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Variability in the accessions from Aravali range assessed for domestication of the Cleomaceae biodiesel plant *Cleome viscosa* Linn.

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In an earlier study at our laboratory showed that the biodiesel derived from the seed oil of the annual herbaceous medicinal weed plant *Cleome viscosa* Linn. possesses properties similar to the commercial biodiesel produced from *Jatropha* seed oil. Here, the possibilities of domestication of *C. viscosa* were examined. With this objective, 15 accessions from Aravali range in North-West India and two from North-East India were evaluated for phenotypic and genetic variability. The accessions were cultivated in four seasons from May to November 2009 at New Delhi by growing them in randomized block design replicated four times. The accessions were studied for 6 qualitative and 13 agronomic characters and significant genetic variability in all the agronomic traits was observed. On the basis of morphological features, the accessions fell into two groups: a small leaved group and a large leaved group. While the small leaved group comprised of accessions from Rajasthan, the large leaved group included accessions from different locations in North-west and North-East India. One of the small leaved accessions called CVR14 was identified as a putative high yielding accession. The July-October (or monsoon-autumn) season of about 13-15 weeks was observed to be the most suitable period for obtaining rainfed crop of *C. viscosa* CVR14. The DNA fingerprinting based analysis of hierarchical relationships between accessions demonstrated that large leaved and small leaved accessions were inter-related. The results indicated that *C. viscosa* accessions from diverse locations perhaps comprised a single complex.

Keywords: Biodiesel, Non-edible oil-seed, Rainfed crop, *Cleome viscosa*, Short-duration plant

IPC code; Int. cl. (2011.01)—A61K 36/00

Introduction

Cleome viscosa Linn., belonging to family Cleomaceae is a annual weed, widely distributed throughout the tropical regions of the world[1-5]. In India it is a weed plant of semi-temperate, subtropical and tropical regions[2-6]. Dense population of *C. viscosa* are found in several parts of Aravali range in North West India encompassing parts of Gujarat, Rajasthan, Haryana and Delhi[2,7]. It is a traditional medicine-cum-vegetable plant, locally called *Hulhul*, *Hurhur*, *Adityabhakta*, *Arkakanta, Hurhuria, Talvani, Ariavila*, *Nayikkadugu*, *Chilakamukkuvithulu*, *Bagro*, *Jakhiya* and *Kukhavominta*[1,2,8,9]. The plant parts are rubefacient, vesicant and sudorific, roots are used as cardiac stimulant and in diabetes. Seeds are used for deworming and extracts of leaves, flowers and stems have antimicrobial activity[6,9-13]. Defatted seeds are used for the industrial extraction of coumarinolignoids, a valuable chemical entity needed by pharmaceutical industries for liver diseases and immunomodulation[14,15]. Defatted seeds are also used as fodder[16] and for production of biogas[17,18].

The seeds of the plant are reported to be rich in oil (23-36.6%). Our previous study demonstrated production of biodiesel from *C. viscosa* oil, which has many of the properties similar to the *Jatropha* biodiesel[19]. Since it has potential as a resource for biodiesel, a question has arisen whether it can be domesticated for possible cultivation. In the present study accessions of *C. viscosa* were collected from different parts of Aravali range and evaluated for their agronomic characteristics. Here we report the results of this study and demonstrate that the collected accessions possess considerable genetic variability and the species can be developed into a short duration crop suitable for cultivation in the summer-monsoon-autumn seasons.

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Materials and Methods

Plant material

Cleome viscosa Linn. accessions were collected during August to October 2008 from the central and the northern parts of Aravali range that extends from Delhi in North East to parts of Gujarat state in West-South (NISCAIR/RHMD/Consult/-2011-12/1889/189). At each location of collection, an area rich in naturally growing *C. viscosa* plants was identified. Mature pods were collected from about 100 plants in a square/quadrant of about 100 m^2. The pods were saved in a muslin bag and were air dried. The dry pods were threshed to obtained seeds which were stored in paper bags for future use. In all 15 accessions were collected from Aravali and two were collected from outside of Aravali, the latter to serve as controls. These were one each from Nalanda in Bihar and Hazaribargh in Jharkhand.

Cultivation conditions

All the 17 accessions were cultivated in 2009 in four summer-autumn seasons: season 1-May to August (summer-monsoon-1), season 2-June to September (summer-monsoon-2), season 3-July to October (monsoon-autumn-1) and season 4-August to November (monsoon-autumn-2).

The accessions were grown in a field plot of Jawaharlal Nehru University (JNU) campus. The field plot was manually prepared after applying farmyard manure at the rate of 30 tonnes/ha. Four lines, each 2.5 m long, were sown for each accession, one/replication. Line to line distance was 0.45 m. The lines were organized in a randomized block design replicated 4 times. A line was sown with 1 g of seeds. At seedling stage, 50 plants were maintained per line and extra plants were removed. The field plot was irrigated as and when required.

Growth studies

The flowering time was recorded as the time period (days) from seed sowing to the time when 50% of the plants had flowered. The maturity time was the number of days from seed sowing to the time when 75% of the plants had turned yellow. A number of observations were recorded at the harvest time.

Accession-wise crop growth dynamics were measured in terms of total dry weight. The accessions were sampled five times at 10-20 day interval from the seedling stage to the harvest time. A sample comprised of five randomly selected plants per accession/replication. The sampled plants were excavated, their roots were washed free of soil, and they were placed in a paper bag and exposed to high temperature and then air dried. The dried plant material was weighed to estimate the dry weight of per plant.

Morphological and agronomic observations

Nineteen morphological and agronomic characters (Tables 1 and 2) were studied in each of the accessions. Seeds placed on slide were photographed using a Nikon SMZ1500 stereo zoom microscope to estimate their size. The leaves, flowers and pods were scanned on hP psc 750 scanner and their photographs were printed on mm^2 paper to estimate their sizes. The leaves and pods for measuring their sizes were taken from the first flowering node. The pigmentation of seeds, stem, leaf and pod was recorded, using the pigmentation standards[20]. The plants were excavated accession and replication-wise along with their roots. The roots were washed free of the adhering soil and separated from the shoot and placed in the paper bags. The shoot parts were separated into stem, leaves and pods. These were placed separately in paper bag. The pods were air dried and the roots, stems and leaves were air dried after exposure to 80°C temperature for 45 minutes. The dried material was weighed using the Mettler AE50 balance. Subsequently, pods were threshed to separate seeds to obtain seed weight. The harvest index was estimated as seed weight ÷ weight of roots+ stem+ leaves+ pods.

To estimate the pollen and ovule fertility, randomly sampled flowers and immature pods were used. Pollen grains were dusted from the flowers onto droplets of dilute glycerol on a slide, stained with acetocarmine and examined under Nikon Eclipse E 100 microscope and photographed with Nikon 8400 digital camera. The empty pollen grain was taken as sterile. To check the fertility of the ovules, the pods were opened out and the growing ovules were placed on slides. Those observed to be shrunken and tiny in size as compared to the normal ovules were counted as aberrant/sterile ovules. The freshly collected flowers were dissected to record the flower structure in terms of calyx, corolla carpel and stamen compositions.

Statistical analysis

Analysis of variance and t-tests were carried out by using the procedures described in Cochran and Cox[21] and Panse and Sukhatme[22].

DNA fingerprinting

Twelve accessions, namely CVR1, CVR2, CVR3, CVR8, CVR10, CVR11, CVR12, CVR14, CVR15,

Table 1—The locations of collection and morphological features in the different genotypes (accessions) of *Cleome viscosa* collected from Aravali range of North India.

S. No.	Accession designation	Location of collection in India		Size of			Non-green pigmentation of		
		City	State	Leaf[b]	Seed	Flower[g]	Stem	Leaf	Siliqua
1	CVR1	Delhi	Delhi	Large	Large[d,f]	Small	Light pink	Nil[h]	Nil[h]
2	CVR2	Delhi	Delhi	do	do	do	do	do	do
3	CVR3	Jaipur	Rajasthan	do	do	do	do	do	do
4	CVR4	Delhi	Delhi	do	do	do	do	do	do
5	CVR6	Delhi	Delhi	do	do	do	do	do	do
6	CVR7	Delhi	Delhi	do	do	do	do	do	do
7	CVR8	Delhi	Delhi	do	do	do	do	do	do
8	CVR11	Delhi	Delhi	do	do	do	do	do	do
9	CVR18	Nalanda	Bihar[a]	do	do	do	do	do	do
10	CVR19	Faridabad	Haryana	do	do	do	Nil[h]	do	do
11	CVR21	Hazaribagh	Jharkhand[a]	do	do	do	Purple	do	do
12	CVR22	Delhi	Delhi	do	do	do	Light pink	do	do
13	CVR24	Delhi	Delhi	do	do	do	do	do	do
14	CVR10	Jaipur	Rajasthan	Small[c]	Small[e,f]	large	Purple	Purple	Purple
15	CVR12	Jaipur	Rajasthan	do	do	do	Nil[h]	Nil[h]	Nil[h]
16	CVR14	Ajmer	Rajasthan	do	do	do	do	Purple	Purple
17	CVR15	Jaipur	Rajasthan	do	do	do	do	Purple	Purple

a–Two accessions were from outside of Aravali range to examine if wide differences existed between Aravali and non-Aravali accessions; b–The leaf size in large leaved accessions was 836±125 mm^2; c–The size of leaf in small leaved accessions was 265±31 mm^2; d–One gram weight of seeds in large leaved accessions contained 1188±90 seeds and the flat side of seed measured 1.36 ± 0.43 mm^2; e–One gram weight seeds in small leaved accessions contained 1998±94 seeds and the flat side of seed measured 0.90 ± 0.16 mm^2; f–The seed color varied from coca brown to leather brown; g–The petal colour in all accessions was yellow, each flower had 4 sepals, 4 petals, 18-28 stamens and one carpel. The pollen grains and ovules were largely fertile (pollen sterility was 0-3.4 % and ovule sterility 3.3-4.2 %); h–no pigmentation.

CVR18, CVR19 and CVR21, were DNA fingerprinted. DNA was extracted from leaf samples using C-TAB method[23]. Randomly amplified DNA polymorphism, inter simple sequence repeat and micro RNA primers were amplified in PCR reactions with each DNA to detect the polymorphism. The amplification products were visualized on 1.5% agarose gel stained with ethidium bromide with the help of Gel-doc UVP. The observations were then used to determine hierarchical relationships between the accessions.

Hierarchical analysis

Tree 4.0 software and unweighted pair group method with arithmetic mean (UPGMA) based on Sneath and Sokal[24] were used for the construction of dendrograms for hierarchical analysis.

Results

Phenotypic variability among accessions

Identification of small and large leaved accessions

Seventeen accessions of *C. viscosa* were characterized for similarities and differences between them in respect of a large number of morphological and agronomic characters (Tables 1 and 2A). For this purpose, the accessions were grown in replicated trials over four seasons from May to November 2009 at the campus of the JNU, New Delhi. Of the accessions, fifteen had been collected from Aravali hills in the states of Delhi, Haryana and Rajasthan and one each from Bihar and Jharkhand. Many of them shared their plant habit characteristics (Table 1). Eleven Aravali accessions (CVR1-CVR4, CVR6-CVR8, CVR11, CVR19, CVR22 and CVR24) and the accessions from Bihar (CVR18) and Jharkhand (CVR21) bore leaves and seeds of large size as compared to a set of four Aravali accessions (CVR10, CVR12, CVR14, CVR15). The small leaved accessions produced flowers of larger size than the flowers formed on large leaved accessions. Except for CVR12, the leaf, stem and siliquae of small leaved accessions were deeply pigmented with purple coloration. The stem of CVR21, the large leaved accession from Jharkhand, also produced shoots bearing deep purple pigmentation. In the other large

Table 2(A)—Agronomic characteristics of different genotypes (accessions) of *Cleome viscosa*, collected from the Aravali range of north India, observed over four seasons in a trial set up in the form of completely randomized design replicated four times, at New Delhi

S.no.	Genotype (Accession)/Season[a]	Height (cm)	Length of root system(cm)	Number of leaves	Shoot weight[b] (g)	Root weight[b] (g)	Total plant weight[b] (g)
A	B	C	D	E	F	G	H
1	CVR1	117.5 ^{m}	19.8 ^{k}	444 ^{klm}	48.6 ^{lm}	3.7 ^{mno}	52.3 ^{kl}
2	CVR2	134.6 ^{n}	20.4 ^{k}	354 ^{jk}	56.7 ^{m}	3.5 ^{lmn}	60.2 ^{l}
3	CVR3	106.8 ^{jk}	15.6 ^{ij}	232 ^{ij}	37.8 ^{jkl}	2.4 ^{jkl}	40.2 ^{jk}
4	CVR4	162.9 ^{o}	19.9 ^{k}	763 ^{pq}	74.7 ^{no}	5.6 ^{p}	80.3 ^{mn}
5	CVR6	131.7 ^{n}	24.5 ^{m}	840 ^{q}	96.9 ^{p}	7.2	104.1 ^{p}
6	CVR7	115.7 ^{lm}	16.5 ^{j}	216 ^{ij}	24.0 ^{ijk}	2.2 ^{jk}	26.2 ^{ij}
7	CVR8	133.1 ^{n}	20.1 ^{k}	521 ^{mn}	62.4 ^{mn}	4.9 ^{op}	67.3 ^{lm}
8	CVR11	168.3 ^{o}	21.0 ^{kl}	512 ^{lmn}	63.0 ^{mn}	5.1 ^{p}	68.1 ^{lm}
9	CVR18	103.8 ^{jk}	20.9 ^{kl}	620 ^{nop}	122.8	8.5	90.19 ^{nop}
10	CVR19	102.7 ^{jk}	20.0 ^{k}	259 ^{j}	35.7 ^{jkl}	2.7 ^{klm}	80.3 ^{mno}
11	CVR21	103.1 ^{jk}	19.7 ^{k}	459 ^{klm}	48.0 ^{lm}	3.6 ^{lmn}	51.6 ^{kl}
12	CVR22	99.8 ^{ij}	14.3 ^{ij}	202 ^{ij}	14.4 ^{i}	0.9 ^{i}	15.3 ^{i}
13	CVR24	109.2 ^{kl}	13.6 ^{i}	84 ^{i}	21.4 ^{ij}	1.3 ^{ij}	22.8 ^{i}
14	CVR10	94.1 ^{i}	19.7 ^{k}	731 ^{0pq}	49.91^{m}	2.8 ^{klm}	52.7 ^{kl}
15	CVR12	119.9 ^{m}	19.7 ^{k}	446 ^{klm}	38.5 ^{kl}	3.2 ^{klm}	41.7 ^{jk}
16	CVR14	138.5 ^{n}	23.9 ^{m}	1132	84.8 ^{op}	4.7 ^{nop}	89.5 ^{nop}
17	CVR15	101.9 ^{j}	23.1 ^{lm}	594 ^{mno}	49.6 ^{lm}	3.1 ^{klm}	52.6 ^{kl}
18	Mean±SD	120.2±21.8	19.6±3.1	494.6±269.5	54.7±28.9	3.8±2.0	58.6±25.2
20	Season 1	139.4	22.6	731.5	84.6	5.8	72.3
21	Season 2	128.8	21.5	629.2	73.3	4.7	75.1
22	Season 3	96.9	15.5	307.0	34.3	2.1	34.2
23	Season 4	115.7	18.4	310.7	41.2	2.8	42.7
24	Mean±SD	120.2±18.3	19.5±3.2	494.6±218.5	58.4±24.4	3.9±1.7	56.1±20.7
25	F value- Genotype(G)	71.96**	14.39**	22.89**	21.18**	21.04**	17.40**
26	F value- Seasons (S)	215.79**	60.91**	63.92**	54.88**	64.35**	50.73**
27	F value- Interaction(GxS)	21.45**	6.79**	6.03**	6.51**	6.61**	5.68**
28	CD at 5% ,Genotype	7.16	2.25	156.97	16.96	1.21	16.66
29	CD at 1% ,Genotype	9.44	2.97	207.17	22.38	1.59	21.98
30	CD at 5% , Seasons	3.47	1.09	76.14	8.23	0.58	8.08
31	CD at 1%, Seasons	4.58	1.44	100.49	10.86	0.77	10.66
32	Mean of 1 to 13	122.2	18.9	424	54.3	4.0	58.4
33	Mean of 14 to 17	113.6	21.6	726	55.7	3.5	59.1
34	$T_{(66)}$ value for comparing 32 and 33 and P value in parenthesis	0.90 (0.37)	1.78 (0.08)	2.77** (0.007)	0.11 (0.91)	0.64 (0.5)	0.062 (0.95)
35	Heritability(variety τ^2/total τ^2 ×100)	23.1	16.8	23.9	24.8	22.0	22.6

leaved accessions, except CVR19, the stems were pigmented with light pink colour. The leaves and siliquae of large leaved accessions, except CVR21, were without pink coloration. There were two accessions, the large leaved CVR19 and small leaved CVR12, in which stem, leaf and siliquae were without pink coloration. The seeds of all the accessions were pigmented similarly with coca brown to leather brown coloration. Further, all accessions produced glands on all the plant organs. Some of the features that

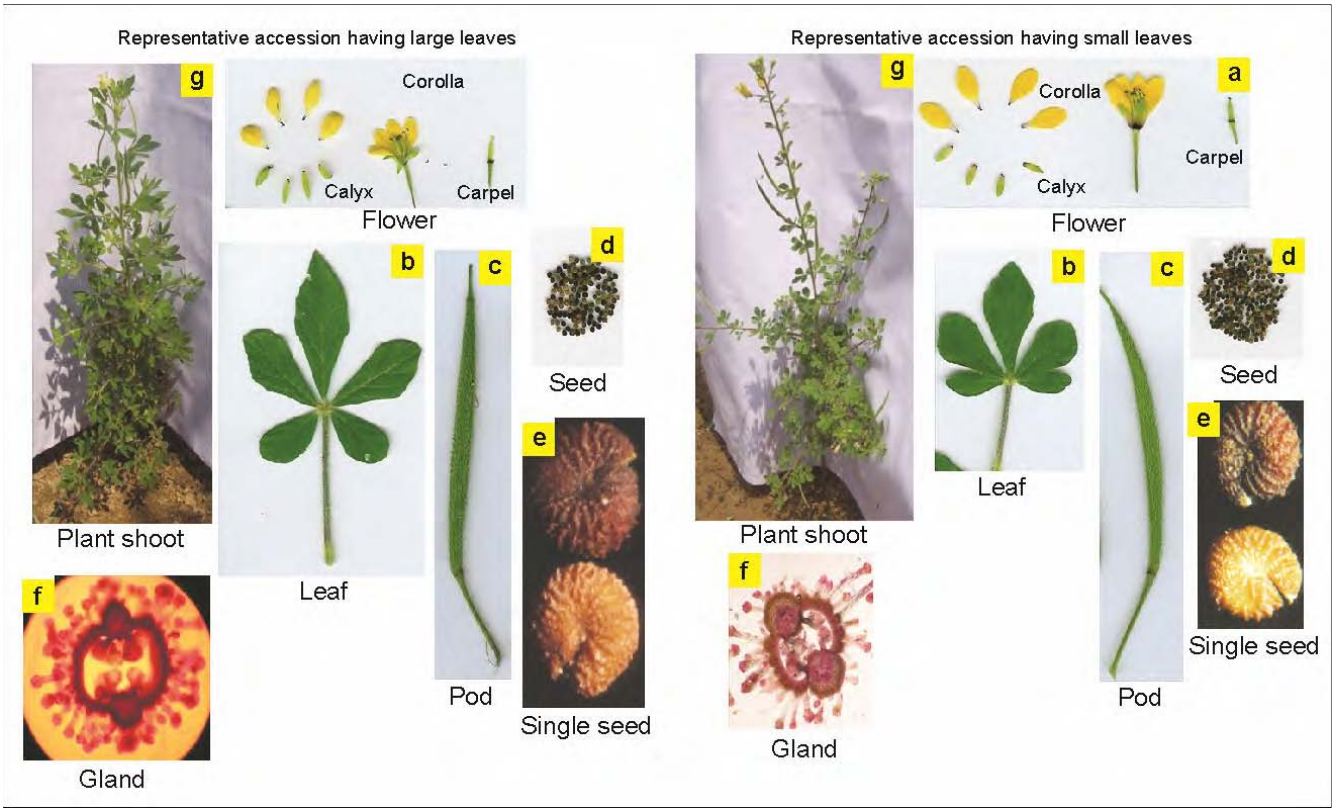

Plate 1—Distinguishing morphological features in the small and large leaved accessions of *Cleome viscosa* from the Aravali range.

distinguished large and small leaved accessions are depicted in the Plate 1.

Table 2B summarizes observations on thirteen quantitative traits among the seventeen accessions. The variances for all the thirteen characters for genotypes, seasons and genotypes x seasons interaction were significant. Among the different seasons: May to August (summer-monsoon -1), June to September (summer-monsoon-2), July to October (monsoon-autumn-1), August to November (monsoon-autumn-2)-on average bases the crops demonstrated better growth and seed productivity in the summer-monsoon-1 and -2 seasons (May to August and June to September) as compared to the monsoon-autumn-1 and -2 seasons (July to October and August to November). This observation was confirmed by the data on the rate of growth of dry matter in the four seasons averaged for all the accessions (Figure 1). However, harvest index was higher in the monsoon-autumn seasons.

The small leaved accessions differed from large leaved accessions by having significantly more number of leaves and pods/plant and by having early flowering. Large leaved accessions on the other hand bore pods of significantly larger size than those in small leaved accessions. Heritabilities of several characters were moderately high (number of pods/plant, seed yield/plant and days to flower).

The results of the comparison of the performance of individual accessions over seasons showed that the small leaved accession CVR14 possessed a combination of characters that made it most productive in terms of seed yield/plant. These characteristics included tall habit, high shoot weight, early flowering, large number of pods/plant, high harvest index and superior performance in late seasons.

Hierarchical relationships between accessions based on morphological and agronomic characters

Figure 2 presents a UPGMA dendrogram of 17 accessions based on their morphological and agronomic traits. It will be seen that the accessions fell into four groups. While the large leaved accessions comprised three groups, the small leaved accessions fell altogether in one group. This confirmed the groupings based on morphological traits, made in the section above, of small leaved accessions into a cluster differing from the large leaved accessions.

Table 2(B)—Agronomic characteristics of different genotypes (accessions) of *Cleome viscosa*, collected from the Aravali range of north India, observed over four seasons in a trial set up in the form of completely randomized design replicated four times, at New Delhi

S.no.	Genotype (Accession)/Season[a]	Number of pods/plant	Length of pods (cm)	Number of seeds/pod	Seed yield of a plant (g)	Harvest index (L/H)	Days to flower	Days to maturity
A	B	I	J	K	L	M	N	O
1	CVR1	104^{ijkl}	5.7^{jklm}	114^{ijk}	6.6^{jkl}	0.15^{ijk}	41^{klm}	95^{ij}
2	CVR2	133^{klm}	6.3^{nop}	152^{n}	10.4^{l}	0.20^{ijk}	43^{mno}	99^{kl}
3	CVR3	86^{ijkl}	5.2^{jk}	96^{i}	6.5^{ijkl}	0.19^{ijk}	42^{lmn}	96^{ijk}
4	CVR4	148^{lm}	6.9^{p}	135^{lmn}	7.6^{jkl}	0.10^{i}	43^{mno}	95^{ij}
5	CVR6	197^{mn}	6.5^{opq}	135^{lmn}	15.1^{mn}	0.14^{ijk}	38^{ij}	91^{i}
6	CVR7	71^{ijk}	6.1^{mnop}	116^{jkl}	3.9^{ij}	0.15^{ijk}	47^{pq}	101^{kl}
7	CVR8	144^{lm}	6.4^{opq}	130^{klm}	14.9^{m}	0.24^{k}	48^{q}	101^{kl}
8	CVR11	125^{jkl}	6.3^{nop}	114^{ijk}	8.4^{kl}	0.13^{ij}	42^{lmn}	96^{ijk}
9	CVR18	262^{mno}	6.3^{nop}	114^{ijk}	20.5^{o}	0.21^{jk}	51^{i}	100^{kl}
10	CVR19	66^{ij}	6.2^{nop}	137^{mn}	7.3^{jkl}	0.37	45	98^{jkl}
11	CVR21	86^{ijkl}	5.8^{lmn}	125^{klm}	7.4^{jkl}	0.18^{ijk}	40^{op}	103^{l}
12	CVR22	53^{i}	5.7^{jklm}	99^{ij}	2.2^{i}	0.15^{ijk}	47^{jkl}	93^{ij}
13	CVR24	67^{ijk}	5.6^{jkl}	138^{mn}	5.0^{ijk}	0.22^{jk}	44^{pq}	103^{l}
14	CVR10	287^{o}	4.5^{i}	126^{klm}	9.5^{l}	0.15^{ijk}	39^{no}	99^{kl}
15	CVR12	119^{ijkl}	5.6^{jkl}	132^{klm}	7.7^{jkl}	0.21^{jk}	41^{jk}	93^{ij}
16	CVR14	529	5.0^{ij}	124^{klm}	19.4^{no}	0.24^{k}	36^{klm}	98^{jkl}
17	CVR15	266^{o}	5.3^{jkl}	154^{n}	8.6^{kl}	0.16^{ijk}	38^{ij}	96^{ijk}
18	Mean±SD	161.4±120.0	5.8±0.6	125.9±16.0	9.5±5.1	0.2±0.1	43±4.0	97±3.5
20	Season 1	199	6.5	136	11.8	0.15	44	95
21	Season 2	225	5.9	128	12.2	0.16	41	95
22	Season 3	121	5.2	103	5.6	0.21	45	104
23	Season 4	118	5.7	135	8.3	0.24	40	96
24	Mean±SD	165.8±54.5	5.8±0.5	125.5±15.4	9.5±3.1	0.2±0.0	43±2.4	97±4.4
25	F value - Genotype(G)	24.44**	14.25**	5.61**	10.95**	3.27**	21.24**	4.46**
26	F value - Seasons (S)	21.95**	46.11**	27.24**	17.57*	6.57**	33.72**	29.93**
27	F value - Interaction(GxS)	4.66**	3.71**	3.10**	3.26**	2.12**	7.11**	3.90**
28	CD at 5%,Genotype	66.71	0.46	18.91	4.31	0.10	2.38	4.49
29	CD at 1%,Genotype	88.05	0.61	24.96	5.69	0.13	3.14	5.92
30	CD at 5%, Seasons	32.36	0.22	9.17	2.09	0.05	1.16	2.17
31	CD at 1%, Seasons	42.71	0.29	12.10	2.76	0.06	1.53	2.87
32	Mean of 1 to 13	118.6	6.1	124.0	8.9	0.2	44	98
33	Mean of 14 to 17	300	5.1	134	11.3	0.2	38	96
34	$T_{(66)}$ value for comparing 32 and 33 and P value in parenthesis	4.58** (0.0001)	4.034** (0.0001)	1.26 (0.211)	1.13 (0.261)	0.15 (0.88)	3.6** (0.0007)	0.62 (0.54)
35	Heritability (variety τ^2/total τ^2 ×100)	45.4	21.5	13.9	33.0	22.9	32.3	11.3

[a] = Season 1, May to August; season 2, June to September; season3 , July to October; season4, August to November; [b] = Dry weight, [c] = The genoytpes that have non-significant differences in the expression of a character have the same superscript (i,j,k,l,m,n,o,p,q,r,s,t,u,v); *= Significant at 5% level; **= Significant at 1% level.

The accession CVR3 comprised a group which was distinct from all other accessions including the small leaved accessions. The other twelve large leaved accessions fell into two groups. A group of six was comprised by four accessions from Delhi and one from Jharkhand. Another group of six was comprised by five accessions from Delhi and one from Bihar. None of the Delhi accessions fell into the small leaved group. Small leaved group was exclusively comprised by accessions from Rajasthan.

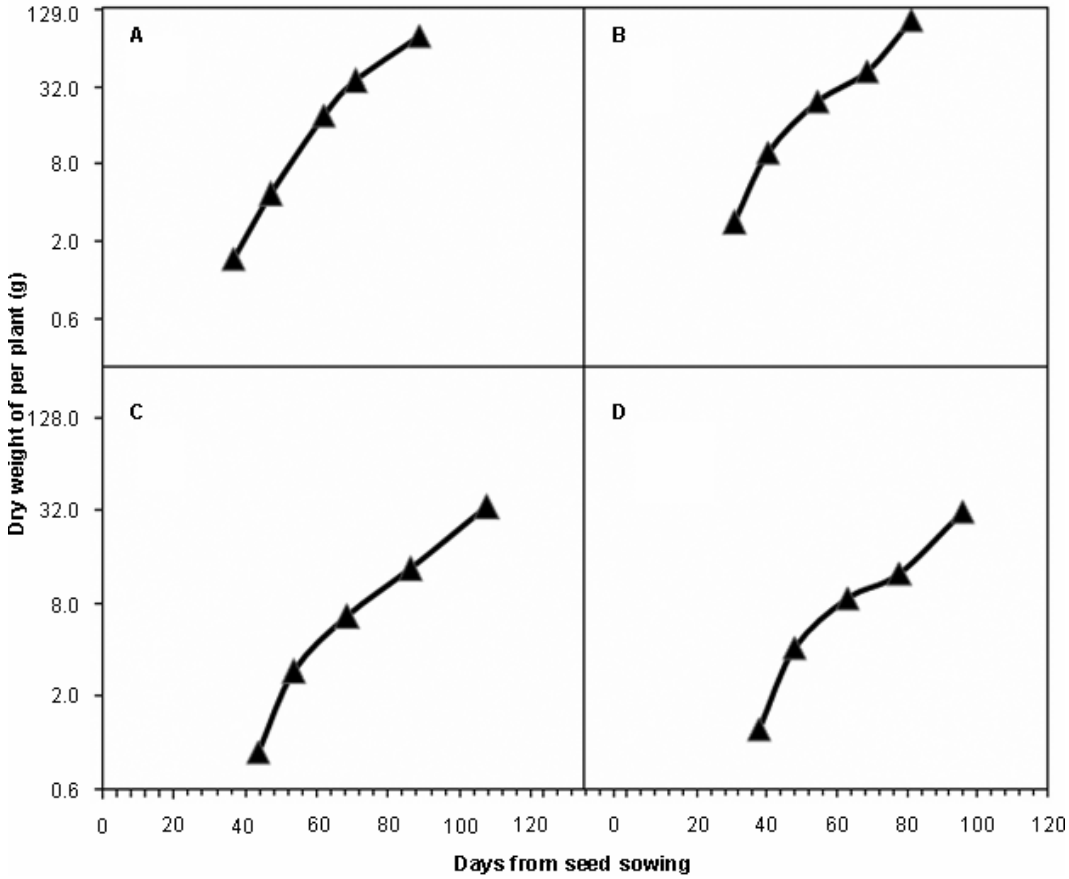

Fig. 1—Patterns of growth in *Cleome viscosa* crops cultivated in four summer-autumn seasons at New Delhi. Growth is shown in terms of dry weight per plant. A = May to August (Summer-Monsoon-1); B = June to September (Summer-Monsoon-2); C = July to October (Monsoon-Autumn-1); D = August to November (Monsoon-Autumn-2).

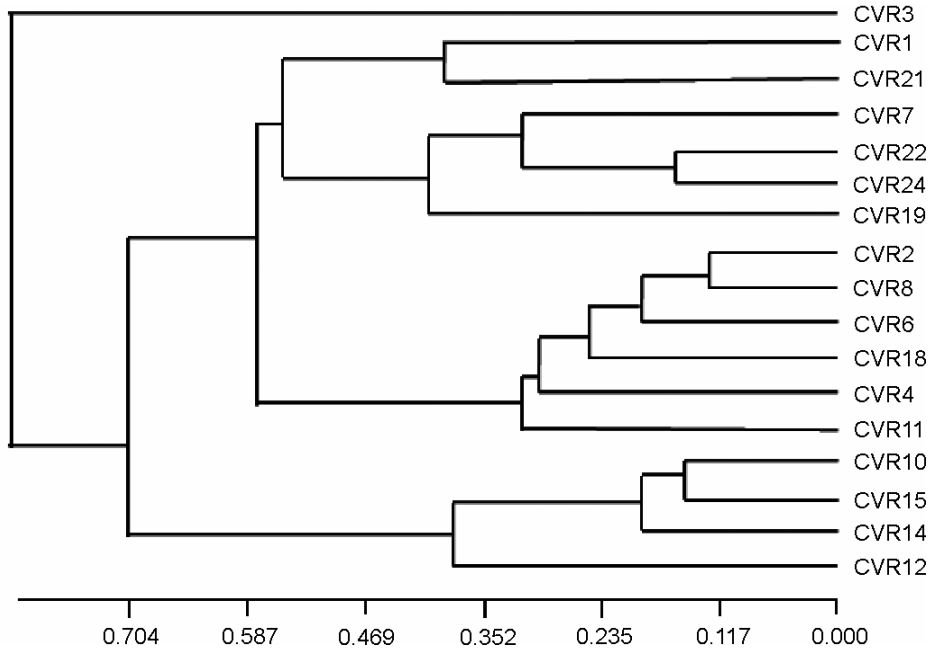

Fig. 2—Dendrogram of 17 accessions of *Cleome viscosa* based on morphological and agronomic characteristics.

Genetic variability among accessions

The polymorphisms demonstrated by accessions for a total of 299 DNA markers were used to bring out the hierarchical relationships between the accessions using the UPGMA (Table 3; Figure 3). In this analysis only twelve accessions were used, all the four small leaved accessions and eight accessions from Delhi, Haryana, Rajasthan, Jharkhand and Bihar which were all large leaved. The accessions fell into five groups. The accessions CVR1 (Delhi) and CVR2 (Delhi) formed single member groups. Other three groups comprised of more than one accession that were from different geographical areas. The accessions CVR15 (Rajasthan) and CVR19 (Haryana) comprised a group of small leaved and large leaved accessions. Another such group was comprised by CVR10, CR12 and CVR3 (all from Rajasthan). The small and large leaved also got included together into fifth group comprised by accessions from Delhi, Haryana, Rajasthan, Bihar and Jharkhand. These results demonstrated that some degree of genetic homology existed between the small and large leaved accessions. These results also indicated that homology also existed between accessions from geographically distant areas.

An UPGMA analysis was also carried out for the morphological, agronomical characters and DNA markers jointly on the twelve accession on which DNA finger printing had been done. These results are shown in the Figure 4. The twelve accessions fell into two groups. One of these comprised of three small leaved accessions from Rajasthan (CVR12, CR14 and CVR15), two accessions from Delhi (CVR8 and CVR11) and one accession each from Bihar (CVR18) and Jharkhand (CVR21). The second group consisted of a small leaved accession from Rajasthan (CVR10), two accessions from Delhi (CVR1 and CVR2) and one from Rajasthan (CVR3) and one from Haryana (CVR19) (all these bearing large leaves). Thus the joint analysis showed that the small and large leaved accessions clustered together into two groups which also had accessions from diverse geographical areas. These results again indicated genetic homology between accessions coming from different geographical locations and also homology between small and large leaved accessions.

Discussion

New non-edible and annual oil seed crops are needed in the era of overall food deficiency and rise in prices arising out of diversion of food grains for biofuel production. Here, the potential of *C. viscosa*

Table 3—Type and number of DNA markers that demonstrated polymorphism between the genotypes (accessions) of *Cleome viscosa*

Type of marker	No. of primer pairs tested	No. of primer pairs that demonstrated polymorphism	No. of markers elicited
ISSR	13	13	80
RAPD	24	20	105
Micro RNA	19	18	114
Total	56	51	299

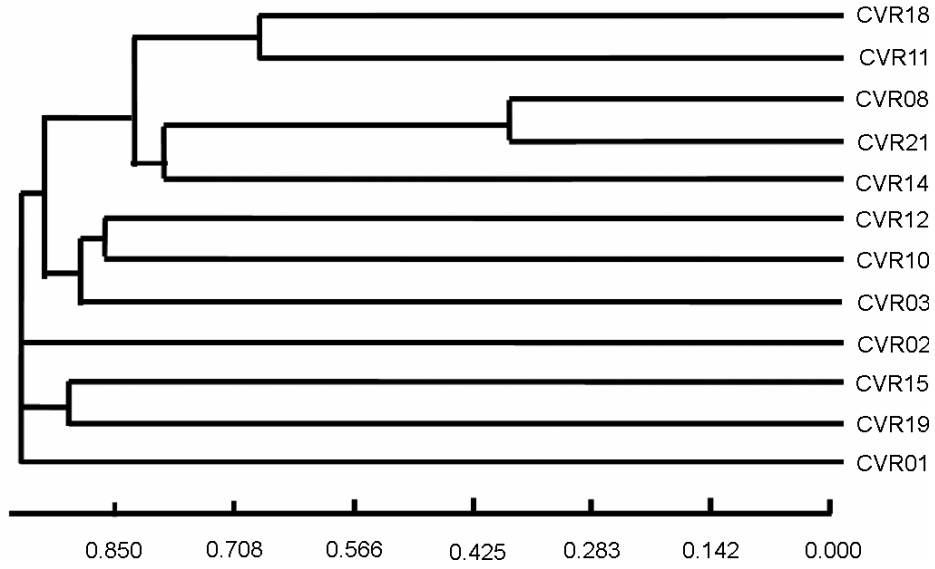

Fig. 3—Dendrogram of 12 accessions of *Cleome viscosa* based on fingerprinting data using UPGMA.

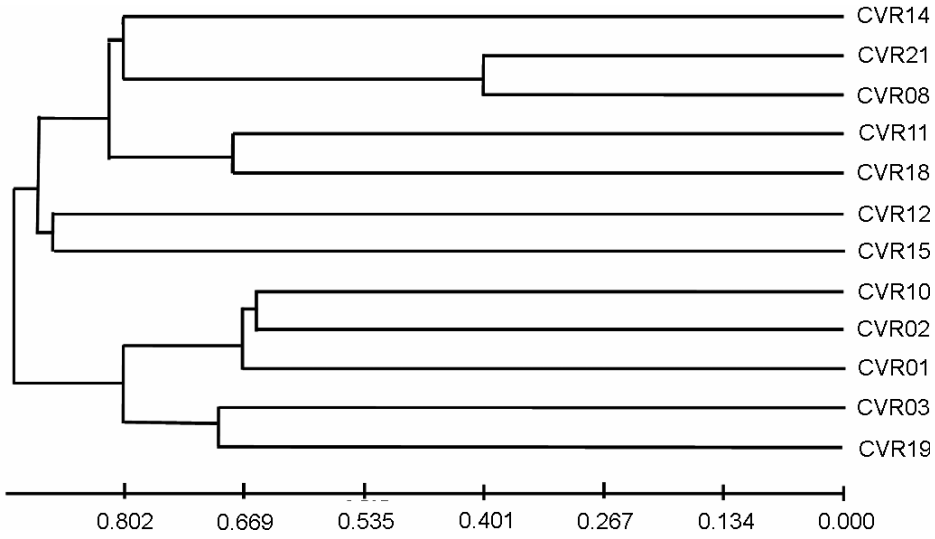

Fig. 4—Dendrogram of 12 accessions *of Cleome viscosa* based on morphological and fingerprinting data.

an annual herbaceous weed species of Aravali area (North West India) has been evaluated as a source of oil seeds, in complementation of a study already carried out showing that *C. viscosa* seed oil gives biodiesel of acceptable quality[19]. The results described above show that the cultivation of *C. viscosa* is possible and relatively high yield of seeds and harvest index are obtainable from certain accession(s) (CVR14) in the monsoon-autumn seasons in about 14 weeks time. Some aspects of the results are discussed below–

The accessions collected from the Aravali areas of Delhi, Haryana and Rajasthan and from Bihar and Jharkhand were morphologically distinguishable into two types: a small leaved group and a large leaved group. The large leaved type accessions were from all the locations of the collection. The small leaved accessions originated exclusively from Rajasthan. The small leaved accessions demonstrated more reproductive growth than the large leaved accessions, as elicited by number of pods/plant. The small leaved accessions demonstrated harvest index equal to that of larger leaved accessions. One of the small leaved accession namely CVR14 out yielded all the accessions indicating its potential for further development into a cultivar.

In the North West India crop rotation system is practiced to cultivate two or more crops in a year. The main winter-spring season crop from November to April is wheat. In the subsequent summer season from April to June/July pulse crop such as cluster bean (and related pulse crops) is cultivated. In the assuredly irrigated areas, the monsoon to autumn season crop is rice. There are some areas where only the rain fed crops such as pearlet millet can be grown. The results of the present study on the effect of seasons on crop behavior show that *C. viscosa* is suitable to cultivate throughout May to November. However, its crop sown in July gave better harvest index. In terms of the crop rotation(s) in vogue and related performance from July to October, *C. viscosa* appears suitable for cropping in the autumn-monsoon season. It is possible to recommend its cultivation as a rain-fed crop in areas where the soil quality is inferior or not suitable for growing the conventional food crop(s).

The accessions of this species studied here demonstrated wide phenotypic variability, between the small leaved accessions and the large leaved accessions on the one hand and between large leaved accessions originating from different geographical areas on the other hand. DNA fingerprinting demonstrated that the genetic variability existed among both types of accessions, small leaved accessions and large leaved accessions. The hierarchical arrangement of accessions based on DNA fingerprinting and DNA fingerprinting combined with morphological and agronomical traits showed that the accessions studied fell into two to five groups and all the multi-accession groups included small and large leaved accessions. The results therefore, indicated that genetically the *C. viscosa* accessions studied in the present work were all related to each other, albeit to different extents. Perhaps its populations growing in different states of India comprise a large interrelated complex. The distantly growing populations may be related on account of wide seed dispersed via interstate vehicular traffic.

Conclusion

In summery, in the present study the potential of *C. viscosa*, an annual herbaceous weed plant, was evaluated for it to be developed into a short duration non-edible oil-seed crop. A comparison of seventeen accessions collected from Aravali and North East India over four summer-monsoon and monsoon-autumn seasons identified an accession which produced high yield of seeds from its crops of 13 to 15 weeks in the monsoon-autumn season. Collectively the accessions demonstrated considerable genetic variability in agronomic characters thus indicating possibilities of selection breeding in *C. viscosa* for developing superior cultivars.

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