Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 157-162
Received: 13-05-2020
Accepted: 15-06-2020

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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

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Abstract
Saraca asoca (Roxb.) de Wilde., (Ashoka) is one of the commercial important medicinal tree species of the Western Ghats that needs conservation attention. In the present study, germplasms from seven different seed sources of Ashoka were collected in the Konkan region of Maharashtra to study the variation in seed traits, germination and seedling vigour. Result showed that there was a significant variation for seed traits (length, thickness and weight) and seedling growth attributes (height, collar diameter, root length and number of leaves). Similarly, seed germination also varied significantly (54.8 to 100%; $P<0.05$) among seven seed sources. Germplasm collected from Dodamarg location resulted in superiority for seed size, germination and seedling growth attributes. Seed length and weight followed normal distribution and significant-positive relationship was recorded between them. The information generated in the study shows that there is potential scope for further improvement and conservation of Ashoka in the Konkan region of Maharashtra.

Keywords: Ashoka, dormancy, germination, *Saraca asoca*, seed traits, seedling growth

Introduction
Saraca asoca (Roxb.) de Wilde. (Ashoka tree; Family-Legumonosae, Sub-family Caesalpinioideae) is one of the ecological important tree species of Western Ghats, India and having potential medicinal use. This plant is mainly distributed in tropical evergreen forests, especially along the stream. This species also occurs in cluster in the sacred groves, traditionally conserved natural forests [1]. The bark is used as major source for medicinal formulations by the pharmaceutical industries, where the bark contains several phyto-chemicals like Epicatechin, Procyanidin B2, 11′ deoxyprocyanidin B2, polymeric procyanidins, saracin and others [2, 3]. *Ashoka-arishtha, Ashoka-kalpa, Mensta, Ashotone* are commercial powder formulations prepared by many pharmaceutical industries using bark of this species. In Ayurveda, the bark is potentially used for curing many women disorders [4].
Due to these potentialities, bark of this species has attracted high commercial value. There is a great demand for dry bark biomass from various pharmaceutical industries. It is estimated that, irrespective of species, the demand for the bark biomass is approximately 10,724 tonnes during 2004-05 with annual growing rate of about 15 per cent [5]. Unfortunately, the present demand for the bark is mostly met from the wild populations. Due to this, natural habitat of this species is depleting year by year and it needs attention for conservation ashoka trees and its natural habitat [6]. Presently, *Saraca asoca* is considered as 'Threatened-Globally' by IUCN (IUCN, 2008) and as 'Endangered-Locally' by Conservation Assessment and Management Planning [5]. Therefore, domestication of this species is essential for commercial harvesting of Ashoka bark for pharmaceutical industrial use as well as conservation.
Mapping and capturing of seed source variation for various commercial traits including reproductive traits are fundamental steps for tree domestication. Further, this data can be used for further selection and multiplication of superior genotypes for commercial plantations [7, 8, 9]. Such information is scanty among the populations of *Saraca asoca* in the Konkan region of Maharashtra. Therefore, the present study was undertaken to understand the seed source variation for seed and seedling morphological attributes among natural populations of Ashoka.

Materials and Methods

Mapping of *Saraca asoca* populations was undertaken in Konkan region of Maharashtra. Total seven distinct locations were earmarked for the study. Due to its conservation status, only required quantity of pods were collected from healthy trees in identified natural populations during June to July 2011. Both laboratory and nursery experiments were undertaken in the College of Forestry, Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra.

After collection, pods were shade dried for a period of seven days and seed-lot wise seeds were extracted from open pods, mixed properly and labelled. Later, these labelled subsets were used to record various seed traits *viz.* seed weight (g), length (mm) and thickness (mm). On other side, germination trial was laid-out at open area by raising nursery bed in the forest nursery. For each population, 200 seeds (50 seeds of 4 replications) were sown following completely randomised design. Germination count was recorded up to 45 days from the date of sowing. Later, location wise seedlings were transplanted from nursery beds to polybags of size 4″x 6″ filled with potting mixture containing soil, sand, FYM in the ratio of 2:1:1. The first seedling growth was recorded at 5 months after transplanting in the nursery. Data were subjected to statistical analysis using statistical software and analysis of variance (ANOVA) was performed for all the studied characteristics. Regression analysis with polynomial type was used to understand the association between seed traits and germination and seedling attributes.

Results and Discussion

Saraca asoca is one of the leguminous tree species bears larger seeds than other legume tree species. Pod maturation occurs during rainy season from June to July. Present study showed a great significant variation (P<0.05) for seed traits, germination and seedling vigour among seven seed sources of *S. asoca.*

Wide range of variation was attributed for seed weight and it ranged from 6.2 to 12.4 g; similarly, seed length was also recorded huge variation with range of 29.4-40.6 mm (Table 1). Interestingly, seed thickness recorded little variation and it was significant among seed sources (20.0-24.1 mm; P<0.05). Morphological observation showed that seed lots collected from Dodamarg source produced relatively larger and bold seeds than other sources. Frequency distribution of seeds according to different size classes were analysed for seed weight, seed length and seed thickness using pooled seed data from all studied sources (Fig. 1). Graphs showed that there was a normal distribution for seed weight (mean and standard deviation of 8.1±3.44 g) and seed length (34.7±5.13 mm). However, seed thickness was slightly skewed towards higher thickness (22.1±3.34 mm; Fig 1). The relationship between seed length with seed weight and thickness was assessed using regression analysis with polynomial type. Result showed that seed length was positively related with seed weight (R^2=0.755) and seed thickness (R^2=0.563; Fig. 2). However, the relationship between seed length and thickness was weak (R^2=0.359).

There was a significant variation for seed germination and it varied from 54.8 to 100 per cent (Fig. 3). Seed lot collected from Chandranagar and Sadawali sources resulted in significantly higher germination (> 95 %) over Phookeri (61%) and Kesari (55%) sources. Seed traits does not show significant influence on seed germination. Significantly higher seedling height and root length was recorded for seedlot collected from Dodmarg source (20.0 cm and 20.7 cm, respectively) over other sources (P<0.5). Collar diameter also showed significant variation and it ranged from 2.0 to 3.5 mm. Even though Dodamarg population recorded lower number of leaves (6.3 leaves per plant), the performance in respect to their growth was superior. Moreover, seedlings raised from Hewale and Sadawali sources produced higher number of leaves per plant than other sources (Table 2).

Morphological variations in seed, germination and seedling growth attributes among the natural population are useful in selection programme for genetic improvement of forest species. Ashoka is one such species that gained commercial value and high traded medicinal tree species of the tropics [5]. Therefore, National Medicinal Plant Board (NMPB) recognized this species for its Research and Development. Patwardhan documented distribution and population status of *Saraca asoca* from Sahyadri–Konkan ecological corridor and they found that the size class distribution of Ashoka from the sacred groves differed both from formally protected sites and private forest that addresses different levels of disturbance problems [6].

Reproductive traits such as seed size play a vital role in assessment of genetic variation among natural population, provenances and/or genotypes and such kind of genetic variability has already been assessed for many tropical tree species [8, 9, 10]. In the present study, seed size variation as influenced by seed source was recorded and this is also true for germination and seedling vigour attributes. This could be due to ecological conditions that shaped the genetic variation in reproductive traits. Such variation in fruit, seed, germination and seedling vigour is recorded in forest tree species of conservation concern *viz.*, *Dysoxylum malabaricum* [11], *Mammea suriga* [12], *Nothapodytes nimmoniana* [13], *Dysoxylum binectariferum* [14], *Garcinia talbotii* [15] and *Sterculia urens* [16].

Deepa recorded significant variation in seed germination among six seed sources of Ashoka in Kerala and it varied from 37.8 to 98 per cent germination [17]. In this study, four out of seven sources recorded about 100 per cent germination within 45 days from sowing date; however, other population achieved more than 50 per cent germination within 45 days. This variation could be due to temporary dormancy as affected by individual seed source and local environment of habitat [18]. Further, un-germinated seeds were examined 60 days after sowing and result showed that some of the seeds were viable and remaining seeds were rotten. Later, these viable seeds were pre-treated with GA_3 solution @ 100 ppm for 6 hrs and sown them in the nursery. Result showed that these pre-treated seeds started germinating within five to ten days and completed 100 per cent germination within 23 days. This clearly shows that physiological dormancy could be the factor that delays seed germination in some of the seedlots. It is noted that secondary or induced dormancy do occurs in many species of Leguminosae, where seeds germinate readily when they young but develop strong seed-coat (physical) dormancy upon drying [18]. This may be the case in *Saraca* also. It is reported that seed size showed positive influence on seedling growth and vigour in *S. asoca* and other species from bulk collection [19, 20, 21]. Since seed source had greater influence on seed germination and seedling vigour; therefore, in the present study, the seed size had no direct relationship with germination and seedling vigour. Hence, seed size cannot be used as a criteria for grading of bulked seed lots of different seed sources/families/clones, as it can narrow down genetic diversity by rejecting small size of seeds [22]. Under this situation, grading of seedlot of individual seed

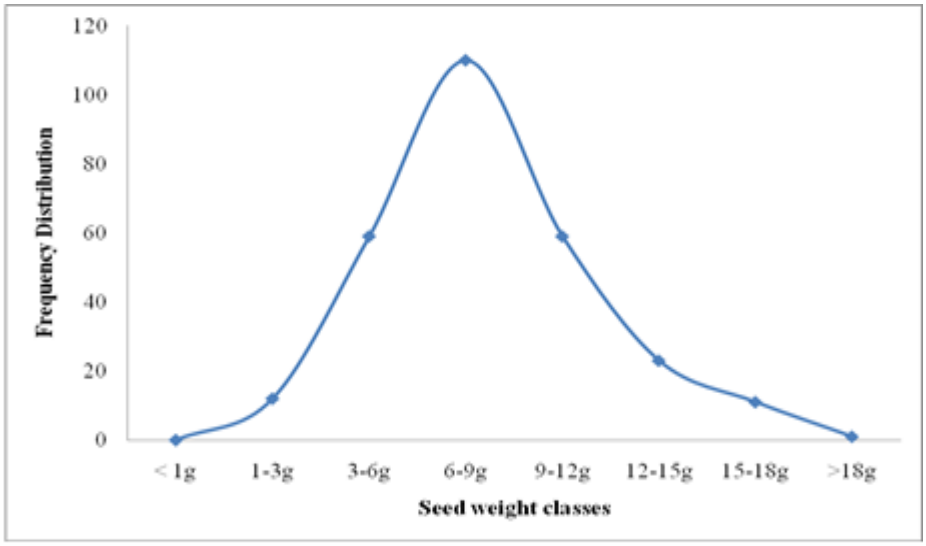
source/family/clones may be followed in order to obtain uniform seedlings in the forest nursery.

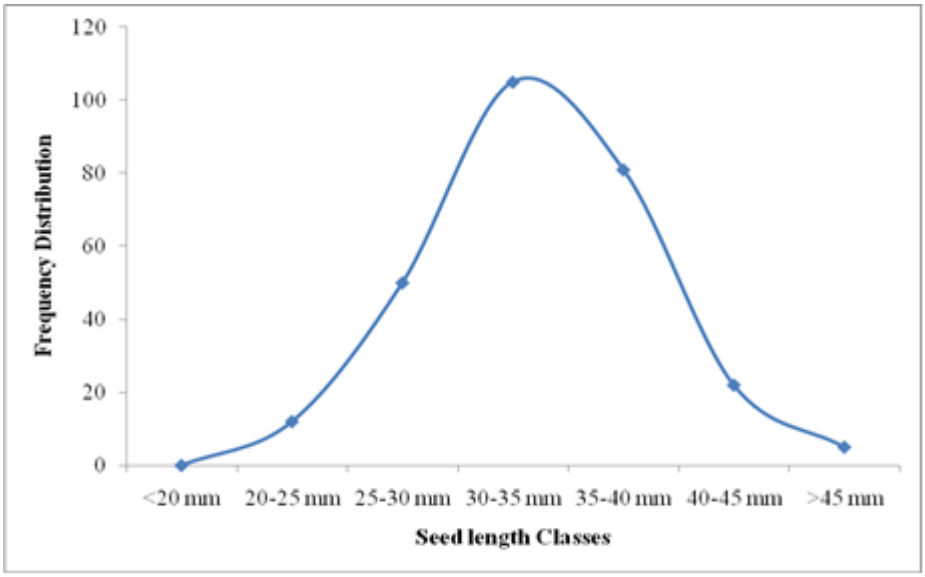
Table 1: Seed morphological variation from seven natural population of *Saraca asoca* in Konkan region of Maharashtra

Natural Population	Seed weight(g)			Seed length (mm)			Seed thickness (mm)		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Sadawali	2.91	13.99	8.1^{b}	24.69	42.26	33.9^{bc}	12.59	26.92	22.0^{ab}
Chandranagar	4.09	14.01	8.3^{b}	23.75	41.51	33.3^{bc}	17.7	26.96	22.0^{ab}
Kudawale	4.93	11.72	8.9^{b}	24.37	42.66	35.8^{b}	18.99	27.61	23.7^{a}
Dodamarg	4.25	19.71	12.4^{a}	32.24	47.38	40.6^{a}	14.1	35.06	24.1^{a}
Hewale	2.37	12.72	7.0^{b}	25.1	41.88	33.3^{bc}	17.13	54.55	22.5^{ab}
Phookeri	1.41	10.02	6.2^{b}	23.21	38.93	32.2^{bc}	13.04	25.46	20.7^{b}
Kesari	2.52	9.91	6.2^{b}	21.52	35.62	29.4^{c}	13.55	22.99	20.0^{b}
Mean			8.16			34.07			22.15
SEm (±)			0.94			1.36			0.83

Table 2: Seedling morphological variation in seven natural population of *S. asoca* in Konkan region of Maharashtra

Name of Population	Plant height (cm)	Collar diameter (mm)	Root length (cm)	No. of leaves
Sadawali	15.2^{b}	3.16^{ab}	12.1^{d}	10.7^{ab}
Chandranagar	13.1^{b}	3.09^{ab}	14.2^{cd}	12.3^{a}
Kudawale	13.2^{b}	2.87^{b}	13.3^{cd}	8.4^{bc}
Dodamarg	20.0^{a}	3.29^{ab}	20.8^{a}	6.3^{cd}
Hewale	14.8^{b}	3.56^{a}	13.8^{cd}	10.5^{ab}
Phookeri	12.0^{b}	2.73^{b}	15.5^{bc}	8.7^{bc}
Kesari	13.1^{b}	2.08^{c}	17.4^{b}	4.8^{d}
Mean	14.5	2.97	15.29	8.81
SEm (±)	1.18	0.18	0.73	1.10





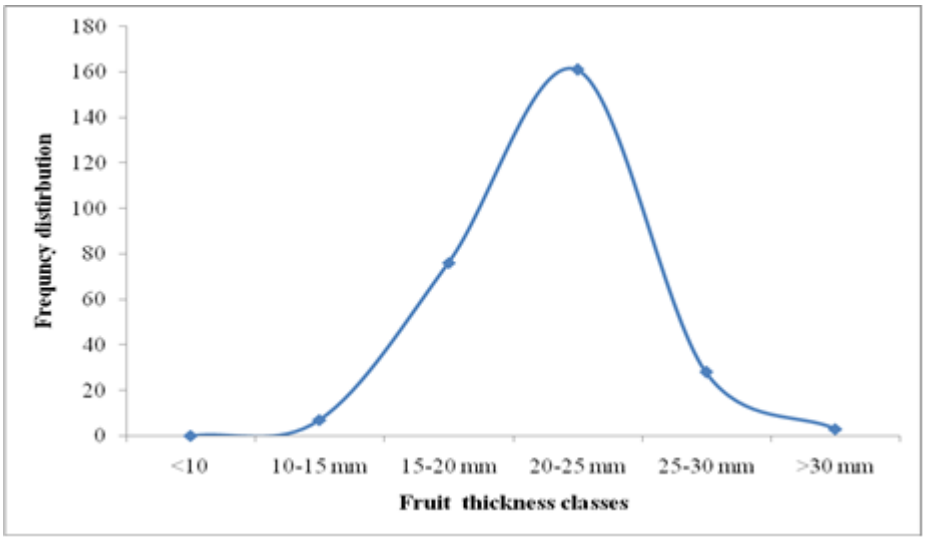
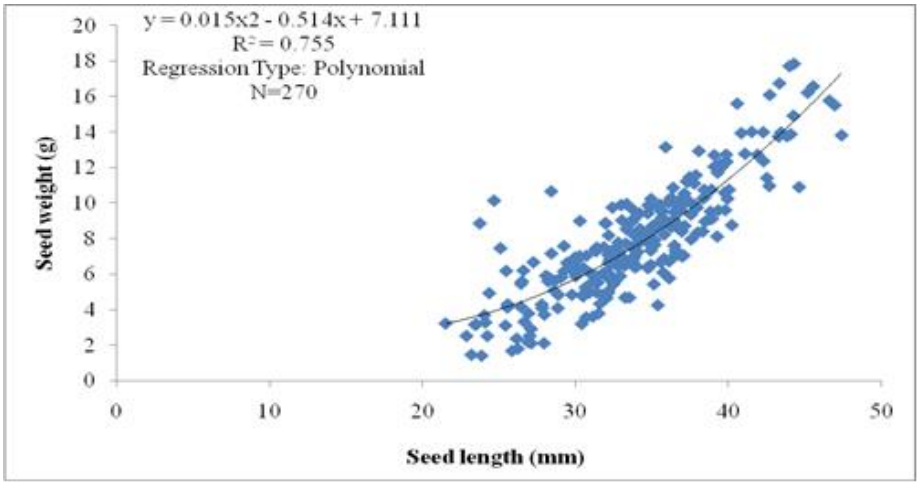
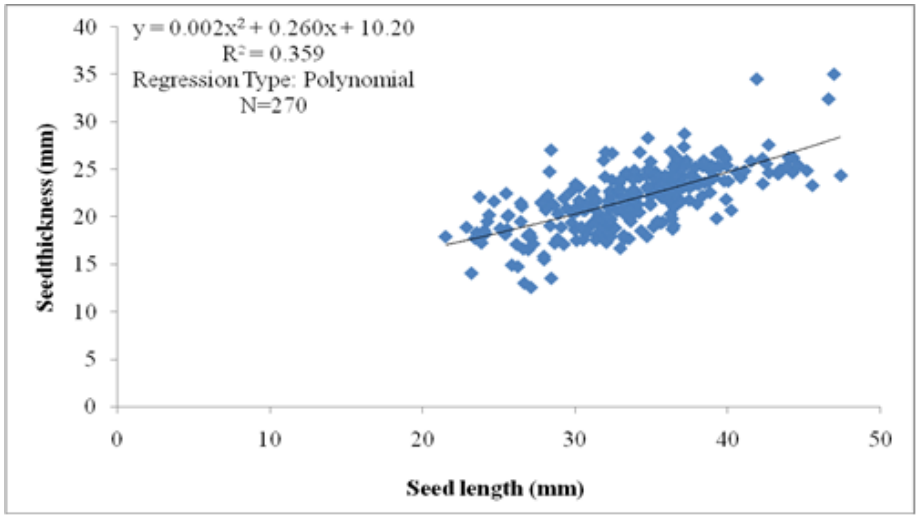


Fig 1: Frequency distibution of seedlot according to differnt seed size classes





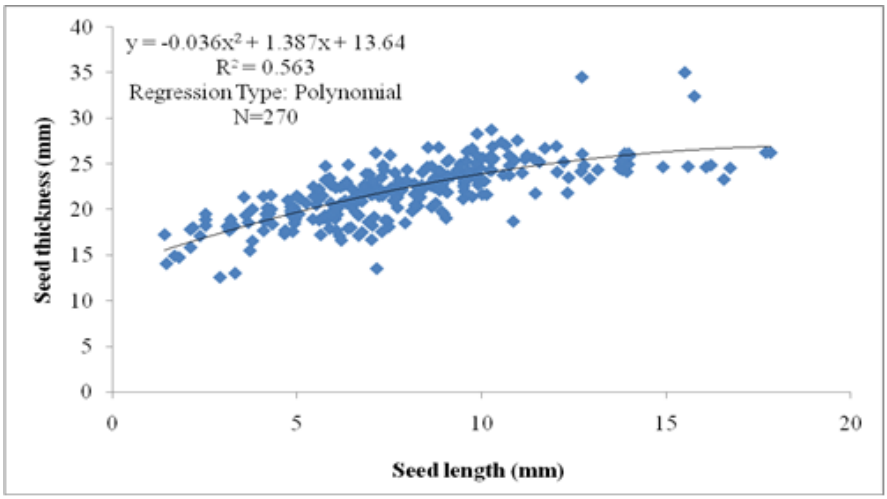


Fig 2: Relationship between various seed traits in *Saraca asoca*

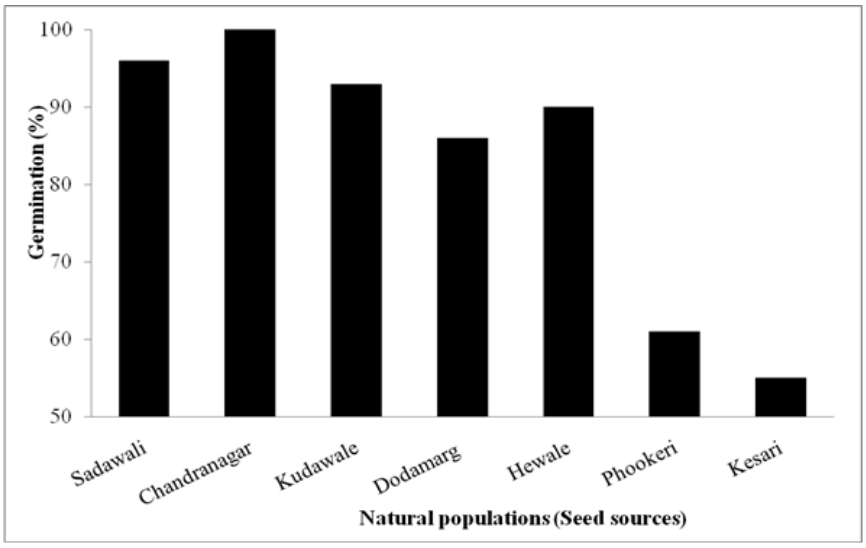


Fig 3: Variation in seed germination among different population of *Saraca asoca*

Plate 1: Mature pods with seeds, Green pods and seeds of *Saraca asoca*

Conclusion

Study showed a significant influence of natural population on seed traits, germination and seedling vigour in *S. asoca*. Dodamarg population recorded superior with respect to seed, germination and seedling vigour attributes. Therefore, this source may be used for large scale plantation programme as well as tree selection. Information generated here would be helpful for nursery entrepreneur as well as researcher for further tree improvement programme.

Acknowledgement

Authors are thankful to Dr. S.G. Bhave, Director of Extension Education and Dr. S.S. Narkhede, Director of Instruction, Dr. BSKKV., Dapoli for their support to carry-out this study at College of Forestry, Dapoli. We also acknowledge Maharashtra Forest Department for their permission and help to collect germplasm.

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