Variation in root yield and ephedrine content of Bala (*Sida cordifolia* Linn.) at differential harvesting under open and shaded situation

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Abstract

A field experiment was conducted under rainfed condition to study the effect of light intensity on yield and quality *of Sida cordifolia* and to find out the optimum stage of harvest of *Sida cordifolia* for higher root yield and quality. The experiment was laid out in randomized block design with three replications. There were nine treatments comprising of stages of harvest from six months after planting to fourteen months after planting at monthly intervals. The experiment on stage of harvest was repeated under direct sunlight and shaded situation to study the effect of shade. Observations on growth characters, yield attributes, fresh and dry yields were recorded. The ephedrine content of the root was estimated by HPLC analysis. The results indicated that vigorous plants and higher fresh and dry root yields were observed under open compared to shaded situation. Under shaded situation, higher root yield was noticed at fourteen months after planting and the yield was very low compared to that under open situation. With respect to ephedrine yield also, higher yield was noticed at hundred per cent sunlight. The maximum root yield and ephedrine content were recorded at eight months after planting and further delay in harvesting showed a sharp decline in both root yield and ephedrine content. The results indicated *Sida cordiflia* preferred open condition for higher root yield and ephedrine content and the optimum stage of harvest is eight months after planting.

Key words : *Sida cordifolia*, Stage of harvest, Dry root yield, Ephedrine content, Open, Shade

Introduction

Sida cordifolia Linn., belonging to the family of Malvaceae, is a species found in tropical and subtropical regions of India. The common names of the crop are country mallow (English), Bala (Hindi), Kurumthotti (Malayalam) and ayurvedic names are *Vatyâlaka, úîtapâki, vâtyodarâhva, bhadraudanî, samangâ, samâmsa* and *svarayastikâ.* According to Nadkarni (1954) a major percent of total alkaloid content in *Sida spp*. is ephedrine. The species has been equated with Bala, one of the most celebrated medicines of Ayurveda (Sivarajan and Balachandran, 1994). The roots of this species are demulcent, diuretic, astringent and stomachic and are used as raw material in many ayurvedic preparations. They are used in the treatment of nervous and urinary diseases, bleeding piles, gonorrhoea and rheumatism. The roots have been used to cure Parkinson's disease and as a food supplement for rapid fat loss. During a survey of different drug markets in the country, most commercial samples were found to be a mixture of other species of Sida, viz. *Sida acuta, Sida cordata* and *Sida rhombifolia* and these were being sold under the same vernacular name 'Bala' by many pharmaceutical companies. Sometimes, because of the non-availability of the roots, the whole plant (95% aerial parts) were also being sold and used as 'Bala'. Domestication and commercialization of cultivation is one of the area to secure the medicinal plant supply of the required quantity to the pharmaceutical companies. Due to high demand of the crop in ayurvedic industry, cultivation has to be encouraged to satisfy the demand. The biosynthesis of secondary metabolites although controlled

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genetically, is affected strongly by environmental influences. Hence it is necessary to study the reaction of *Sida cordifolia* to light intensity and to find out the optimum harvesting stage for maximum root yield and quality. This would help to supply uniform quality roots of *Sida cordifolia* according to market demand.

Considering the medicinal value of *Sida* spp., its increasing demand and meagre information on the agronomic management, the present investigation was undertaken to study the reaction of *Sida cordifolia* to light intensity and to find out the optimum stage of harvest for higher root yield and ephedrine content in *Sida cordifolia*.

Materials and Methods

A field experiment was conducted at All India Co-ordinated Research Project on Medicinal and Aromatic Plants, Kerala Agricultural University, Vellanikkara, Kerala under rain fed condition during 2008-09 and 2009-10. The soil of the experimental field was sandy clay loam laterite with pH of 5.78 and EC of 0.41 dSm^{-1}. The nutrient status of the soil was 277.76 kg/ha of available nitrogen, 25.92 kg/ha of available P_2O_5 and 440.50 kg/ha of available K_2O. The experiment was laid out in randomized block design with three replications. There were nine treatments comprising of stage of harvests from six months after planting to fourteen months after planting at monthly intervals. The experiment on stage of harvest was repeated under open condition in direct sunlight and under shade in a D x T hybrid coconut garden aged over 30 years. The light availability in the interspaces of coconut garden was measured as 60 percent using Luxmeter. The different stages of harvest were fixed based on the data of root yield from the preliminary observational trial conducted for growth analysis. The seeds of *Sida cordifolia* were sown in the nursery by April 15th and one month old seedlings were transplanted under open and shaded situation at a spacing of 50 x 25 cm. Plots of 10 m^2 were prepared and two seedlings were planted per hole in beds so that there were 80 plants per plot. Farmyard manure at the rate of 10 t/ha was applied basally and incorporated in the soil. The total rainfall during the experimental period was 2159.8 mm and 2638.3 mm in 2008 and 2009 respectively. Five plants from each plot were selected for recording growth and yield parameters. The plants from net plots were uprooted at different stages of harvest as per the treatment. Observations on fresh yield and fresh biomass production were recorded. They were oven dried at 70^{o}C for recording dry yield and dry matter production. The root yield was recorded as g/plot and expressed in kg per hectare. The ephedrine content of the root was estimated by HPLC analysis (Narasimhan *et al.*, 2009). The extracted roots were treated with 5 mL 10% ammonia solution for 10 min, to enable complete soaking and then extracted with methanol (4 × 25 mL, each time for 10 min) under reflux on a water bath . The extracts were filtered and concentrated under reduced pressure. The extracted samples were filtered through 0.4µ filter paper and used for analysis in HPLC (C18 reverse phase column and UV-Vis detector of 210 nm). 20µl of extracted sample was injected into the system with a flow rate of 1ml/minute using Acetonitrile-water (65:35) as solvents. Retention time of ephedrine was 2.8 minutes. The ephedrine yield per hectare was calculated from ephedrine content of the root and root yield. The statistical analysis of the data of individual years and pooled data were done by adopting standard procedures of Sukhatme and Amble (1985).

Results and Discussion

Effect of light intensity

The plants grown under open condition consistently showed higher root length, number of roots and root: shoot ratio (Table 2). The plants were found to be lanky under shade compared to open situation (Table 1). The yield attributing characters viz. number of roots and root length were also considerably higher with full sunlight compared to shade. The R:S ratio was maximum at eight months after planting with plants grown under open and was almost four times

Table 1. Effect of harvesting stage on growth characters of *Sida cordifolia* under open and shade

Treatments	Height (cm)						No of branches					
	Open			Shade			Open			Shade		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
6MAP	29.10	29.43	29.26	75.06	76.60	75.83	10.40	11.40	10.9	8.76	9.40	9.08
7MAP	46.80	47.46	47.13	86.66	87.60	87.13	16.13	17.13	16.6	17.33	18.33	17.83
8MAP	76.86	77.81	77.33	92.37	93.30	92.83	28.20	29.20	28.7	18.83	19.86	19.34
9MAP	89.93	80.01	84.97	109.38	113.66	111.5	23.97	24.97	24.4	10.17	11.16	10.66
10MAP	46.33	47.33	46.83	96.60	97.90	97.25	24.40	25.73	25.0	14.90	15.90	15.4
11MAP	32.33	33.00	32.66	85.87	85.20	85.53	23.53	24.53	24.0	14.03	15.03	14.53
12MAP	26.42	27.42	26.92	90.50	78.13	84.31	7.20	9.66	8.43	14.15	14.83	14.49
13MAP	29.92	31.05	30.48	92.92	93.92	93.42	11.50	12.50	12.0	18.90	19.70	19.30
14MAP	31.08	49.86	40.47	58.63	59.93	59.28	6.70	7.70	7.2	6.07	7.03	6.55
CD(0.05)	12.16	10.26	11.56	12.16	10.30	12.03	4.00	3.06	2.31	4.00	3.96	2.01

MAP- months after planting

Table 2. Effect of harvesting stage on root characters of *Sida cordifolia* under open and shade

Treatments	Root length (cm)						No of roots					
	Open			Shade			Open			Shade		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
6MAP	13.06	29.86	21.46	28.86	14.06	21.46	3.66	18.30	10.98	17.20	4.65	10.92
7MAP	18.16	48.60	33.38	47.60	19.16	33.38	10.13	19.30	14.71	18.36	10.86	14.61
8MAP	20.23	50.27	35.25	49.26	21.23	35.24	10.50	19.70	15.1	19.03	11.50	15.26
9MAP	20.60	32.26	26.43	31.96	21.60	26.78	10.73	20.83	15.78	19.83	11.73	15.78
10MAP	14.63	20.53	17.58	19.23	15.63	17.43	10.66	14.56	12.61	13.56	11.66	12.61
11MAP	15.26	23.56	19.41	22.26	16.26	19.26	5.06	13.63	9.345	12.63	6.06	9.34
12MAP	21.93	21.83	21.88	20.83	22.68	21.75	7.63	9.45	8.54	8.80	8.53	8.66
13MAP	13.50	18.93	16.21	17.93	14.52	16.22	7.03	15.00	11.01	14.00	8.06	11.03
14MAP	17.73	16.33	17.03	15.33	20.00	17.65	2.53	11.26	6.895	10.26	3.53	6.89
CD(0.05)	5.89	4.19	4.91	5.89	4.19	5.01	2.67	1.96	2.42	2.67	1.96	2.39

MAP- months after planting

Table 3. Effect of harvesting stage on root shoot ratio and dry matter production of *Sida cordifolia* under open and shade

Treatments	R:S ratio						Dry matter production (g plant^{-1})					
	Open			Shade			Open			Shade		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
6MAP	0.104	0.112	0.108	0.058	0.090	0.074	41.23	49.56	45.39	12.36	18.56	15.46
7MAP	0.169	0.180	0.174	0.064	0.083	0.073	42.62	50.21	46.41	35.64	38.69	37.16
8MAP	0.298	0.311	0.304	0.063	0.081	0.072	61.02	65.63	63.32	30.65	34.65	32.65
9MAP	0.146	0.160	0.153	0.059	0.103	0.081	28.64	30.21	29.42	23.65	28.26	25.95
10MAP	0.125	0.141	0.133	0.063	0.111	0.087	19.58	21.52	20.55	18.65	22.35	20.5
11MAP	0.081	0.090	0.085	0.0636	0.096	0.079	15.30	12.26	13.78	18.26	20.36	19.31
12MAP	0.040	0.149	0.094	0.182	0.215	0.198	22.32	20.36	21.34	20.35	19.58	19.96
13MAP	0.176	0.231	0.203	0.157	0.187	0.172	20.51	28.65	24.58	22.35	29.35	25.85
14MAP	0.250	0.238	0.244	0.240	0.219	0.229	23.65	26.39	25.02	22.35	26.35	24.35
CD(0.05)	0.08	0.11	0.102	0.08	0.11	0.102	8.95	11.51	7.86	8.95	6.58	7.64

MAP- months after planting

higher compared to the plants of the same age under shade (Table 3). The shoot yield was also found to be higher at eight months after planting as evident from higher number of branches and biomass production. Higher R:S ratio under open condition signified that proportionally more photosynthates were allocated to root tissue under full sunlight. It was reported that to utilize available photosynthetic photon flux density efficiently the plants maximize production of photosynthetic tissues by redistributing dry matter (Regnier *et al.*,1988). The fresh as well as dry root yields also followed the same trend of higher values under open condition (Table 4). On the contrary, higher R:S ratio with increasing shade intensities was reported in *Adathoda beddomei* by Neerakal *et al.*(2009). The dry root yield under open condition was nearly 6.8 times higher compared to that under shade at eight months of age. Under shade the plants took more time for the allocation of dry matter to roots and the highest dry root yield was recorded at fourteen months after planting and it was 2.42 times lower compared to that under open situation (Fig.1). With respect to ephedrine yield also the same trend of higher ephedrine yield at hundred per cent sunlight was noticed (Table 5). As the dry matter partitioning was slower under shade, the quality development was also in the same pace and highest ephedrine yield was observed at eight months after planting and was very low compared to that under open situation (Fig.2). The maximum yield and ephedrine content was noticed under open condition.

Effect of harvesting stage

Significant variability on account of differential harvesting was noticed with respect to growth and yield components of *Sida cordifolia* during both years (Table 1 and 2). The different harvesting stages produced remarkable influence on height of the plant, number of branches and dry matter production. The dry matter production under open was significantly higher with the crop harvested at eight months after planting which was thirty six and one hundred and fifteen per cent higher compared to harvesting at seven months and nine months after planting respectively (Table 3). Significantly higher root yield recorded at eight months of age was due to higher dry matter production and consequently increased partitioning of photosynthates to the roots (Table 3). This might have contributed to increased root length and more number of roots at eight months of age which resulted in higher root yield compared to other stages of harvest (Table 2). The fresh root yield at eight months of age was nearly seventy per cent higher than that at nine months of age (Table 4). Further increase in the fresh root yield from twelve months of age was due to reflushing and regrowth of the plant due to the receipt of South West Monsoon and seasonal variations. The dry root yield also followed the same trend (Table 4). The variation

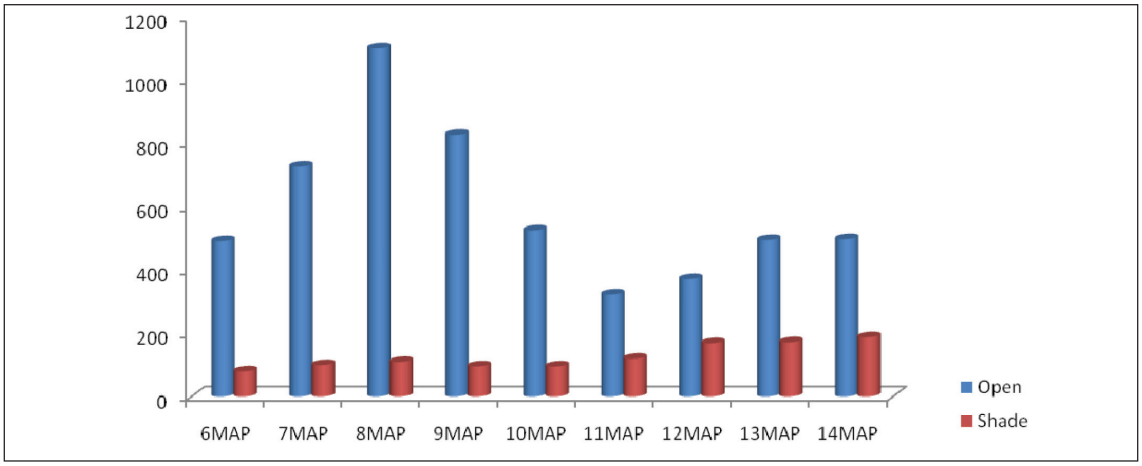

Figure 1. Variation in root yield at different stages of harvesting under open and shaded condition

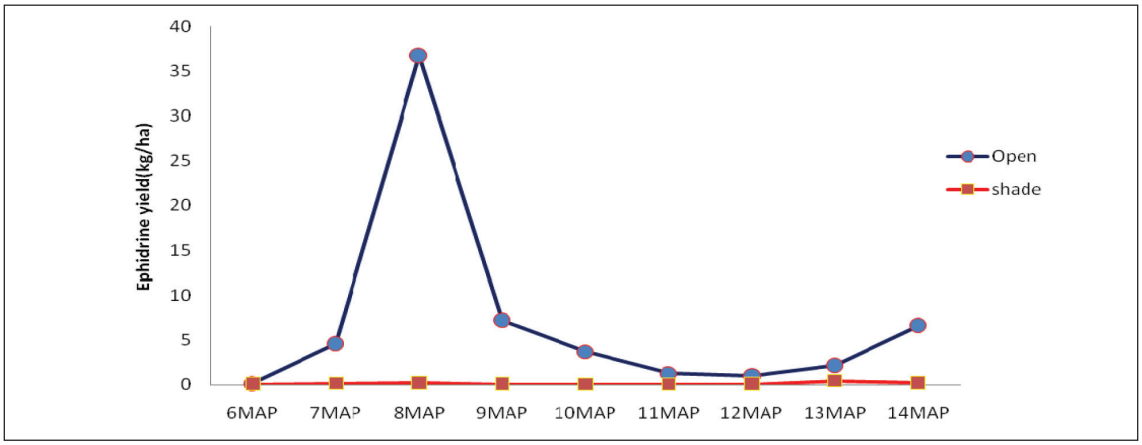

Figure 2. Variation in ephedrine yield at different stages of harvesting under open and shaded condition

in dry root yield with stage of harvest is depicted in Fig.1. The result indicated an increase in dry root yield up to eight months of age and after that a decline was noticed. Similar results of variation in yield due to difference in stages of harvest was reported in *Withania somnifera* [Kubsad et *al.*(2009)], *Thymus vulgaris* [Badi *et al*.(2003) and *Chlorophytum borivilianum* [Kothari *et al*.(2009)] . The ephedrine content also followed the same trend and was found to be maximum at eight months of age. The data on ephedrine yield estimated from ephedrine content and root yield also indicated that the ephedrine yield was maximum at eight months after planting and further delay in harvesting after eight months showed a large decline in the ephedrine yield (Table 5). The ephedrine yield was progressing up to eight months after planting and a further decline was noticed from 9th month onwards(Fig.2). The variation in distribution of dry matter production with differential harvesting time might have also resulted in changes of secondary metabolite production and their accumulation. The increment in root yield as well as ephedrine content over months of growth indicated a peak at eight months of age which gives an indication of the correct stage for harvesting the crop. Similar findings were noticed in andrographaloid content of *Andrographis paniculata.* [Wankhade *et al.* (2005)], essential oil composition of *Thymbra spicata* [Memet *et al*.(2011)] and oil content in *Thymus vulgaris* [Badi *et al*.(2003)].

Table 4. Effect of harvesting stage on fresh and dry root yield of *Sida cordifolia* under open and shade

Treatments	Fresh root yield (kg ha^{-1})						Dry root yield (kg ha^{-1})					
	Open			Shade			Open			Shade		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
6MAP	433.73	754.33	594	192.3	268.33	230.31	233.3	590.0	411.65	160.0	144.4	152.20
7MAP	914.93	1485.0	1199	205.6	418.33	311.96	573.5	1058.1	815.80	162.5	161.0	161.75
8MAP	1058.4	1998.3	1528	200.6	431.25	315.92	621.7	1703.2	1162.4	166.5	171.4	168.95
9MAP	784.00	1015.0	899	182.6	225.36	203.98	418.9	726.8	572.85	93.53	111.8	102.66
10MAP	693.07	935.66	814	162.3	261.32	211.81	298.7	638.4	468.55	78.72	192.0	135.36
11MAP	423.66	865.32	644	169.3	278.45	223.87	230.0	519.2	374.60	95.00	190.6	142.80
12MAP	414.06	836.00	625	173.0	424.33	298.66	280.0	470.0	375.00	100.0	205.4	152.70
13MAP	584.86	1019.6	802	196.5	441.33	318.91	294.0	629.1	461.55	120.2	300.2	210.20
14MAP	666.80	1123.3	895	207.2	425.32	316.26	434.2	751.8	593.00	128.3	361.1	244.70
CD(0.05)	97.66	86.14	86.14	76.65	68.69	58.48	58.45	79.12	68.12	42.36	79.12	46.98

MAP- months after planting

Table 5. Effect of harvesting stage on ephedrine content and ephedrine yield of *Sida cordifolia* under open and shade

Treatments	Ephedrine content (%)						Ephedrine yield (kg ha^{-1})					
	Open			Shade			Open			Shade		
	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled	2009	2010	Pooled
6MAP	0.0003	0.0002	0.0002	0.0009	0.0016	0.0012	0.0006	0.0011	0.0008	0.0014	0.0023	0.0018
7MAP	0.0008	0.0091	0.0049	0.0032	0.0028	0.0030	0.0045	0.0960	0.0399	0.0052	0.0045	0.0048
8MAP	0.0460	0.0480	0.0470	0.0052	0.0068	0.0060	0.2859	0.8175	0.5463	0.0086	0.0116	0.0101
9MAP	0.0210	0.0190	0.0200	0.0014	0.0011	0.0012	0.0879	0.1380	0.1145	0.0013	0.0012	0.0012
10MAP	0.0080	0.0081	0.0080	0.0007	0.0008	0.0007	0.0238	0.0517	0.0374	0.0005	0.0015	0.0009
11MAP	0.0040	0.0056	0.0048	0.0006	0.0005	0.0005	0.0093	0.0290	0.0179	0.0005	0.0009	0.0007
12MAP	0.0030	0.0030	0.0030	0.0004	0.0003	0.0003	0.0084	0.0141	0.0112	0.0004	0.0006	0.0004
13MAP	0.0050	0.0063	0.0056	0.0008	0.0009	0.0008	0.0147	0.0396	0.0258	0.0009	0.0027	0.0001
14MAP	0.0190	0.0189	0.0189	0.0017	0.0018	0.0017	0.0824	0.1420	0.1120	0.0021	0.0064	0.0041
CD(0.05)	0.0002	0.0003	0.0002	0.0018	0.0003	0.0002	0.0014	0.0019	0.0018	0.0014	0.0019	0.0018

MAP- months after planting

Cultivation of shade loving medicinal plants in the homesteads which is a unique system prevalent in Kerala holds promise as a source of subsidiary income. This in turn necessitates identification of appropriate species for cultivation under different light regimes. The reaction of *Sida cordifolia* to light intensity indicated that it preferred open condition for higher root yield and ephedrine content and hence is not suitable for cultivation in the homesteads of Kerala.

Thus it can be concluded that *Sida cordifolia* preferred open condition for higher root yield and ephedrine content. The optimum stage for the harvest of *Sida cordifolia* is eight months after planting under open condition, at which maximum root yield and ephedrine content was noticed.

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