	1083
3	Vandavasi, TN	12°46′15.4″N	79°53′46.5″E	45	28.3	1083
4	Villiampakkam, TN	12°45′07.2″N	79°55′56.6″E	40	28.3	1083
5	Pondi, Puducherry	12°03′59.4″N	79°52′04.6″E	9	28.3	1171
6	Puthupattu, TN	12°03′29.0″N	79°52′04.4″E	11	28.3	1171
7	Mangalam, TN	11°54′10.2″N	79°44′23.2″E	13	28.3	1171
8	Kizhoor, TN	11°53′12.4″N	79°40′50.9″E	27	28.3	1171
9	Karukkai, TN	11°44′38.1″N	79°28′51.4″E	52	28.2	1252
10	Otteri, TN	11°44′21.1″N	79°42′27.0″E	14	28.2	1252
11	Vallathirakottai, TN	10°19′36.9″N	78°52′59.6″E	76	28.6	910
12	Kothakurichi, TN	10°18′53.5″N	78°54′46.2″E	104	28.6	910
13	M. Kottai, TN	10°18′21.2″N	78°53′59.3″E	78	28.6	910
14	Thiruvananthapuram, Kerala	10°32′52.3″N	76°16′45.3″E	50	26.7	1774

followed as per Ramachandran and Vasudeva (2020). Seed germination traits and seedling growth performance were studied in separate experiments, the seed collection made in 2016 and 2015 respectively. Observations on phenology and sex ratio were taken on 60 reproductively mature lianas in the common garden (i.e. part of the experimental material for seedling growth performance), belonging to seven seed sources viz. Thiruvananthapuram, Villiampakkam, Mangalam, Kizhoor, Kothakurichi, Otteri and M. Kottai.

Seed germination trial

The seeds from eight sources viz. Karukai, Pazhaiyasivaram, Pazhaiyasivaram (b), Pondi, Puthupattu, Vandavasi, Villiampakkam and Walajabad, collected in the 2016 collection expedition were sown in root trainers containing a potting medium of sand and soil in 2:1 ratio to study the germination characteristics at the nursery of the College of Forestry, Sirsi (14.6196 °N, 74.8441 °E, 792 m above MSL). The site has an annual precipitation of 2528 mm, and an annual mean temperature of 24.4 °C (Source: https://en.climate-data.org/asia/india/karnataka/sirsi-24141/). The coldest month is December, and the warmest month is May. Each seed source was represented by 50 seeds (10 seeds in 5 replications). No pre-treatment was given to the seeds as a precaution against the confounding of the genetic potential by external factors in the seed source trial. Adequate water was sprinkled every day. No supplemental fertilizers were used. In order to provide warm and humid conditions congenial for germination of the seeds, the root trainers were placed inside a poly-house. Germination readings were taken daily. The following germination traits were recorded/ calculated:

1. Germination per cent = (Number of seeds germinated until the 120nd day after sowing ÷ Number of seeds sown) × 100
2. Germination capacity = (Total number of seeds germinated irrespective of time ÷ Number of seeds sown) × 100

Seedling growth trial

In the common garden experiment, growth performance of one-year old seedlings in terms of ground diameter (mm), shoot length (cm), internodal length

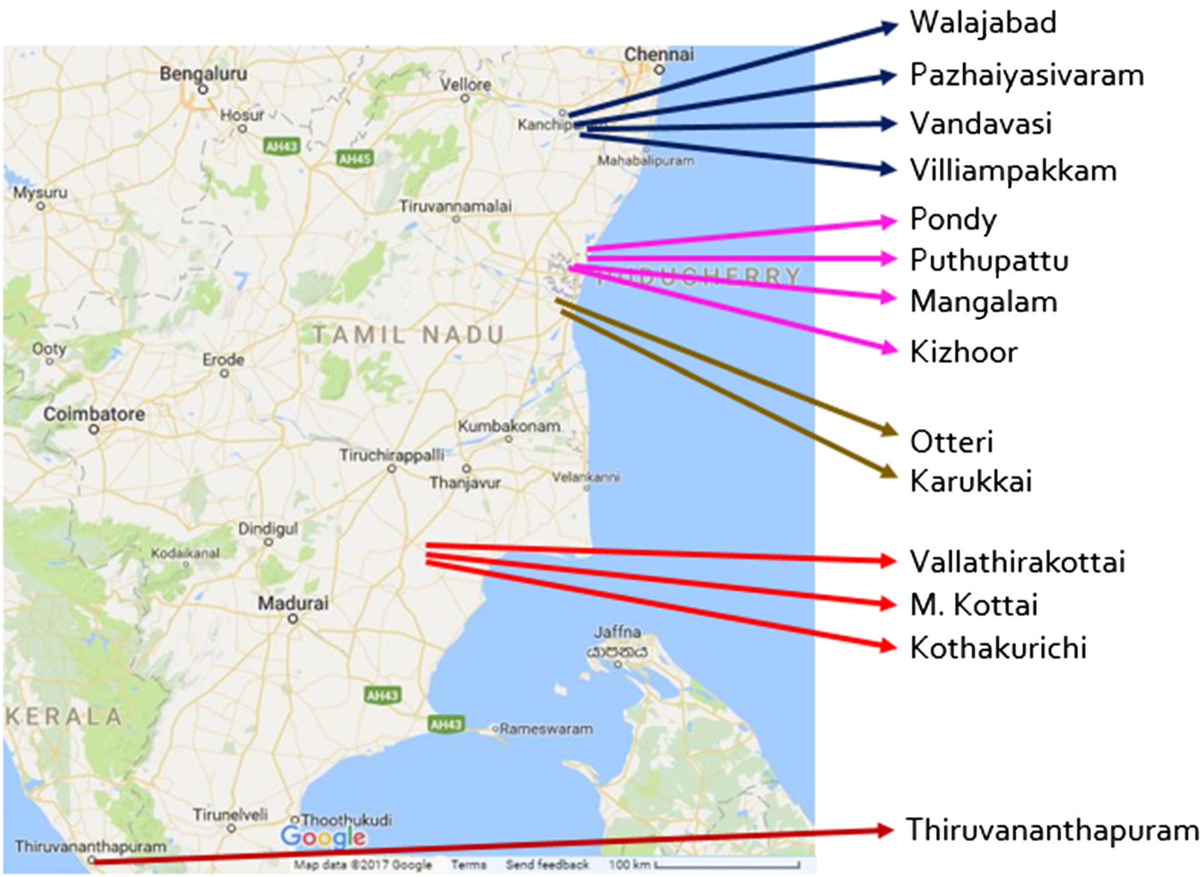

Fig. 1 Locations from which experimental material has been collected for this study

(mm), number of leaves and number of branches were recorded every 3 months starting from March 2016 (subsequently in July 2016, November 2016 and March 2017). Sturdiness index as suggested by Roller (1976) was computed as the ratio of shoot length (cm) to ground diameter (mm). The seedlings for this experiment were obtained from a collection in February 2015. There were seven seed sources (Thiruvananthapuram, Villiampakkam, Mangalam, Kizhoor, Kothakurichi, Otteri and M. Kottai) for this experiment designed in a Completely Randomised experimental Design (CRD) with three replications. Seedlings were maintained in cement pots (0.24 m $\times$ 0.24 m $\times$ 0.4 m) spaced 0.3 m apart and grown in the nursery. Seeds obtained from five of these seed sources were used for comparison of CPT yield from seed.

Comparison of seed sources for CPT yield traits

Five seed sources (Kizhoor, Mangalam, M. Kottai, Otteri, Villiampakkam) from the common garden experiment were selected for comparison of CPT content of whole seed. These sources were selected based on precocity of flowering/ fruiting (fruit set in the first year itself) and availability of adequate quantity of seeds (minimum ten per plant). The data on the whole seed CPT content of seed sources Puthupattu, Vallathirakkottai and Pazhaiyasivaram was not used for the discussion on seed CPT yield because enough seeds could not be retrieved from those sources. High Performance Liquid Chromatography (HPLC) technique was employed to quantify the CPT present in the whole seeds of *P. volubilis* as per Suma et al. (2014, 2017). The chromatographic conditions provided for the HPLC (LC-20 AD, Shimadzu, Japan) analysis were: Phenomenex RP-C18 (250 $\times$ 4.6 mm, 5 μ size), SPD-M20A photodiode array detector, 254 nm wavelength for CPT, 1.5 mL/min flow rate, 20 µL injection volume, mobile phase consisting of 25% acetonitrile (Pump A) and 75% water +0.1% trifluro-acetic acid (Pump B) in an isocratic mode. 1 mgL^{-1} CPT (Sigma grade pure) stock was prepared in methanol and DMSO (3:1 *v*/v) and serially diluted for standard curve calibration. 100 mg finely powdered whole seed samples were carefully mixed with 5 mL of 61% of ethanol. The solution was kept in a water-bath at 60 °C for 3 h, with intermittent, vigorous shaking of the tubes every 30 min. At the end of 3 h, the tubes were kept still to allow the fine powder to settle. 1 mL of the supernatant was pipetted out into 2 mL Eppendorf tubes. Overnight cooling was done at 12 °C. The samples in the Eppendorf tubes were thawed and centrifuged (Mini Spin Plus, Eppendorf) at 23 °C for 10 min at 10,000 rpm. The HPLC columns were cleaned and run with ethanol as control. Sample was then used to clean the loop without loading. The samples were then injected one by one and then loaded to get the peaks in the Post Run Analysis software. The standard of CPT was run and the peak was compared to the peaks in the samples for the same retention time (11 min) and flow rate (1.5 mL/min). The areas of the peaks were calculated using the Post Run Analysis software by comparison with CPT standard regression curves. Based on the areas, the % CPT was estimated using the standard formula:

$$\begin{aligned}\%\mathrm{CPT} = {} & (\text{Sample Area}/\text{Standard Area}) \\ & \times (\text{Standard Weight}/\text{Standard Dilution}) \\ & \times (\text{Sample Dilution}/\text{Weight of sample}) \\ & \times \text{Purity of Standard}.\end{aligned}$$

The descriptive statistics for germination traits, correlation analysis between seed weight and seedling growth traits and Analysis of Variance (ANOVA) of the CRD experiment were done with the use of MS Excel.

Results and discussion

Our experience in the field over 5 years suggest that the natural populations of *P. volubilis* bear fruits during September–October in Kerala state (along the west coast of the southern Indian peninsula) and during December–January in Tamil Nadu state (along the east coast). However, the 60 reproductively mature lianas grown in common garden experiments at the College of Forestry, Sirsi, which is situated 2 degrees north of the natural distributional range, showed extended periods of flowering and fruiting throughout the year. There were two peaks for flowering during January–February and September–October. There were also two peaks in fruiting during the months of March–May and November–December with sporadic reproductive activity during other months throughout the year. It was also observed that the male flowers were initiated prior to female flowers but remained for the whole of the flowering period. The plants showed a sex ratio of 10:9 (female:male), the sample set being the 60 reproductively mature plants grown for the common garden experiment. We also report for the first time, the observation of one monoecious individual (Fig. 2).

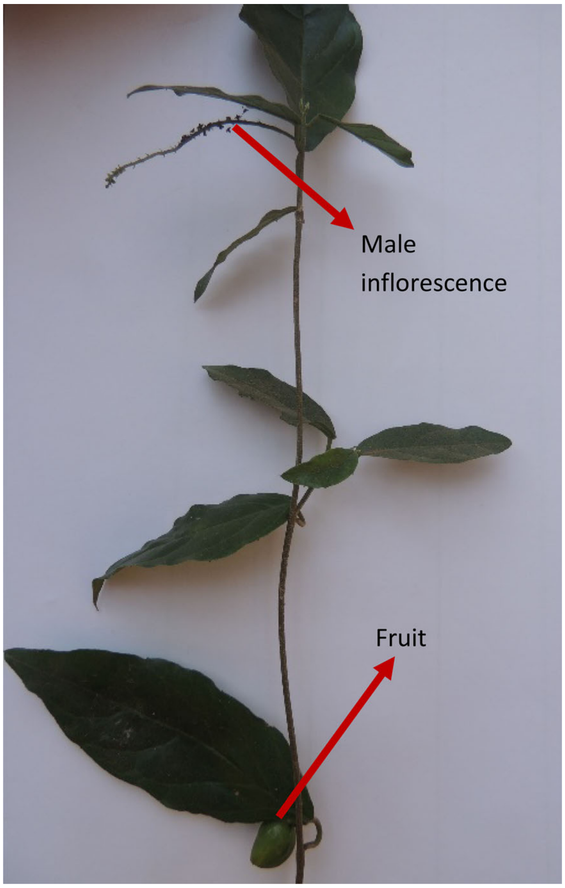

Fig. 2 A monoecious plant of *Pyrenacantha volubilis*

Seed source variations for germination traits

The best performing source with respect to seed germination characteristics (Table 2) was Pazhaiyasivaram (70% germination percentage and 80% germination capacity). Another sub-population of Pazhaiyasivaram i.e. Pazhaiyasivaram (b), a dust-ridden patch, scored least for all the germination related parameters. The second-best performing seed source was Villiampakkam (37.22% germination percentage and 40.65% germination capacity). Hence, Pazhaiyasivaram population is undoubtedly the best seed source for production of large number of seedlings for mass propagation of the plant. This experiment did not involve the use of any pre-germination treatment, which could have confounded the genetic potential of the populations for better germination. Pownitha (2017) has reported the maximum germination characteristics in a bulked seed lot (source unknown) of *P. volubilis* by soaking seeds in GA_3 (100 mgL^{-1}) for 12 h. Hence, further trials involving Pazhaiyasivaram and Villiampakkam seed sources treated with GA_3 can improve germination traits.

Seed source variations for seedling vigour traits

Seedlings obtained from Villiampakkam source were better than other sources for ground diameter (2.99 mm), number of leaves (24) and number of branches (2.95). Mangalam seed source yielded the longest (51 cm) and sturdiest (20.28 cm/mm) seedlings. Thiruvananthapuram source produced seedlings with the lankiest growth, with an average internodal length of 29.41 mm (Table 3). Shoot length, number of leaves and number of branches varied seasonally showing consistent rise during November (north-east monsoon) to March which coincided with the rainy season of the seed sources (Supplementary material: S1-S5). Seed weight showed statistically significant positive correlation with ground diameter (Karl Pearson's correlation coefficient, r = 0.37; number of data points, *n* = 51), shoot length (r = 0.29, n = 51), number of leaves (r = 0.34, n = 51) and number of branches (*n* = 0.35, n = 51) in the seedlings (Figs. 3, 4, 5, 6). Thus, seed weight could act as an efficient morphological marker for selecting seedlings superior for growth traits as has been reported in diverse taxa such as *Hordeum vulgare* L. (barley), *Vicia sativa* L., *Artocarpus heterophyllus* Lam. and *Prunus jenkinsii* Hook. f. & Thomson (Boyd et al. 1971; Acikgoz and Rum-Baugh 1979; Khan 2004; Upadhaya et al. 2007).

Seed source variations for CPT

HPLC analysis of powdered whole seeds revealed that maximum CPT (1.06% *w*/w) is accumulated by the Puthupattu population, followed by Pazhaiyasivaram (1.02% w/w), M. Kottai (0.91% w/w), Villiampakkam (0.85% w/w), Otteri (0.66% w/w), Vallathirakottai (0.66% w/w) and Mangalam (0.51% w/w) sources while the least content was from Kizhoor (0.48% w/w) population (Fig. 7). This clearly showed the genotype-dependent effect on CPT content in *P. volubilis* seeds. Similar genotype-dependent variation in CPT accumulation has been described in *Camptotheca* (Liu et al. 2002; Wang et al. 2014) and *Nothapodytes nimmoniana* (Padmanabha et al. 2006).

Table 2 Germination characteristics of seed sources of *P. volubilis*

Seed source	Test weight (g)	Germination per cent (%)	Germination capacity (%)[a]
Karukkai	162.9	23.33	26.67
Pazhaiyasivaram	136.9	70.00	80.00
Pazhaiyasivaram (b)	136.6	6.67	6.67
Pondi	149.7	15.88	25.40
Puthupattu	125.7	20.00	21.11
Vandavasi	127.9	26.67	40.00
Villiampakkam	143.5	37.22	40.65
Walajabad	134.4	20.00	40.00
Mean	–	27.05	35.06
S.Em. ±	–	6.76	6.42
C.D. at 5%	–	20.49	19.48

[a]Number of seeds germinated until the 180th day after sowing

Table 3 Growth parameters of seedlings of various seed sources of *P. volubilis*, 24 months after sowing

Seed source	N (number of seedlings)	Ground diameter (mm)	Shoot length (cm)	Internodal length (mm)	Number of leaves	Number of branches	Sturdiness index (cm/mm)
Kizhoor	25	2.41 ± 0.09	22.71 ± 1.68	13.53 ± 1.16	16.83 ± 0.41	1.85 ± 0.14	9.47 ± 0.84
Kothakurichi	25	2.63 ± 0.18	29.44 ± 7.04	16.33 ± 1.30	17.68 ± 3.00	1.90 ± 0.37	10.97 ± 1.90
Mangalam	25	2.57 ± 0.15	51.00 ± 7.91	21.86 ± 2.92	23.33 ± 1.94	2.38 ± 0.29	20.28 ± 4.01
M. Kottai	25	2.41 ± 0.14	31.16 ± 6.03	13.46 ± 1.60	22.77 ± 1.61	2.21 ± 0.14	12.76 ± 1.82
Otteri	25	2.85 ± 0.14	30.29 ± 7.02	17.40 ± 4.16	17.61 ± 1.34	1.47 ± 0.29	10.90 ± 3.10
Thiruvananthapuram	25	2.17 ± 0.05	35.15 ± 2.70	29.41 ± 2.25	11.63 ± 0.80	2.70 ± 0.13	16.22 ± 1.46
Villiampakkam	25	2.99 ± 0.26	49.11 ± 4.68	20.62 ± 2.17	24.00 ± 2.01	2.95 ± 0.66	16.46 ± 1.10
Mean		2.58	35.55	18.94	19.12	2.21	13.87
S.Em. ±		0.16	5.84	2.56	1.81	0.32	2.31
C.D. at 5%		0.51	17.99	7.90	5.59	1.00	7.11

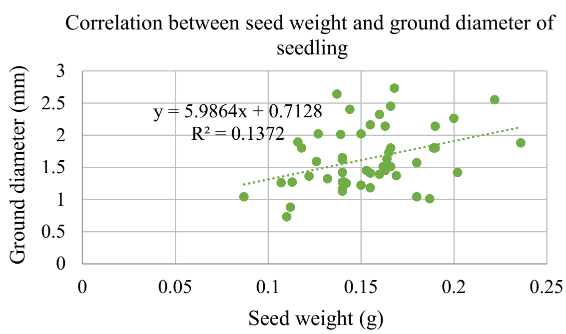

Fig. 3 Correlation (r = 0.37) between seed weight (g) and diameter (mm) of seedling at ground level

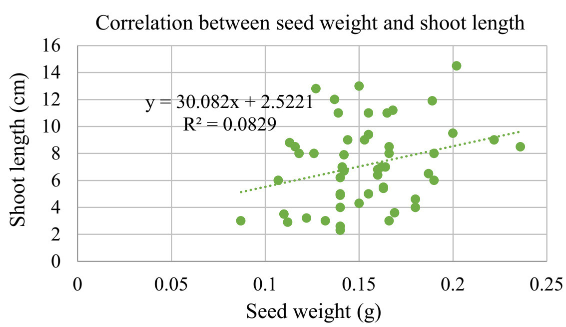

Fig. 4 Correlation (r = 0.29) between seed weight (g) and length (cm) of seedling from ground

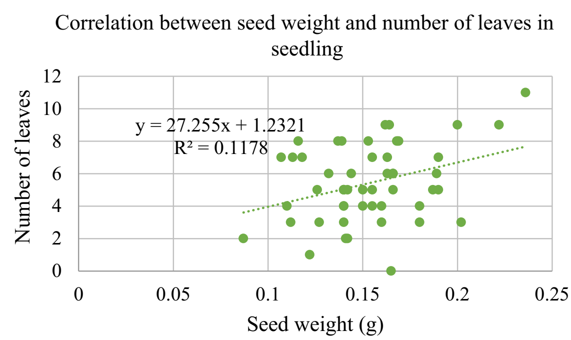

Fig. 5 Correlation (r = 0.34) between seed weight (g) and number of leaves in the seedling

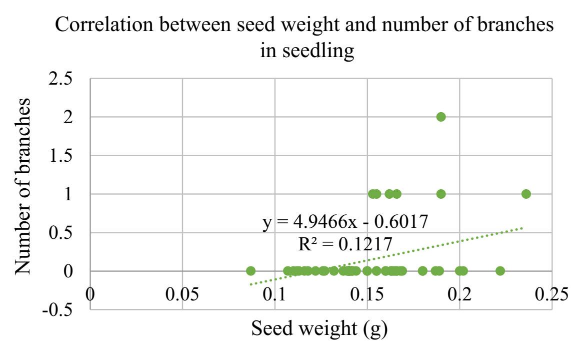

Fig. 6 Correlation (r = 0.35) between seed weight (g) and number of branches in the seedling

Clinal variations were observed for most of the characters studied. Southern populations were found to have larger fruits, seeds and greater pulp yield compared to the northern populations. In terms of the other characters like germination percentage, ground diameter, shoot length and whole seed CPT content (Fig. 8), the northern populations were found to be better (Ramachandran 2017).

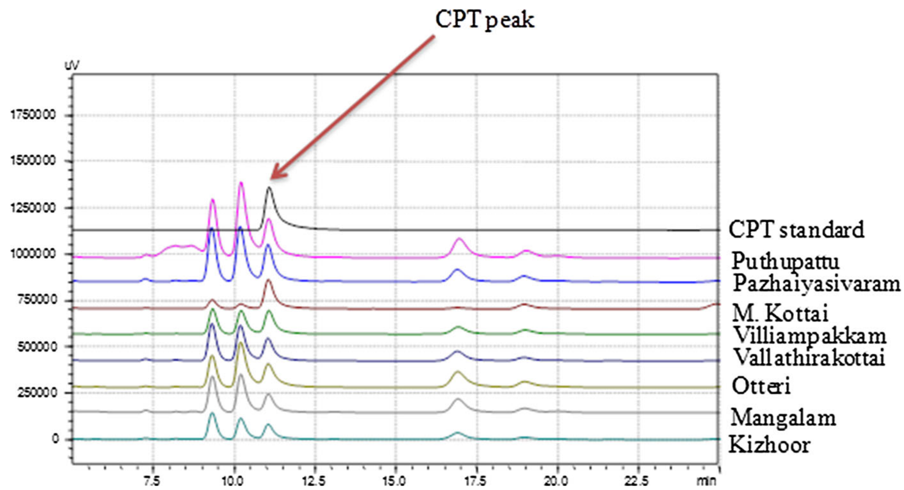

Fig. 7 HPLC chromatogram of whole seed samples of various seed sources with standard CPT curve

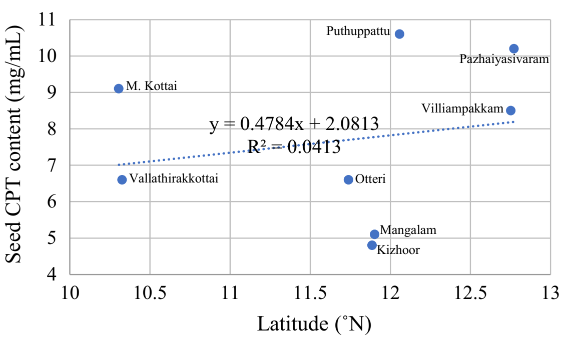

Fig. 8 Clinal variation for CPT content of whole seeds of *P. volubilis*

Domestication of *P. volubilis* and expected CPT yield

P. volubilis is a perennial liana (woody climber), tolerant to shade, making it a potential component in multi-tier agroforestry systems such as tropical home gardens and a suitable intercrop in shaded monocultures such as rubber and coconut plantations (Pownitha 2017; Ramachandran 2017). It is a versatile plant responsive to the training given, i.e. it can grow as a climber and as a creeper depending on the availability of support. *P. volubilis* is tolerant to drought, being able to survive under minimal water conditions as long as the soil has adequate organic matter content, although luxuriant vegetative growth can be observed at field capacity (Pownitha 2017). *P. volubilis* is a good coppicer, hence amenable to frequent pruning operations. This ability can be utilised to repeatedly harvest the shoot biomass to sustainably harvest maximum amount of CPT from the shoots as well as seeds and fruit pulp (Ramachandran 2017).

The results of the seed source test provided for the first-time, an evidence for variations in CPT concentrations of *P. volubilis* under a common garden experiment. Almost three times variation was identified in the test. Earlier, significant seed source differences for fruit and seed traits in *P. volubilis* were reported by Ramachandran and Vasudeva (2020). It was shown that Kizhoor seed source was superior to other seed sources tested, in terms of test weight (171.5 g) (Ramachandran 2017), while Villiampakkam seed source was better in terms of total yield per plant (Table 4). The product of seed yield per plant, average CPT content in seeds and the total number of plants per hectare (ha) provides an estimate of the total CPT yield per ha from different seed sources. Planting at a spacing of 1 m × 1 m and assuming that only half of the 10,000 plants in a hectare bear fruits (1:1 sex ratio), the variation in per ha CPT yield among seed sources can be realized by harvesting only the seeds from the third year is shown in Table 4. Since total CPT yield is a product of seed production and the associated CPT concentration, seed sources that have larger seed yield per plant and higher CPT concentration are desirable. In this regard, seed source Villiampakkam showed the highest potential of 223.125 g CPT / ha, while the lowest yield of 81.9 g per ha was observed in M. Kottai seed source. Despite M. Kottai being the richest source, which provides seeds with the highest concentration of CPT (0.91% *w*/w), its abysmally low seed yield (1.8 g dry weight per female plant), makes its potentiality poor with respect

Table 4 Estimated camptothecin yield from different seed sources

Seed source	CPT content in seeds (% w/w)	Seed yield per female plant (dry weight in g)	CPT yield based on laboratory extraction procedures (g per ha)
Kizhoor	0.48	4.76	114.24
Mangalam	0.51	4.32	110.16
M. Kottai	0.91	1.8	81.9
Otteri	0.66	3.06	100.98
Villiampakkam	0.85	5.25	223.125

to net CPT production (81.9 g/ha) per unit area of the farm. Villiampakkam seed source is hence best in terms of seed yield per plant. Further, per ha CPT yield from the fruits would increase every year with growth making it sustainable. Since it has already been shown that CPT content is highest in mature but unripe fruits (green colour) compared to young or fully ripened fruits (yellowish colour) in *P. volubilis* by Suma et al. (2017), it is also important to harvest the seeds when it is at mature but unripe green stage.

CPT extraction from fruits/ seeds of domesticated populations of *P. volubilis* is more sustainable than the bark harvesting currently advocated for the wild populations of *N. nimmoniana* (Ramesha et al. 2008). Additionally, being a shade tolerant plant, a good coppicer and being amenable to training and pruning, *P. volubilis* could be grown at closer spacings or incorporated into other compatible cropping systems like *Myristica fragrans* Houtt., to realise net yields much greater than that computed in this study (Ramachandran 2017). Hence, there is a huge opportunity for the incorporation of this species into multi-tier agroforestry systems and to be grown along bunds and farm boundaries. Incorporation of *P. volubilis* into the cropping systems is not at the expense of the existing crops. Hence, the returns obtained are only cumulative to the existing corpus of the farmers' income.

In the Western Ghats region in order to isolate 1000 kg of CPT, about 1–1.5 million kg of *N. nimmoniana* wood chips is required to be harvested from natural population. About 0.5–0.7 million kg of wood chips of *N. nimmoniana* are harvested from wild sources annually for domestic consumption and export (Patwardhan 2006). This has led to a declined forested area and tree individuals by 50–80% in the last decade alone (Kumar and Ved 2000; Ved et al. 2016). In this backdrop, promotion of extraction of CPT from seeds through captive cultivation of the best seed source of *P. volubilis* is a significant step towards mitigating the irrational harvest of other CPT yielding species such as *N. nimmoniana*. Hence promoting sustainable harvesting of seeds of *P. volubilis* to isolate CPT could be a promising alternative. Since CPT is an important, naturally occurring compound for the production of several semi-synthetic CPT drugs required in large quantities by the pharmaceutical industry, it is important to maximise sustainable CPT yield. In this regard, growing Villiampakkam seed source at a spacing of 1 m × 1 m and harvesting seeds from the third year onwards would be optimal. Pre-sowing treatment should be advocated to achieve maximal germination characteristics so as to ensure availability of enough planting material (Pownitha 2017). Application of biofertilizers has been found to increase the vegetative growth as well as seed yield (Pownitha 2017). We would like to caution the readers on an important caveat that the Genotype x Environment effect of the genotype on the effects of pretreatments and fertilisers has not been explored in our study. Weeding is an important intercultural operation that could aid the growth and seed yield of *P. volubilis* (Pownitha 2017). Pruning can be done with the onset of pre-monsoon showers to increase the number of branches (thereby potential inflorescence bearing nodes) of the plants (Pownitha 2017; Ramachandran 2017). The key to successful cultivation of *P. volubilis* is to never expose the plants to full sunlight.

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Complaince with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This paper complies with all the ethical standards mentioned in the "Instructions to Authors" section of the journal's homepage.

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