

# Chapter 1

## Work carried out on screening of crops for Salinity tolerance

I joined the Discipline of Sea water Irriculture on 26<sup>th</sup> May 1981. At that time mainly work was being carried out on screening of crop plants for sea water tolerance. Therefore I was assigned to work on salinity tolerance of crop plants and undertake breeding work in Bajra for screening of salt tolerance of mother as well as hybrids because the problem of salinity is widespread in the arid and semi-arid regions and the coastal belts in India. During first two years efforts were made to screen different crop varieties for tolerance to salinity and improve the varieties for increasing tolerance and yield.

Use of highly saline water and seawater has emanated from the pioneering experiments conducted in the desert of Eliat (Israel) using seawater from East Mediterranean Sea, the North Sea and the Caspian sea having 4.3, 2.2 and 1.1 % salt respectively, wherein sugarbeet and barley were found tolerant upto a salt concentration of 22 g/l (Boyko and Boyko, 1959). However, these experiments were conducted on coastal dune sand. In Germany, Meijering (1966) reported successful growth of *Agropyrum junceum* using seawater containing 32 g/l salts. No adverse effects have been observed when seawater of 35,000 ppm was applied to lettuce in sandy loam soil in United States (Lunin and Gallatin, 1960).

In Spain, a variety of crops viz. food, forage and vegetables were grown irrigating directly with seawater (35 g/l) by adopting the system of hydroponics combined with traditional agriculture (Gomez, 1964). The composition of sea water has been shown in Table-1.

**Table 1.1: Seawater composition (Total dissolved salts 34.73 g/L)**

Elements	mg/liter
Chloride(Cl <sup>-</sup> )	18.980
Sodium (Na <sup>+</sup> )	10.556
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	2.649
Magnesium (Mg <sup>2+</sup> )	1.262
Calcium (Ca <sup>2+</sup> )	0.400
Potassium(K <sup>+</sup> )	0.380
Bicarbonate(HCO <sub>3</sub> <sup>-</sup> )	0.140

In India, efforts have been made to grow crops by use of seawater on coastal dune sand for the last two decades. A brief review has been presented on the success of the work carried out by Central Salt and Marine Chemicals Research Institute, Bhavnagar. The seawater based culturing and cultivation of plants attempted by Epstein revealed conceptable phenotypic plasticity and genotypic variability in large number of varieties using the criteria of 50% reduction in yield in high concentration of seawater.

#### *Early seedling growth*

Many of the crop varieties succumb to high salinity at the germination and early seedling growth stages. However, differential tolerance of crops and their varieties to salinity was observed both during germination and early seedling growth when seawater of different salinity was used (Iyengar et al. 1968, 1977).

The tolerance during the early seedling growth was taken as criteria for selection and acclimatization to higher salinity induction of crop varieties. With a view to grow crops under direct irrigation with seawater, initial establishment was done with rain or fresh water and later supplemented with seawater.

The germination being the most critical stage of plant growth, evaluation was made of different species and varieties of crops to various grades of salinity of seawater. It was observed that the rate of imbibition is inversely proportional to the salts present in water of different salinity and the percentage germination was lower in high salt concentrations (Iyengar et al. 1968, 1977; Kurian and Iyengar, 1965). Many of the varieties of crop species showed good germination percentage upto 10,000 ppm of seawater while only tolerant varieties could germinate in sea- water of 15,000 to 20,000 ppm.

Few crops were evaluated during my work for salinity tolerance and taking the criteria of 50% reduction in yield over control, crops were classified as tolerant; moderately tolerant and susceptible. The salinity tolerance of a variety can be adjudged by (i) the minimum percentage reduction in yield over control and (ii) the highest level of salinity that a variety can tolerate and can complete the life cycle. The tolerant varieties of various crops which showed less than 50% reduction in yield at 20,000 of ppm of seawater are summarized in Table 1.2.

**Table 1.2: Percentage reduction in seed yield of different crops due to sea water irrigation**

Name of the crop	Total number of varieties screened	Varieties tolerant above 20,000 ppm	% reduction in yield
Wheat	21	Kharchia, NP-824, Rupsal, RPC-1-35	30
Barley	27	BG-131, DL-3, CS-3	40-45
Bajra	38	Babapuri local-2	25
Ragi ( <i>Elusinecoracana</i> )	20	VL-120, VL-123 and VL-110	40-45

The reduction in yield in the crops has been mainly attributed to the reduction in number of leaves, number of tillers, number of branches, ear length, percent grain filling, days to flowering and days to maturity.

In our experiments, graminaceous crops were found to be more tolerant to seawater as compared to others i.e. oil seeds, pulses, vegetable crops as earlier reported by Ayers and Westcot (1977) and Mass and Hoffman (1977). When local selection of bajra var. Babapuri and wheat var. Kharchia were compared for seawater tolerance with other varieties including hybrids, the local varieties performed better as compared to others. The tolerant variety of bajra when crossed with female lines presuming to transfer the character, the hybrids showed improved yield over the parent variety. Carter (1975) and Epstein and Norlyn (1977) reported similar type of variation under different salinity treatments. Rush and Epstein (1976) also contended that selections from seashore plants have inherited trait of salt tolerance and are transferable.

Ragi (*Eleusine coracana*) varieties were also evaluated for seawater (5,000 ppm) tolerance and few varieties were identified to have less than 50% reduction in yield.

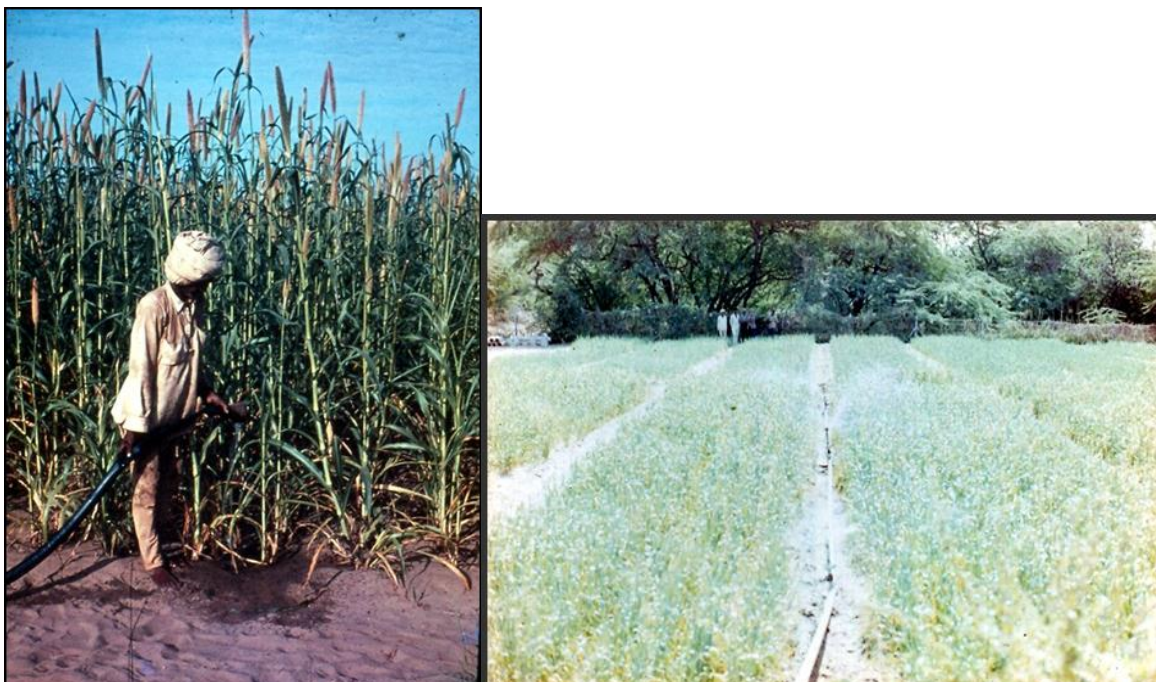
Under "All India Coordinated Project on Barley for Salinity" 13 genotypes were studied for yield potential under definite EC of seawater concentrations. The work was taken up with yield as the final parameter for delineating the salinity tolerance in the genotypes. Seeds of 13 varieties of barley viz. CS-21, CS-46, DL-200, K-324, BH-98, DL-165, CS-80-2, Karan-Iv, DL-3, CS-54, Karan-18, CS-3 and CS-8, obtained from Central Soil Salinity Research Institute, Kamal, were grown in randomized block design with three replications on coastal dune sand at Hathab field station during Rabi season of 1981-82. The plot size

was 8.0 m x 1.0 m and row spacing was 25 cm. The plants were allowed to establish under fresh water upto 30 days. Later on, diluted seawater of 10,000 ppm (predecided for comparison with soil salinity of other centers) was given as irrigation water on each third day. The salinity of dune sand was also estimated for fresh water plot and saline water plots after the experiment at different depths.

On 10 plants of each variety randomly selected from each replication, observations were recorded for different quantitative characters, viz. plant height, ear length, number of spikelet per ear, number of grains per ear, grain yield per plant, days to maturity, 1,000 grain weight, total straw production per plot, percent reduction in seed setting. Plant samples collected at the harvest were dried and utilized for mineral analysis in different plant parts.

Varieties DL-3 (48.48%), Karan-19 (36.36%), CS-3 (53.01 %) showed about 50% reduction in yield under seawater irrigation and regarded as tolerant as compared to other varieties where reduction was found to be as high as 82% (Table 2).

Ayers et al. (1952), Giriraj et al. (1976), Maas and Haffman (1977) and others also suggested that less reduction in yield potential (50%) at particular salinity level of a genotype indicates its tolerance.



**Field trials of Bajra and Wheat on coastal sand dunes at Hathab, Bhavnagar**

**Table1.3: Effect of seawater irrigation on different morphological and yield contributing characters under fresh water (C) and sea water (S).**

S. no.	Characters	Plant height (cm)	Ear length (cm)	Number of spikelets/ear	Number of grains/ear	Grain yield/plant (g)	1000 grain weight	Days to maturity	Total straw production /plot (g)	% reduction in grain setting/ear
	Varieties									
1	CS-21 C	51.80	15.13	27.80	19.87	0.600	30.91	100.66	4426.67	--
	S	34.33	11.87	14.47	8.80	0.190	21.95	86.67	2200.00	55.71
2	CS-46	52.33	16.07	27.87	25.93	0.990	38.92	97.33	2433.33	--
		35.07	13.33	17.53	15.00	0.340	24.01	94.66	1640.00	42.15
3	DL-200	53.06	15.23	33.80	29.67	0.810	30.48	97.33	2633.33	--
		34.13	11.13	17.87	10.20	0.200	19.35	96.33	1400.00	65.62
4	K-324	52.07	15.73	23.80	1.07	0.720	34.70	100.67	3200.00	--
		32.07	11.47	10.93	7.40	0.160	22.10	95.33	1580.00	64.82
5	BH-98	53.47	16.50	27.63	23.93	0.880	37.55	106.33	1553.00	--
		34.13	12.07	17.87	5.93	0.150	28.39	98.67	1700.00	75.22
6	DL-165	51.73	16.00	27.67	20.20	0.760	33.67	107.00	3000.00	--
		35.23	13.13	17.07	91.53	0.230	25.80	95.33	1646.67	52.82
7	CS-80-2	46.47	14.40	22.07	18.67	0.540	30.09	105.67	3280.00	--
		33.83	11.27	17.00	8.53	0.210	26.01	88.00	2713.33	54.31
8	Karan-19	40.87	12.40	19.87	10.20	0.220	23.44	103.67	2633.33	--
		32.17	11.00	17.80	6.67	0.140	21.13	94.33	2240.00	34.61
9	DL-3	52.60	16.00	2533	23.73	0.660	27.08	101.66	3080.00	-
		39.73	14.00	20.93	14.27	0.340	21.67	95.00	2400.00	66.29
10	CS-54	44.67	15.07	23.33	18.20	0.590	35.93	103.66	3726.67	-
		36.20	12.80	22.27	8.73	0.200	24.32	93.00	1920.00	52.03
11	Karan 18	46.70	13.17	22.40	14.80	0.340	34.92	105.67	2913.33	-
		29.07	10.60	16.47	7.10	0.140	22.75	94.33	1140.00	52.03
12	CS-3	55.67	15.87	25.67	23.07	0.830	36.67	105.33	2700.00	-
		39.87	13.80	22.67	13.40	0.390	29.27	87.67	2687.67	41.92
13	CS-8	51.87	14.80	26.80	2227	0.560	27.33	10767	3480.00	-
		40.47	13.07	17.13	8.40	0.210	25.64	95.33	2820.00	62.28
	CD at 0.01%									
	Variety	1.00	0.62	0.80	088	NS	0.88	139	91.45	
	Salinity	1.74	1.70	2.14	2.27	NS	2.27	3.55	233.88	
	Interaction	3.48	2.40	1.53	3.20	007	3.19	500	329.76	

The reduction in yield under seawater irrigation or salinity has been worked by a number of workers in different crops particularly in barley by Epstein and Norlyn (1977). It is probably either due to greater salt build up near the root zone accompanied by low external water potential as reported by Bernstein and Hayward (1958) and Kumar et al. (1981) or due to poor seed setting caused by pollen sterility (Ota et al. 1956). The reduction in seed setting is found to be maximum in BH-98 (75%) while it was minimum in Karan – 19 (34.6%). As earlier observed by Pearson and Bernstein (1959), during the present studies also 1000 grain weight was adversely affected in almost all the varieties. The straw yield was also influenced under seawater irrigation in all the varieties except BH-98 (whereas increase of 9.47% over control was observed) probably due to adverse effect on transpiration and pH of the cell sap.

Total ion accumulation has also been studied in all the varieties in different plant parts, which is found to be more under seawater irrigation as compared to control. Low K/Na ratio indicates more accumulation of  $\text{Na}^+$  in stem as compared to leaves while rest of the cations found to be more in leaves due to high export from shoot. The varieties like DL-3, Karan-19 and CS-3 which have shown about 50% reduction in yield found to accumulate relatively more cations as compared to other varieties perhaps due to ion compartmentation. On the contrary, variety K-324 which showed 77% yield reduction also accumulated highest amount of cations in plant parts. Therefore, each genotype behaves differentially to withstand high salinity. Earlier Greenway and Munns (1980) also observed that in rice one tolerant variety had low  $\text{Na}^+$  in its leaves, while another tolerant variety was high in  $\text{Na}^+$ . Similarly, in maize, it was observed that varieties which differed substantially in salt tolerance had similar ion concentrations in leaves. The findings also indicate that among the genotypes differences in tolerance to salinity are associated either in their behavior as excluder or accumulator.

The salinity of soil increased almost double after irrigating with seawater, however, pH of the soil was not influenced to the greater extent. Due to leaching of salts to deepest layer of soil, salt concentration increased with the increase in soil depth during successive irrigations in dune sand.

**Table 1.4: Mineral composition of different plant parts under treatments.**

Sr. No.	Varieties	Total cation accumulation in stem & leaves	K <sup>+</sup> /Na <sup>+</sup>
1	CS-21 C	193.89 ( 191.27)*	0.42 (0.54)*
	S	286.39 (293.72)	0.20 (0.30)
2	CS-46	188.36 (149.95)	0.50 (0.77)
		263.32 (287.87)	0.22 (0.31)
3	DL-200	187.39 (183.97)	0.86 (1.36)
		292.99 (268.83)	0.24 (0.27)
4	K-324	198.30 (208.40)	0.21 (0.32)
		353.16 (342.95)	0.21 (0.26)
5	BH-98	153.01 (141.18)	0.73 (1.12)
		230.47 (231.74)	0.38 (0.51)
6	CS-80-2	110.67(140.21)	0.63 (0.67)
		291 31 (294.81)	0.26 (0.28)
7	DL-165	156.45 (150.31)	0.52 (0.83)
		286.36 (273.54)	0.26 (0.48)
8	Karan-19	215.56 (181.26)	0.17 (0.30)
		303 56 (302.46 )	0.09 (0.20)
9	DL-3	165.87 (195.43)	0.41 (0.84)
		293.28 (349.38)	0.28 (0.31)
10	CS-54	164.66 (168.56)	0.17 (0.36)
		294.71 (321.10)	0.11 (0.23)
11	Karan-19	122.52 (164.01)	0.59 (0.93)
		310.37 (285.55)	10 (0.27)
12	CS-3	192.05 (166.67)	0.73 (0.88)
		346.07 (360.41)	0.22 (0.24)
13	CS-8	136.48 (200.51)	0.37 (0.54)
		298.97 (338.74)	0.21 (0.19)

On the basis of results it could be concluded that genetic differences occur widely among varieties for salt tolerance. As the growth or reduction in yield is a quantitative character, measurements of salinity tolerance should also be assessed quantitatively. Therefore, the absolute salt tolerance denoted by yield potential of a genotype at a particular salinity level was used as main criteria for identification of tolerant varieties. Such evaluation was also made for a number of crops and compiled by Maas and Hoffman (1976). The successive acclimatization of tolerant varieties to higher dilutions of seawater will be utilized for improvement in genotypes and their yield potential since it is contended to have a permanent alteration in the genome probably stimulated by the environment in due course of time (Epstein et al. 1980, Shannon 1982).

**Table 1.5: Accumulation of salts in control and saline plots at different depths after experiment**

Treatment	0-15 cms		15-30 cms		30-45 cms	
	EC	pH	EC	pH	EC	pH
Control	0.70	8.5	0.85	8.05	0.96	8.02
Saline	1.22	8.25	1.49	8.0	1.69	7.70

### **Salt-affected soil of U.P.**

A survey of Amethi soils was conducted to evaluate for the suitability of Jatropha cultivation. Under the study two profile samples and eleven surface samples collected representing sodic (User) soil and were analyzed for salinity appraisal and for fertility status. The organic carbon contents were very low. These soils were non-calcareous in nature and CEC varied from 8.6 to 13.91 cmol kg<sup>-1</sup> in profiles whereas, it varied from 4.52 to 8.17 cmol kg<sup>-1</sup> in surface soils. In profiles, CEC values corroborated well with clay distribution. The exchange complex was dominated by Na followed by Ca, Mg and K. The ESP was varied from 64.69 to 89.98 in profiles, whereas it varied from 57.71 to 83.97 with a mean value of 71.96. The pH ranged from 9.9 to 10.3 in profile, whereas it varied from 8.2 to 10.4 in case of surface samples. The ECs ranged 4.2 to 37.3 dS m<sup>-1</sup> in profiles and gradually decreased with depth. In surface samples ECs varied from 1.2 to 47.1 dS m<sup>-1</sup> with a mean value of 24.2 dS m<sup>-1</sup>. The salts of carbonates and bicarbonates of sodium followed by Ca, Mg and K were dominant in saturation extract. These soils were very low in available N, and medium to high in available P and high in K. (Agropedology2006).

## **Chapter 2**

### **Improvement and multiplication of Jojoba and other important desert economic plants**

I was assigned to work on selection and genetic improvement in Jojoba from 1984 onwards to generate basic data on adaptability and seed yield.

#### **A. Jojoba (*Simmondsia chinensis*)**

Jojoba (*Simmondsia chinensis*), a desert shrub endemic to Sonoran desert of Mexico, Arizona and California belongs to family Simmondsiaceae. It has attracted worldwide attention of scientists as potential renewable source of liquid wax which is a substitute to sperm whale oil. Jojoba is a natural wood, dioecious, wind pollinated, evergreen, sturdy shrub of grey-green colour, reaching a height of ten to twelve feet. It exhibits great morphological heterogeneity in the populations raised from seeds and said to live for 100-150 years.

The jojoba seed oil is as unique in the plant kingdom as the sperm whale oil in the animal kingdom. It stands out singularly distinct as the only non-triglyceride vegetable oil. It is not a fat but a liquid wax. The oil is soluble in common organic solvents such as benzene, ether, chloroform, carbon tetrachloride, carbon disulfide and not in alcohol & acetone.

It grows well in the area of less rainfall (12.5-40 cm) and low soil fertility. Temperature is critical factor in growing jojoba. An annual rainfall of low and medium with temperature above 6°C is well suited for jojoba cultivation. High temperatures not exceeding 50°C are said to have no adverse effect. It thrives well on variety of soil types from porous rocks to clays. However, well aerated and well drained soils are the ideal substrate for its growth. It cannot grow on wet lands and marshy areas. But it can be grown on well drained non-saline soils and dune sand of various states of the country viz. Gujarat, Orissa, Andhra Pradesh, Tamil Nadu etc.

#### **1. Section and improvement in Jojoba**

Jojoba (*Simmondsia chinensis*) exhibits tremendous variability in almost all the botanical traits including yield potential. Being dioecious in nature, male plants outnumber female plants in plantations raised from seeds and yield per plant is very erratic during different years.



**Jojoba experimental field at Zanjmer, Tal. Talaja, Bhavnagar**

#### **Morphological variability:**

Genetic improvement programme was initiated in Jojoba and for the purpose morphological variability was studied both in male and female plants in the population. Jojoba was initially introduced for domestication purpose on sand dunes in the remote coastal village of Zanjmer (Dist. Bhavnagar, Gujarat), having a semi-arid climate. Seeds for this purpose were obtained in 1980 from M/s. Juncas's Jojoba Oil and Seed Inc., Arizona, U. S. A. Initially plants were established in randomized block design on 1 hectare area. The population raised from the seeds showed approximately 1:1 ratio of male and female plants.

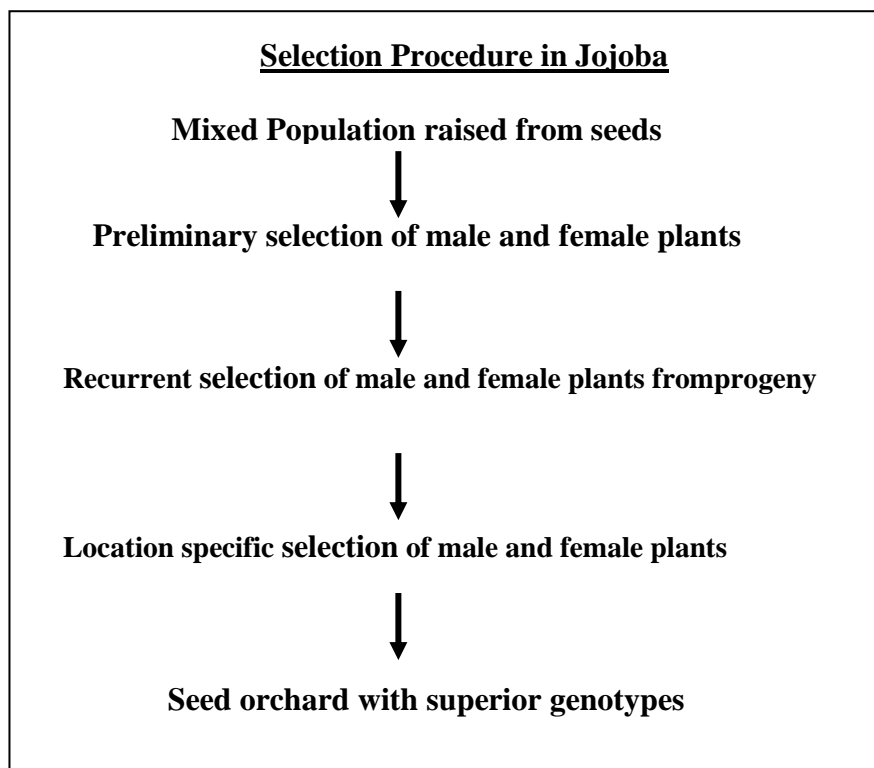
A population of 1150 plants was established on the coastal sand dunes in the semiarid region of Gujarat. Various morphological characters were measured to study the magnitude of variability in this population. Both male and female plants were classified into three categories on the basis of height and spread: i) plants with less than 1 m height and less than 3 m spread, ii) plants with 1-2 m height and 3-6 m spread and iii) plants with more than 2 m height and more than 6 m spread. Various statistical parameters of variability (SE and CV) were worked out to assess the extent of variability in 579 male and 571 female plants grouped in the above three categories (Table 2.1).

**Table 2.1: Variability in plant height and plant spread in various categories of male and female plants of Jojoba**

Category	Character	Plants in each category (%)		Range		Mean $\pm$ SE		CV	
		Male	Female	Male	Female	Male	Female	Male	Female
0-1	Height	8.5	1.0	0.44-0.95	0.40-0.80	0.76 $\pm$ 0.02	0.71 $\pm$ 0.07	52.1	19.7
0-3	Spread	3.0	4.0	0.60-2.84	0.65-2.80	1.95 $\pm$ 0.11	2.14 $\pm$ 0.15	41.2	32.7
1-3	Height	75.4	76.8	1.10-2.00	1.00-1.90	1.84 $\pm$ 0.04	1.62 $\pm$ 0.26	20.1	81.4
3-6	Spread	39.8	30.8	3.22-6.00	3.40-5.86	4.55 $\pm$ 0.18	4.37 $\pm$ 0.08	23.1	26.5
$\geq 2$	Height	16.1	10.0	2.20-2.90	2.11-2.90	2.56 $\pm$ 0.24	2.30 $\pm$ 0.40	15.4	9.7
$\leq 6$	Spread	57.1	62.2	6.14-6.60	6.08-6.45	6.22 $\pm$ 0.10	6.35 $\pm$ 0.18	29.1	22.5



**Variability in seed characteristics and tepals in Jojoba**



## **Selection**

Plants were further reclassified into three categories on the basis of spread (S) and height (H) ratio as follows: i) erect, with S/H ratio less than 1.5, ii) semi-erect, with S/H ratio between 1.5- 3.00 and iii) prostrate with S/H ratio above 3.00. The mean, range, SD and CV were calculated for plant height, plant spread, plant volume, internode length, pollen fertility, pollen grain size, and duration of flowering for 30 male plants; and plant height, plant spread, plant volume, internode length, seed size, seeds/plant, seed

yield/plant, and 100-seed weight for 30 female plants in the three plant types separately (Table 2.2 and 2.3). Plant volume (m<sup>3</sup>) was calculated from the maximum crown diameter and height according to the following equation

$$V = 0.67 \pi r^3 + \pi r^3 (h-r-0.35)$$

The population raised from seeds exhibited marked variability in vegetative growth especially in case of plant height and plant spread. Some of the plants were about 3m in height, while others grew only to the height of 40 cm. The plant spread also varied from 60 cm to more than 6m (Table 2.1).

**Table 2.2 Variability in morphological characters in various categories of male plants of Jojoba**

Sr. No.	Character	Erect type	Semi-erect type	Prostrate type
1	Plant height (m)	0.7-3.0 2.07 34.6	0.8-2.2 1.64 32.3	0.62-1.70 a 1.13b 32.9 c
2	Plant spread (m)	0.7-4.4 2.57 44.1	2.0-5.5 4.27 27.1	2.00-5.50 4.43 24.7
3	Plant volume (m)	0.7-14.1 5.08 105.4	2.5-23.8 12.09 58.9	9.7-16.5 12.28 39.3
4	Internode length (cm)	2.5-3.50 3.04 9.60	2.40-3.33 2.82 9.80	2.40-3.30 2.91 10.40
5	Pollen fertility (%)	82.9-98.00 96.85 5.30	90.8-97.7 94.14 2.9	81.80-94.2 87.57 4.3
6	Pollen grain size (µm)	34.0-44.2 39.44 6.7	23.5-40.9 33.45 13.9	29.9-39.3 34.22 9.7
7	Flowering duration (days)	22.8-36.0 31.80 7.7	28.0-34.0 29.40 8.22	29.0-34.0 30.50 5.1

a = range, b = mean and c = C.V. %

Earlier Yermanos (1977) also reported considerable variability in plant height and plant diameter in Jojoba. Among male plants, maximum variation was observed among the erect type plants for plant volume, plant spread, Plant height and pollen fertility (96.8%), diameter of pollen grain (39.4 µm), duration of flowering (31.8 days). The semi erect plants showed sufficient variability for pollen diameter and duration of flowering only. The

mean values for these traits in this group were lowest as compared to other plant types.

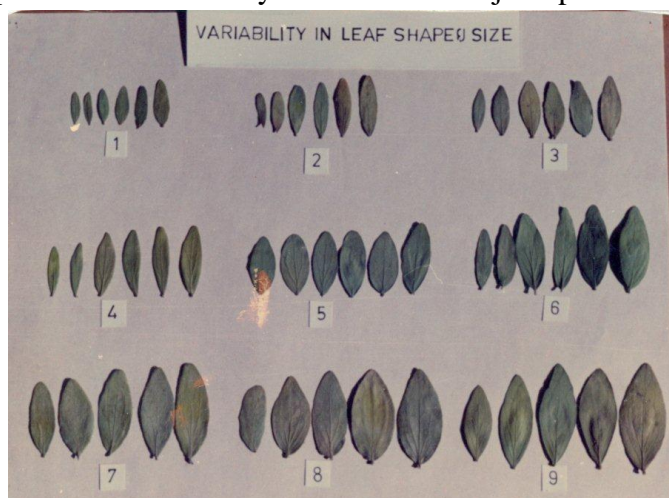
The prostrate plant type was intermediate between the two categories in most of the characters; although the means were high for plant spread (4.43 m) and plant volume (12.84 m<sup>3</sup>) where, pollen fertility (87.57%) and plant height were found lowest in these plants (Table 2.2).

Reviewing the performance of 30 selected female plants of jojoba under semiarid conditions, more or less similar values of CV were obtained for plant height and plant spread amongst the three types while considerable variation was observed for plant volume all the seed characters (Table 2.3). The CV values for yield and its contributing characters were high in the semi-erect group, especially for seed size and seeds/plant, while the prostrate plants exhibited maximum variability for 100-seed weight and seed yield per plant. Wide range of variation was recorded for seeds/plants, but the maximum variability (500-3051 seeds/plant) was recorded in the group of prostrate plants. The CV for this attribute was highest in the semi-erect group (74.36), although maximum average seed number (1257.70) was recorded in the prostrate group of plants. Thus, this character may be helpful in the improvement of this plant species.

**Table 2.3 Variability in morphological characters in various categories of female plants of Jojoba**

Character	Erect			Semi erect			Prostrate		
	Mean	Range	CV (%)	Mean	Range	CV (%)	Mean	Range	CV %
Plant height (m)	1.99	1.0-2.8	29.1	1.80	0.9-2.9	28.9	1.71	1.4-2.9	25.7
Plant Spread(m)	2.22	1.1-3.2	29.3	3.86	1.9-5.1	23.3	5.62	4.3-8.8	24.6
Plant Volume (m <sup>3</sup> )	7.38	0.4-13.8	61.1	11.53	0.8-35.6	84.1	4.22	4.8-67.3	127.9
Internode length (cm)	3.00	2.0-3.7	19.7	2.7	1.9-3.7	21.0	2.80	2.5-3.2	8.1
Seed size (cm)	1.62	1.5-1.9	7.4	1.66	1.4-2.5	18.1	1.58	1.3-1.7	8.6
No. of seeds/plant	180.7	101.0-396	50.7	780.7	236.0-1929.0	74.4	1257.7	500.0-3051.0	60.1
100-seeds weight (g)	56.42	42.24-78.5	20.1	67.06	38.3-82.5	17.3	64.67	47.1-99.9	21.8
Seed yield/ plant (g)	109.09	50.5-300.6	70.3	512.14	194.6-1302.6	77.8	771.43	348.3-1794.9	55.4

Leaf variability and estimation of leaf area in Jojoba selections was estimated in Jojoba population. Leaf area, is an important parameter to compute the photosynthetic area of leaf, contributing ultimately towards the yield. Leaves exhibit a wide range of variability in their size, shape and area among various genotypes of male and female plants. Therefore, it was proposed to study leaf variability in both male and female plants so as to develop a rapid, accurate and non-destructive method for leaf area estimation in jojoba and find out the difference, if any, in both types of plants. For the purpose, ten male and ten female plants were randomly selected from Jojoba plantation raised in 5 ha area on coastal sand dunes at village Zanjmer, Gujarat.



Twenty five leaf samples from each type taken from 5<sup>th</sup> inter nodes were considered for recording observations. Leaf length was measured to the nearest point from the tip of the

petiole while leaf width was taken at the widest region across the leaf. Area of individual leaf was measured using graph paper by counting the squares. Leaf area was also obtained by length x breadth which was higher in each case than the actual area of individual leaf obtained from graphical method. Therefore, the values of length and breadth of the leaf were multiplied by a constant, which was obtained by dividing the sum of actual leaf area (graphic values) of all the leaf samples by the sum of products of length and breadth of leaves.

In jojoba, two types of leaves i.e. oval and lanceolate were identified in the samples of both male and female plants. Leaf samples of both types when analyzed statistically showed that the mean values of leaf length and leaf area were more in female plants where as leaf index was more in male plants, however, leaf width was almost equal in both the types. Male plants exhibited a wide range in leaf length (2.66-5.59 cm) whereas in females it ranged 3.61-4.36 cm. The actual leaf area varied from 2.412 - 11.072 cm<sup>2</sup> in male plants (Table 2.4) whereas in females, area ranged from 4.721- 10.995 cm<sup>2</sup> indicating that female plants are having slightly larger leaf area (Mean 6.693) than male plants (Mean 6.075) in jojoba (Table 2.5). However, no relation was observed between the shape of the leaf and leaf area as reported in cashew (Bhagavan and Mohan, 1988). Maximum leaf area was observed in male genotype E

13-5 (11.072 cm<sup>2</sup>) and female genotype E14-1O (10.995 cm<sup>2</sup>) whereas lowest leaf area among female plants was recorded in the genotype E 4-11 (4.721 cm<sup>2</sup>) and also in male genotype E 16-4 (2.412 cm). The differences between the leaf areas calculated from graph and estimated with the help of formula ranged from 0.006-- 0.066 both for male as well as female genotypes. It was interesting to note that differences between these two estimations were lower in case of males (0.006 to 0.378) than in females (0.022- 0.663) and the differences obtained for all genotypes were within the permitted range of experimental error.

In male plants C.V. values for both the methods were found to be almost equal. On the contrary in female plants the mean values of actual and estimated area varied up to some extent and the C.V. values for both the methods were also different. Degree of association between the estimated leaf area and the actual leaf area was also estimated for accuracy. Regression equations were developed to estimate the leaf area on the basis of length and breadth of leaves separately for both males and females. Coefficient of determination (R<sup>2</sup>) values was also highly significant for male (0.994) as well as for females (0.985).

**Table 2.4: Variability in leaf size, leaf area and leaf shape in various male genotypes of Jojoba.**

S. No.	Genotype	Leaf size		L x B (in cm <sup>2</sup> )	Leaf index	Leaf area (cm <sup>2</sup> )		Difference in estimated & actual	Leaf shape
		Length	Width			Actual (Graphic)	Estimated (LxBxK)		
		(L)	(B)						
1	E16-4	2.663	1.211	3.224	2.199	2.412	2.530	0.118	Oval
2	E15-5	4.400	2.932	12.900	1.501	10.001	10.126	0.125	Oval
3	E27-3	4.850	2.372	11.504	2.045	8.289	9.030	0.741	Lanceolate
4	E24-2	3.790	1.600	6.064	2.369	4.346	4.760	0.414	Lanceolate
5	E18-6	4.262	1.774	7.560	2.402	5.555	5.935	0.380	Oval
6	E42-1	3.880	1.630	6.324	2.380	4.906	4.964	0.058	Oval
7	E2-7	4.527	1.352	6.120	3.348	4.734	4.804	0.070	Oval
8	E13-5	5.590	2.700	15.093	2.070	11.072	11.848	0.776	Lanceolate
9	E18-6	3.836	1.782	6.835	2.153	5.002	5.365	0.363	Lanceolate
10	W41-1O	4.050	1.421	5.755	2.850	4.429	4.518	0.089	Oval
	Mean±	4.184	1.877	8.137	2.332	6.074	6.388		60% Oval
	S.E.	±0.24	±0.19	± 1.18	±0.16	±0.88	±0.93		40%
	C.V.	18.42	31.38	45.95	21.03	45.63	45.00		Lanceolate

**Table 2.5: Variability in leaf size, leaf area and leaf shape in various female genotypes of Jojoba.**

S. No.	Genotype	Leaf size		L x B (in cm <sup>2</sup> )	Leaf index	Leaf area (cm <sup>2</sup> )		Differences in estimated and actual	Leaf shape
		(L)	(B)			Actual (Graphic)	Estimated (LxBxK)		
1	E14-13	3.992	1.972	7.872	2.024	6.179	6.813	0.634	Oval
2	E14-10	4.935	2.730	13.472	1.808	10.995	12.072	1.077	Oval
3	E8-19	3842	1.589	6.1049	2.418	4.792	5.534	0.742	Oval
4	E8-17	4.800	1.592	7.641	3.015	6.990	7.339	0.349	Oval
5	E5-17	4.722	1.600	7.555	2.951	5.930	6.878	0.948	Lanceolate
6	E5-15	4.224	1.600	6.758	2.640	5.805	6.430	0.625	Lanceolate
7	E4-II	3.611	1.666	6.105	2.167	4.721	5.737	1.016	Lanceolate
8	E4-4	4.930	1.867	9.204	2.641	8.225	9.013	0.788	Lanceolate
9	E2-19	4.356	2.011	8.759	2.166	7.275	8.111	0.836	Oval
10	E2-4	4.227	1.753	7.409	2.411	6.016	7.124	1.108	Oval
	Mean ±	4.363	1.838	8.078	1.838	6.693	7.505		60% Oval
	S.E.	±0.15	±0.11	±0.68	±0.12	±0.59	±0.60		40%
	C.V.	10.17	20.47	26.45	16.12	27.67	25.40		Lanceolate

**Variability in yield parameters:**

From experimental plot at Zanjmer Field Unit, Bhavnagar, yield of all the female plants was recorded individually for the last 18-20 years (Table 2.6). Seeds from selected plants were harvested to raise their progenies in plant-row design for further assessment of segregation of characters and yield performance.

Yield behaviour of female plants of 1 ha. plantation during different years has been shown separately. It shows that the percentage of plants bearing fruits varied during different years. Based on various morphological and yield characters initially 31 female plants were selected (Chikara et al. 1991) and rest of the plants were considered as general ones. Later few plants are short-listed on the basis of yield performance. To assess the effect of temperature and rainfall on yield correlation were calculated and it was found that only the former affected the yield. Later 7 high yielding female plants were identified and their yield during different years has been demonstrated (Table 2.7).

**Table 2.6: Yield of Jojoba (Junco plantation) at Zanjmer Field Station during different years**

<b>S. No.</b>	<b>Year</b>	<b>Total no. of female plants</b>	<b>Plants bearing fruits (%)</b>	<b>Mean seed yield per plant (g)</b>
1	1982	758	34.30	6.76
2	1983	754	50.27	10.12
3	1984	746	37.0	16.33
4	1985	741	32.25	18.93
5	1986	733	62.35	63.29
6	1987	733	32.20	22.30
7	1988	735	35.10	08.63
8	1989	735	3.13	07.26
9	1990	735	0.26	15.78
10	1991	725	10.07	42.35
11	1992	720	29.03	43.30
12	1993	705	24.39	112.15
13	1994	660	37.72	48.27
14	1995	620	33.38	35.75
15	1996	575	17.21	43.81
16	1997	520	37.11	94.64
17	1998	503	28.42	151.20
18	1999	480	23.12	65.97
19	2000	480	44.40	251.57
20	2001	456	41.01	267.98
21	2002	444	27.88	156.77
22	2003	444	32.65	234.89
23	2004	416	23.83	211.43
24	2005	347	25.67	98.56
25	2006	339	20.12	55.61
26	2007	324	30.44	49.68

**Table 2.7: Performance of 7 Identified Productive Plants of Jojoba during 1982-2000**

Date of transplantation March 1980

spacing: 3 x 2 M

Area: 1ha. (Total plants: 1600)

No. of Female plants: 443/760

S. No.	Year	ZE 19-1	ZW 39-3	ZW 12-8	ZE 21-14	ZE 24-8	ZE 9-13	ZE 18-5
1	Pooled yield 1982-1987	1392.66	1378.44	627.04	1020.62	1812.85	1136.83	1118.06
2	1988	26.27	314.69	32.57	23.58	16.19	22.00	13.26
3	1989	25.36	-	41.38	12.58	14.45	16.88	13.82
4	1990	137.86	162.05	62.95	369.80	11.15	24.25	45.90
5	1991	886.00	13.30	-	166.45	25.35	373.76	12.00
6	1992	364.60	-	680.00	20.48	34.89	154.77	15.98
7	1993	2882.99	166.48	1621.68	965.82	76.86	24.73	332.58
8	1994	47.92	12.37	-	18.96	-	21.69	12.70
9	1995	427.22	33.56	44.53	116.56	14.12	-	-
10	1996	75.77	24.97	24.70	14.57	52.84	-	82.44
11	1997	810.95	339.27	870.14	1288.62	886.30	614.58	60.34
12	1998	122.94	8.09	19.59	8.39	48.47	25.70	32.06
13	1999	660.41	48.92	175.79	472.88	270.07	-	732.20
14	2000	383.80	193.12	681.92	135.00	25.00	263.49	670.40

During 2000-2002 an analysis was carried out to compare the seed yield of Jojoba plantation of CSMCRI and data was compared with the production potential of other plantations in the world. It can be seen below that the seed yield of CSMCRI elite plants was comparable to other plants in the world.

**Data on Worldwide Jojoba Cultivation and Seed Production along with data on relative productivity of CSMCRI elite plants during 2000-2002**

<b>Sr. No.</b>	<b>Country<sup>1</sup></b>	<b>Total Area<sup>1</sup> (ha)</b>	<b>Total Seed Production (MT)</b>	<b>Productivity (Kg per ha)<sup>2</sup></b>	<b>Yield per Plant (kg)</b>
1	Israel	600	1000	1666.66	1.67
2	USA	2240	1455	649.55	0.72
3	Mexico	310	90	290.32	0.32
4	Argentina	3400	950	279.41	0.31
5	Peru	300	75	250.00	0.28
6	Egypt	140	15	107.14	0.12
7	Australia	400	8	20.00	0.022
8	CSMCRI <sup>3</sup>		0.02153 <sup>4</sup>	1195.58	1.54 <sup>4</sup>
			0.00276 <sup>5</sup>	153.38	0.20 <sup>5</sup>
			0.02152 <sup>6</sup>	1195.00	1.54 <sup>6</sup>

<sup>1</sup> Figures are from International Jojoba Export Council (IJEC) for the year 2000;

<sup>2</sup> Figures calculated based on the data given by IJEC;

<sup>3</sup> Figures given are for the 14 elite genotypes in CSMCRI's Zanjmer progeny plot (date of transplantation, June 1987) on coastal sand dune, with bare minimum agronomic input;

<sup>4</sup> Data for the year 2000;

<sup>5</sup> Data for the year 2001;

<sup>6</sup> Data for the year 2002.

Since the water of the well at field station became saline and is not suitable for irrigating Jojoba, the plants are being maintained with no irrigation. In between the data on production potential could not be recorded due to several reasons. Again since last couple of years, data on seed yield was collected for only selected female plants which are given below (Table 2.8 and 2.9). It can be seen that even under rain fed conditions the seed yield per plant in some of the plants exceeded one kilogram during 2009 and 2010. The assessment of seed yield is being continued.

**Table 2.8: Ten highest seed producing plants of Jojoba during 2009 harvest**

S. No.	Plant number	Number of Seeds	Seed Yield (g)
1	P3 E 1-11	2212	1890.2
2	JE 13-3	1471	890.7
3	JE 18-5	1355	739.9
4	P1 5-3	1350	829.1
5	JW 12-8	1170	801.3
6	P4E 4-7	1125	573.6
7	JE 13-12	1100	524.3
8	JW 15-2	1072	616.1
9	P3 E 2-9	1045	225.8
10	JE 17-2	852	493.5

**Table 2.9: Ten highest seed producing plants during 2010**

S. No.	Jojoba-Top-10 2010	Seed yield (in g)
1	Progeny-3-E-14-6	1909
2	Progeny-1-W-8-23	1408
3	Progeny-1-W-12-24	1317
4	Progeny-1-W-18-22	1079
5	Progeny-1-W-17-23	945
6	Progeny-1-W-7-23	939
7	Progeny-3-E-3-10	890
8	Progeny-1-W-22-5	873
9	Varietal-E-5-2	741
10	Progeny-1-W-2-24	732

Initially study of production indices was made for 65 female plants taking into account the average yield of five years (Table 2.10). It is clear from the table that maximum percentage of plants (35.38%) exhibited seed yield between 16.37 g to 97.02 g followed by 27.69% plant yielding 100-200 g seed yield per plant. 20% plant performed better with average yield of 200-400g, while rest of 17% plants exhibited average yield between 500 g to 1 Kg. of seeds. High C.V. of 43.90 and 37.08 were recorded in the first and sixth category respectively, thus exhibiting vast variation in these plants. Highest and lowest yield per plant recorded was 1795.12 g and 16.37 g respectively.

**Table 2.10: Index for 65 productive plants on the basis of seed yield per plant.**

S. No.	Range of yield (in g)	No. of % of plants Plants		Range	Mean	C.V. %
1	<100	23	35.58	16.37 -97.02	57.18	43.90
2	100-200	18	27.69	106.37-199.76	160.40	17.74
3	200-300	6	9.23	205.91 - 282.75	236.76	12.04
4	300-400	7	10.77	300.64 - 398.30	350.32	9.94
5	400-500	2	3.08	411.05 -476.39	443.72	10.41
6	500 &>	9	13.85	624.29- 1795.12	1031.93	37.08

The 100 seed weight for 65 selected female plants was estimated separately to select the plants on the basis of 100 seed weight. 60% of these plants are having 100 seed weight between 50-75 g. Approximately 29% plants have 100 seed weight between 75-100 g while one plant had 100 seed weight of about 103 g The plants having higher seed weight were also taken into consideration for the breeding purpose (Chikara et al., 1991).

**Table 2.11: Correlation coefficients between various plants attributes in 65 plants of Jojoba.**

Plant height (cm)	Plant spread (cm)	Plant volume	Intermodal length (cm)	Seed size (cm)	Seed yield per plant (g)	No. of seeds per plant	100 seed weight (g)
	0.558**	0.820**	-0.044	-0.187	0.360**	0.308**	0.315**
		1.000**	0.055	-0.221	0.512**	0.505**	-0.031
			0.0006	0.127	0.460*	0.413*	0.121
				0.124	-0.088	-0.069	0.077
					-0.079	-0.068	-0.002
						0.984**	-0.143
							-0.254

- \* Significant at 5% level
- \*\* significant at 1% level

A critical perusal of the data on correlation response between different components showed a significant positive correlation between seed yield and morphological characters (Chikara et al 1991). Plant height showed a significant positive correlation with plant spread, number of seeds per plant and 100-seed weight (Table 2.11). A significant positive correlation coefficient between plant spread and number of seeds per plant was also recorded( $r= 0.505^{**}$ ). Number of seed per plant was found to be negatively significant with 100 seed weight ( $r= -0.254^{*}$ ).

Maximum positive correlation was noticed between seed yield and number of seeds per plants ( $r= 0.984^{**}$ ). Correlation between plant height and plant spread( $r= 0.558^{**}$ ) and plant spread with seed yield per plant ( $r= 0.512^{**}$ ) and plant spread with

number of seeds per plant ( $r= 0.505^{**}$ ) were also positive and highly significant.

It may be stated that attributes showing significant positive correlation with yield and oil content in seeds were helpful in further improvement and identification of elite clones for commercial exploitation. Forti et al. (1985) reported inverse correlation between plant volume and fruit yield in jojoba. Earlier and Yermanos (1983), Abramovich et al. (1976) and Ramonet and Mores (1985) reported that none of the morphological characters has significant correlation with total yield. While recently Remonet (1988) reported high correlation between number of seed per plant and seed yield which is confirmed during present studies

Later seed from the 30 selected plants were harvested and progeny plants were raised in plant- row design so as to assess the segregation and yield performance among these plants (Table 2.12). The progeny plants were also raised at other location i.e. and Kakarva, Kutch besides Zanjmer, for assessing the performance and comparison of yield. The average yield at Zanjmer and Kakarva was recorded as 100 g and 121 g per plant respectively in the third year (after planting) which was far more than the parent plants grown at Zanjmer experimental plot. From these progeny plants again selection of desirable genotypes was made which are being used for multiplication. A comparative study was made between general, selected and progeny plants for seed yield per plant during different years and shown below.

**Table 2.12: Comparison of average yield potential of general population, selected plants and progeny plants during different years**

Years after planting	General population (in g.)	Selected plants (Av. of 31 plants, in g)	Progeny plants (Av. of 110 plants, in g)
I Year	----	---	---
II Year	10.12	15.78	---
III Year	16.33	20.62	32.46
IV Year	18.93	19.24	58.00
V Year	63.29	72.11	36.87
VI Year	22.30	28.30	80.20
VII Year	08.63	27.84	49.86
VIII Year	17.26	36.64	139.27
IX Year	15.78	38.38	---
X Year	42.35	110.24	---
XI Year	43.30	83.73	---
XII Year	24.53	282.61	---

Normally solitary fruits are observed on female plants but during the course of studies few female plants were identified having a bunch of fruits instead of single fruit. Some of these plants showed erratic behaviour in this character while a few continuously showed this feature. Since this is a desirable character to



enhance the seed yield this plants were selected for further multiplication. Besides these, some plants showed hermaphrodite behaviour with both male and female plants in the same bunch. The female buds did not mature to a fertile fruit, leading to abortive fruiting.

During the studies few abnormal plants were identified in the mother plantation as well as in nursery at experimental plot on coastal sand dunes at Zanjmer (Bhavnagar  $21^{\circ} 47' N$   $72^{\circ} 09' E$ , Gujarat). Various morphological characters of these plants are compared with the normal plants and given in table 2.13. The height of normal plant ranged from 1.50-3.0 m, mutant ranged only approx. 0.75 - 1.20 m. Significant differences were recorded in the size of internode, length and breadth of leaves of mutant and normal plants. The mutant has been with small leaves of oval to long or lanceolate type. The total DNA and RNA contents of mutant plants were found to be almost one third of the normal plants.

Though, the number of chromosomes is same in both the types ( $2n= 52$ ) the variation in DNA content was due to differences in the size of the chromosomes as reported in several other plants. Flowers of this mutant are quite smaller in size as compared to the normal plants. The frequency of these plants in the population recorded to be approx. 0.001-0.002%. Mutants of spontaneous origin were also reported in pea, sawa and cotton. The seeds gave rise to the true type plants in the next generation. The plant is being multiplied through micropropagation as it will be useful to raise high density plantations on marginal lands or as a hedge plant along with existing other oil seed crops. These types of spontaneous mutants are rare in nature and can be exploited in conventional breeding programmes to assess the mode of inheritance of various characters in the existing germplasm of Jojoba (Chikara et al., 2001).

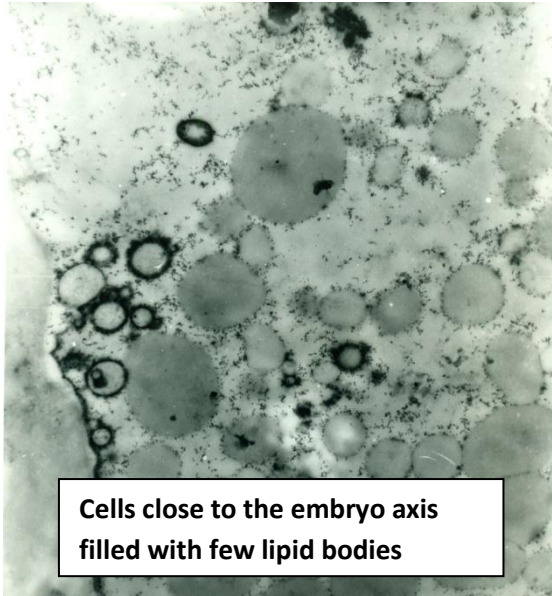
**Table 2.13: Comparison of morphological and yield characters of Jojoba mutant with normal plants.**

Characters	Normal	Mutant
1. Plant height (in m)	1.54 - 2.90 (1.60)*	0.80-1.10 (9.32)
2. Spread (in m)	7.66 - 35.61 (8.80)	1.20-2.85 (1.90)
3. Internode length (in cm.) (1.56)	1.87 - 3.33 (2.61)	0.80 - 2.00
4. Leaf length (in cm.)	1.70 - 3.00 (2.31)	1.15 - 2.10 (1.45)
5. Leaf width (in cm.)	1.15 - 2.10 (1.45)	0.70 - 1.10 (0.89)
6. No. of male flowers/ bunch	8-11 (10.07)	4 - 8 (5.67)
7. Seed yield (in g) (65.43)	348.30 - 794.90 (621.92)	15.27 - 79.41
8. 100 seed weight (in g) (55.00)	38.35 - 103.71 (71.08)	14.00 - 67.87
9. DNA content ( $\mu\text{g/g}$ ). (66.06)	119.44-164.50 (138.25)	64.76-68.09
10. RNA content ( $\mu\text{g/g}$ ) (1894.31)	834.75-912.09 (878.44)	1716.57-1932.63

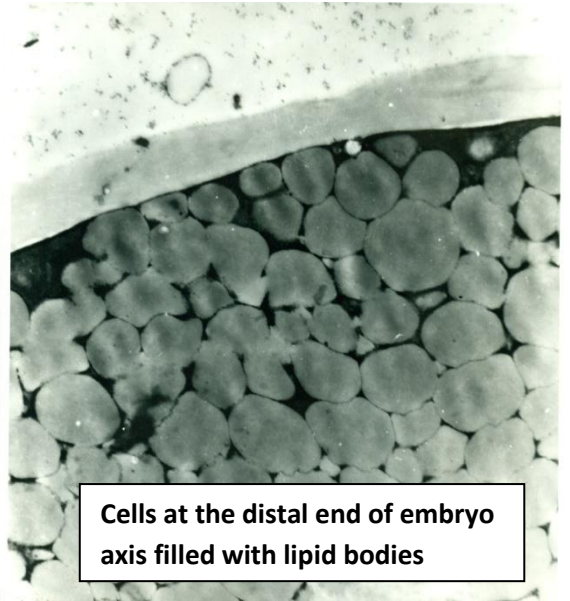
\* Figures in parenthesis are mean values of at least 10 values.

#### **Ultra-structural Studies:**

During the CSIR-CNRS exchange programme program work was carried out to study the ultrastructure of cells in the cotyledons of Jojoba and *Salvadora*. Seeds were germinated in water and later cotyledons were processed for carrying out cytological studies. The samples were fixed in 2% glutaraldehyde in cacodylate buffer 0.1 M pH 7.2 or 7.4 for 1 to 4 hrs at room temperature (normally 2 minutes). It is better to put the sample in vacuum for better penetration. Samples were washed with buffer 2 or 3 times 10 to 20 minute each. Later post fixation was carried out in 1-2% osmium tetra oxide dissolved or mixed in buffer for 2 hrs. The samples were washed in double distilled water 10 minute each for 3 times. Dehydration was carried out in ethanol and propylene oxide series followed by embedding in pure araldite. The ultra-thinsections were cut on ultra-microtome, fixed on grids and observed in HITACHI HU IIE ELECTRON MICROSCOPE. The pictures were taken on EG 17 0.18 mm thickness x 8.8 x 11.8 cm film.

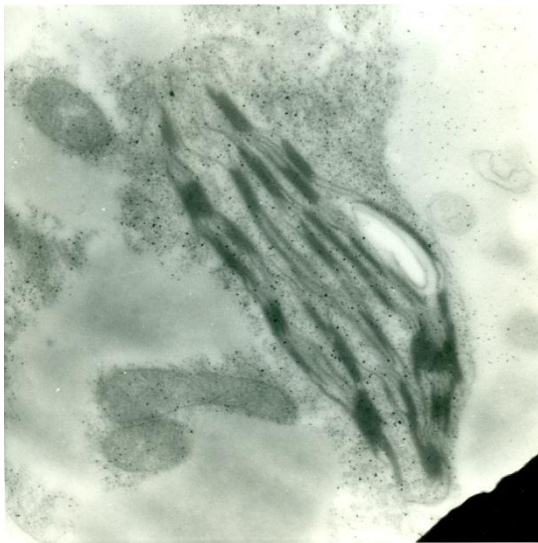


**Cells close to the embryo axis filled with few lipid bodies**

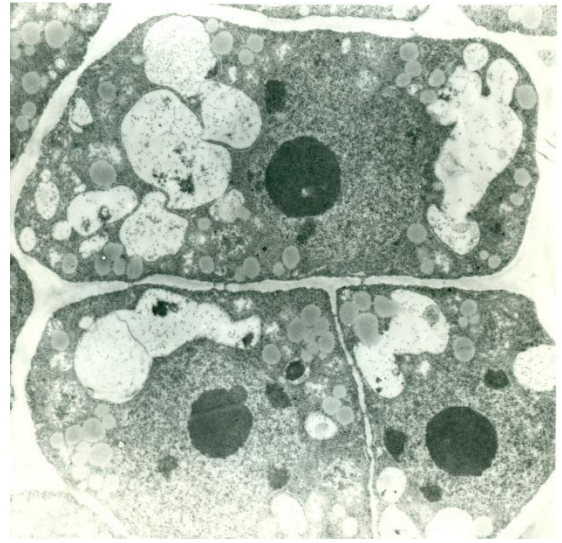


**Cells at the distal end of embryo axis filled with lipid bodies**

It can be seen from the pictures that the cells in cotyledons of Jojoba which were closure to the embryo were totally filled with lipid bodies while cells which were closure to the embryo were having few lipid bodies.



**Chloroplast in *Salvadora persica* cotyledon**



**Meristematic cells in *Salvadora persica* cotyledon**

### ***Salvadora* Shoot:-**

*Salvadora* seeds were soaked in water and kept for germination at 25 °C. The cotyledonary tissue was viewed after germination. The section of cotyledon apex shows meristematic dividing cells with small plastids. The work was carried out just to learn the technique of electron microscopy during the stay in France.

### **Breeding studies:**

Aruna Kumari et al. (1991), and Chikara et al. (1991) reported wide diversity in phenotypic and yield characters, oil content and fatty acid composition due to dioeciousness nature. This naturally existing variability can be exploited for genetic manipulations through intraspecific hybridization. The identification of correct parents to be used in crosses for specific objectives is of prime importance so as to achieve the genetic advance. Successful completion of pollen-pistil interaction is an essential prerequisite for fertilization and seed set.

Therefore, efforts were made to identify desirable genotypes based on their morphological and yield performance and to explore the possibility of intraspecific hybridization and to establish the well-defined technique of artificial pollination for the development of desirable hybrids of jojoba in future. It is therefore, crosses were made in different combinations between these selected parents.

Four male plants were selected after studying various aspects of their floral biology e.g. duration of flowering, time of flower bud emergence, time of maximum blooming, stamen maturation, anther dehiscence, and viability of the pollens and mode of pollination etc. While four female plants were selected on the basis of stigma receptivity, yield performance and seed oil characters etc. before attempting artificial hybridization. Yellow colored anthers were collected during morning hours and kept in a glass petri dish in sunlight for 20-30 minutes so as to facilitate their dehiscence and get sufficient quantity of pollen grains liberated for conducting controlled pollination. Due to lack of nectaries, the identification of stigma maturity or receptivity or maturity was a difficult task. Hence the female flowers were manually opened based on visual observations.

Female flower buds were bagged during initial growth phase and regular observations were recorded till stigma reached maturity at the desirable stage of receptivity. The female flowers were manually opened based on visual observations and pollination was carried out with the help of camel-hair brush by dusting sufficient quantity of pollen and thereafter buds were again covered with paper bags. They were

kept for a couple of days to ensure the prevention of undesirable or foreign pollen. Pollination was carried out between two sets of male and female plants for obtaining single cross combinations. Paper bags were removed from the fertilized buds after ascertaining the completion of crossing process and allowed them to grow under normal conditions. In the first set a single male plant M2 was crossed with four different female plants namely F1, F2, F3 and F4. While in the second set female plant F4 was crossed with four different male plants viz. M1, M2, M3 and M4.

In one combination one female was pollinated with all the four males separately while in another combination one male was kept common to pollinate all the four females. Seeds obtained from each cross were analysed for single seed weight, oil content and fatty acid composition by gas chromatography (GC-9A, Shimadzu, Japan). Seeds obtained from open pollination were treated as control for comparison. Seeds were collected from each cross individually after maturation along with naturally pollinated plants and observations were recorded on number of buds operated per harvested seed. Phenotypic seed characters including seed weight of crossed as well opened pollinated plants were compared in the experiment. Percentage fruit set and self-compatibility was worked out for each cross.

$$\text{Fruit set (\%)} = \frac{\text{No. of filled fruits}}{\text{No. of female flowers used}} \times 100$$

$$\text{Self-compatibility (\%)} = \frac{\text{Seed set with hand pollination}}{\text{Seed set under open pollination}} \times 100$$

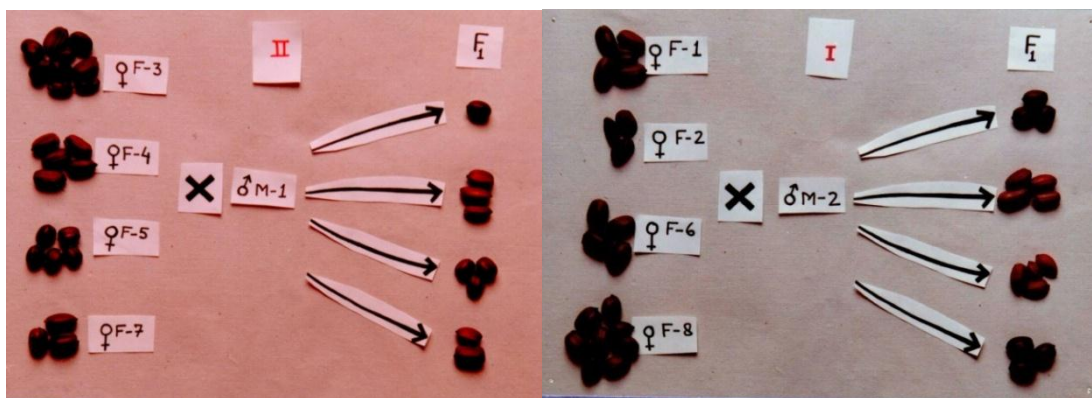
Though, there were no satisfactory short cut methods to determine stigma receptivity, the visual observation showed that in female flower emergence of style indicated the receptivity of stigma, which remained receptive for 2-3 days. The receptivity was observed between 9.00 a.m. to 3.00 pm. and was more during the initial 2-3 hours of opening of floral bud and it remained receptive effectively for approx. 36 hours. These timings were confirmed based on the number of fruit set obtained by pollinating different female buds at different intervals. With the maturation of stigma, it became flaccid and colour started changing from greenish yellow to pale yellow which followed by swelling of ovary. The position of stigma becomes flat so as to facilitate

the pollination. Variations were observed in style length within the same plant and female flowers with sufficiently long style appeared to be better as indicated by more number of fruits setting. In case of very short style length, fruit setting was very poor. As soon as crossing was over, the stigma loses turgidity and gradually started turning light brown and senescing. Though, opening of staminate flowers was observed during morning hours particularly from 8.00 to 11.00 a.m., peak dehiscence of pollen was recorded only during 10.30 pm to 2.30 pm.

Yellow colored fresh anthers suitable for pollination were available between 8.30 a.m. to 10.00 a.m. The ideal timing for isolating the anthers was fixed after testing the suitable stage and proper dehiscence of pollen grains from the male flowers. Crosses made between four identified male and female plants showed the influence of selected genotype on seed characters in terms of fruit set and single seed weight in all the attempted crosses. When characteristics of these seeds obtained through crosses compared with the seeds collected from open-pollinated plants, they showed variability for most of the parameters in all the crosses.

In the present experiment, when 4 selected females (F1-F4) were crossed either with one single selected male parent (M2) or one single selected female (F4) was pollinated with four different male plants (M1 to M4) separately, it was observed that true to type characters of parental seeds were maintained in all the crosses except pointedness which was not obtained in F1 seeds (Table 1&2). It can be seen from the table that out of 8 crosses attempted, there was increase in single seed weight in 5 crosses while it slightly decreased in rest of the 3 crosses.

Self-compatibility percentage in cross C4 (128.57) was maximum followed by C6 (114.28) and C7 (100.00) on the contrary cross C2 showed lowest % of compatibility (55.55). A close perusal of the data revealed that though, compatibility % varied in all the crosses due to different male sources, all the female parent plants almost breed true to type in F1 generation for seed characters irrespective of male parent used in various crosses. It indicated that the extent of trait expression was dependent upon the relationship and compatibility between the parental genotypes involved in crosses.



Significant improvement was evident in most seed samples over control seeds. Approx. 75% of the crossed seed samples showed a significant increase in single seed weight while 62% seed samples indicated higher oil content as compared to open pollinated seeds from same female plant.

Maximum average single seed weight (1.08 g) was recorded in F4 x M4 cross whereas minimum single seed weight (0.85 g) was observed in F I X M2 cross. Maximum oil content was observed in the seeds of Cross between FI x MI (54.35%) while minimum in F2 x M2 (42.47%). The oil was extracted using hexane as a solvent in a soxhlet apparatus. The extraction was continued for three hours at 60°C. The extraction procedure was repeated to achieve complete extraction of oil. The residual hexane was removed by heating the flask on water-bath. To remove traces of solvent the flask was placed in an air-oven at 80°C temperature till the sample reaches to a constant weight.

Methyl esters were prepared from the oil by reacting with methanolic sodium methoxide and extracting the esters from the aqueous phase with hexane. The fatty acid methyl esters were analyzed on a Shimadzu GC-9A gas chromatograph equipped with dual flame ionization detector and 2.00 mm x 3mm ID x 4 mm OD. stainless-steel column packed with dexsil 300 (Carborane methyl phenyl silicone) on 80 /100 mesh W. Injector and detector temperature was 350° C and the column was programmed from 120°C to 210°C at 5°C / min. with an initial hold period of 2 min. and final hold period of 5 min. with a nitrogen carrier gas flow rate of 60 mL/ min. Each analysis of fatty acid ester required 25 min. run period. Methyl esters of fatty acids obtained from Sigma Chemicals Company was used as standard and quantified the Jojoba methyl esters.

Data obtained for fatty acid composition was analysed statistically and pooled Critical Difference (CD) values were calculated for all the four fatty acids. Out of 16

crosses, 11 crosses showed significant increase in major fatty acids as compared to open pollinated seeds. Even in the same female plant significant alteration in fatty acids was achieved due to different male plants. Results revealed that significant differences were obtained only for Eicosenoic (C 20:1) and Docosenoic (C 22:1) fatty acids while oleic acid (C 18:1) was found significant at 5% level. Differences in Palmitic acid (C 16:0) were however, not significant which shows that different combination of male and female did not affect its composition. In the case of oleic acid (CI81) cross between F4 x M2 resulted in the highest concentration whereas C20.1 was highest in F2 x M3 combination. Maximum oil concentration was recorded in Cross FI x M3. The preliminary experiment shows that improvement in particular fatty acid content can be achieved in jojoba by using particular male-female genotype combination.

## **Multiplication of selected plants of Jojoba**

### **a. Micropropagation**

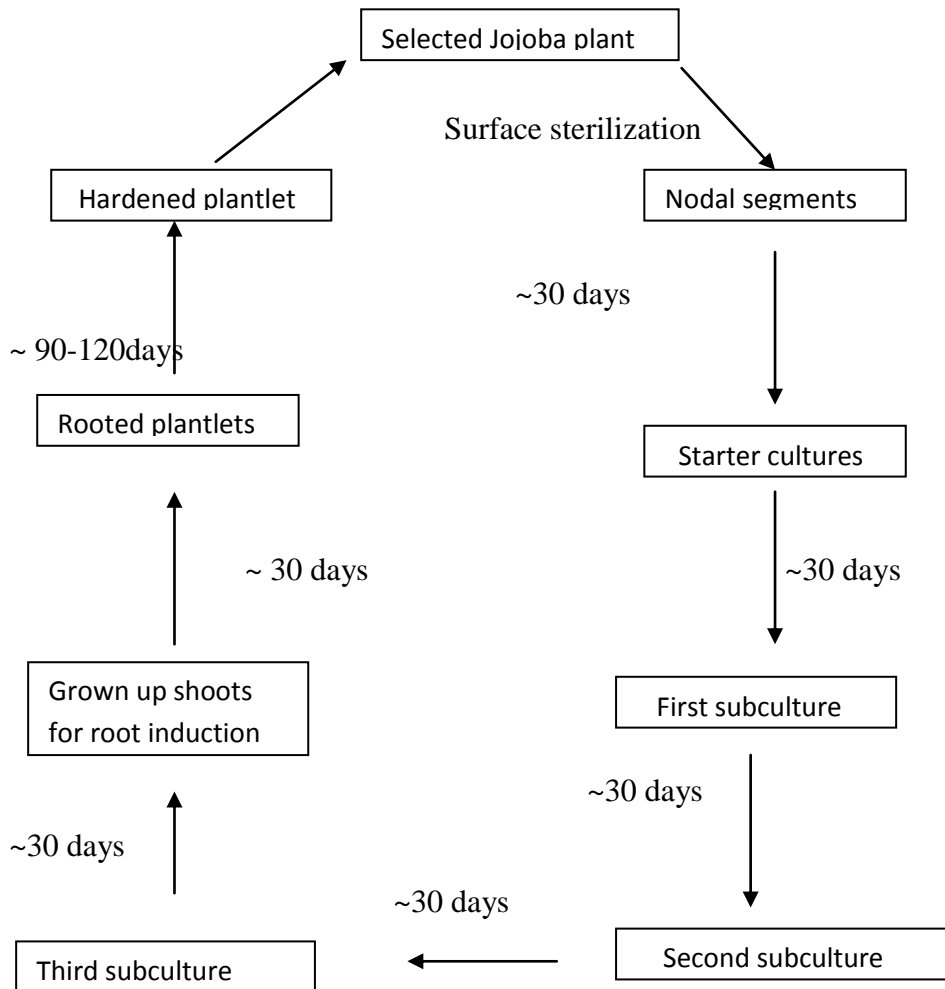
In the population raised from seeds, ratio of males to females remains unbalanced as males outnumber the females. Sex determination in the species at early stages (before flowering) is very difficult. To overcome these problems techniques like micropropagation is highly advantageous not only for the generation of known sex seedlings but also multiply high yielding female plants. It will reduce the heterogeneity in the future populations and stabilize the yield to some extent.

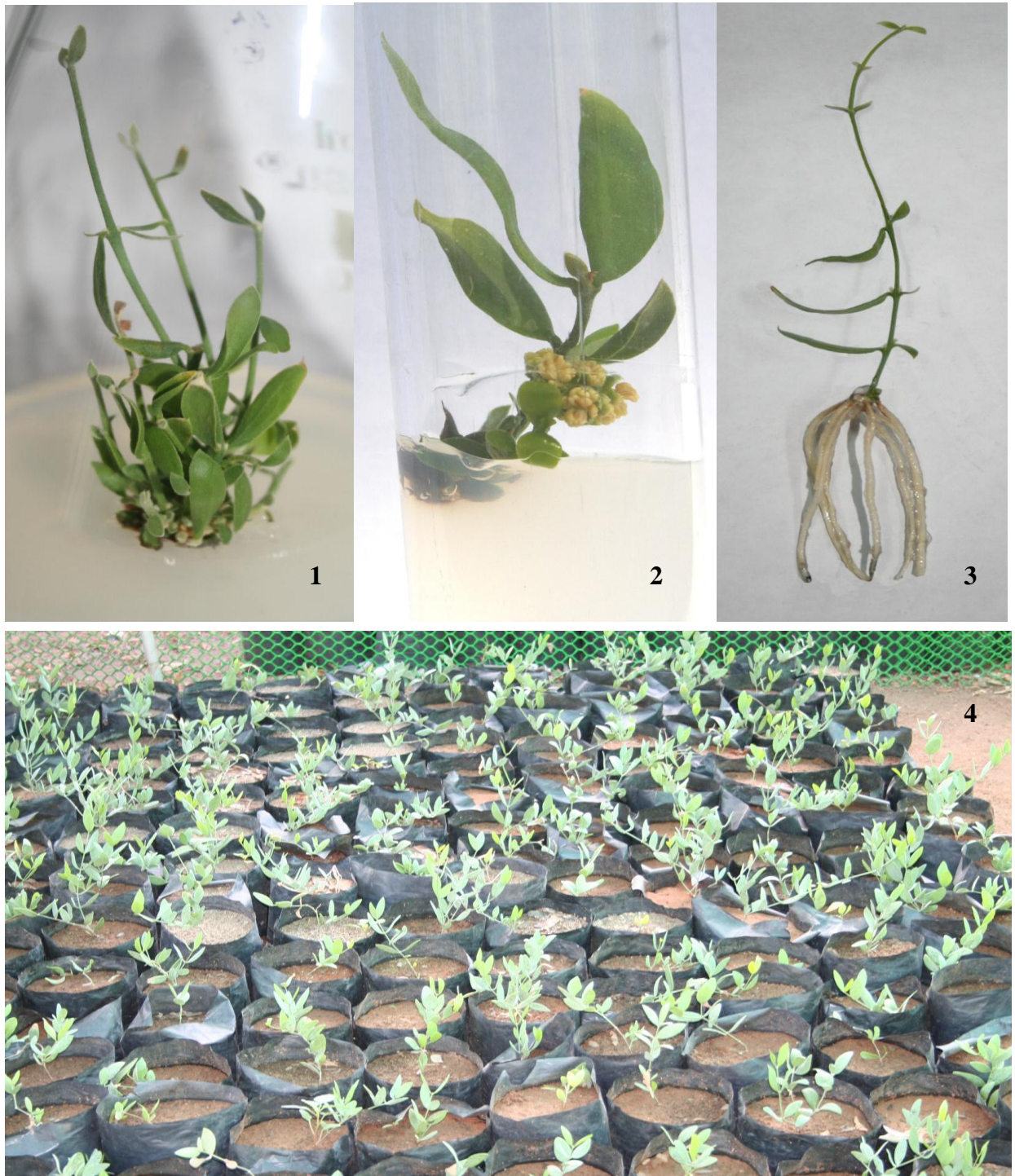
The selected genotypes were attempted to multiply through micropropagation. The explants or nodal segments (1.0 - 2.0 cm long) were collected from growing branches of mature selected plants in early hours of the day. These were surface sterilized and cultured on modified M.S. media supplemented with 0.5 - 1.5 mg/L BAP and 10-15 mg/L adenine. The proliferated shoots were further sub-cultured at 30-40 days intervals to obtain multiple shoots. July to December period was not found suitable for initiation of axenic cultures due to high rate of fungal infection.

After 3-4 subcultures, rooting was induced in elongated shoots having 3-4 pairs of well expanded leaves through pulse treatment with a mixture of root inducing hormones i. e. IAA, IPA, IBA and NAA for 48 hours and then sub-culturing on modified M.S. media for rooting. Approximately 50% rooting was observed after 20-25 days in these shoots. These rooted plantlets were transferred to polybags (filled with sterilized nursery mixture) moistened with nutrient solution (MS ¼ salts) and maintained at 30°C + 2°C temperature in hardening unit. Initially relative humidity was

maintained between 70 - 80% which was gradually reduced to approximately 50%.

### Scheme for Micro propagation of Jojoba





Micropropagation of jojoba Fig 1-4.

1) Multiple shoots formation. 2) Flowering in male culture. 3) Rooted shoot. 4) Hardened plants in the nursery.

The survival percentage could be achieved up to about 80% during hardening process. The plantlets were irrigated with MS  $\frac{1}{4}$  salts every third day. After 8-9 weeks of hardening period, seedlings were taken out and once again transferred to bigger

polybags in nursery. Seedlings thus raised were hardened and provided to farmers for field trial both in Gujarat and Ahmednagar in Maharashtra.

During the course of multiplication, effect of different combinations of root inducing hormones was studied both male and female plants and it was observed that there was no significant difference in the rooting percentage of male and female plants. The pretreatment with IBA (10 mg.) and IPA (10 mg.) induced tap roots while addition of NAA (1.0 mg) induced branching in the roots. Thus suitable combination of growth hormones has been developed for proper rooting.

#### **b. Clonal propagation through stem cuttings:**

Clonal propagation of Jojoba was also undertaken through stem cuttings. After pruning freshly grown branches (3-4 month old) were excised and collected from the field during early hours of the day when leaves are fully turgid. The basal node of the 8- 10cm. long cuttings (with 3-4 nodes) were dipped in a mixture of root inducing hormones ( i.e. a mixture of auxins) for 2-3 minutes and kept in small poly bags filled with sand and vermiculite at a constant temperature of ca. 25% with high humidity. After rooting which takes 30-35days to occur, rooted cuttings were gradually taken out to the higher temperatures.

#### **Rooting mixture**

IBA-1.00 g

KCl- 1.00 g

Boric acid- 1.00 g

Ascorbic acid- 50 mg

Mix in 100 ml of distilled water and use

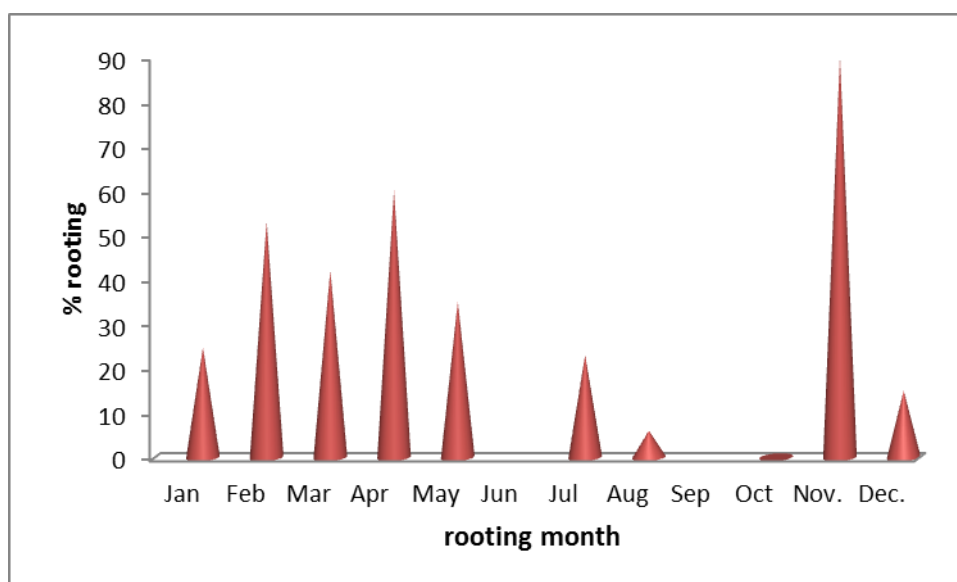


**Effect of rooting hormones on rooting pattern in Jojoba 1) IAA, 2) KIBA, 3) RM1 and 4) IPA**

The rooting percentage varied from 40%-80%, depending upon the genotype and season. It can be seen that winter months are better for generation of rooted cuttings in Jojoba.

#### Percentage rooting in Jojoba cuttings during different months

Genotypes	No. of cutting kept	No. of rooted cuttings	Percentage rooting
1. E 21-14	164	89	54.3
2. E 24-8	145	75	52.0
3. E 12-8	150	95	63.3
4. E 16-10	111	88 "	79.3
5. E3-11 (Male)	38	16	42.0



Results of rooting mixture were also compared with root inducing hormone obtained from Israel. The results obtained at AJORP, Jaipur were as follows.

Genotypes	No. of cutting kept	No of rooted cuttings	Percentage rooting
1. pooled cuttings from females treated with Israel hormone	1553	699	45.0 %
2. Pooled cuttings from females treated with CSMCRI hormone	442	177	40.0 %

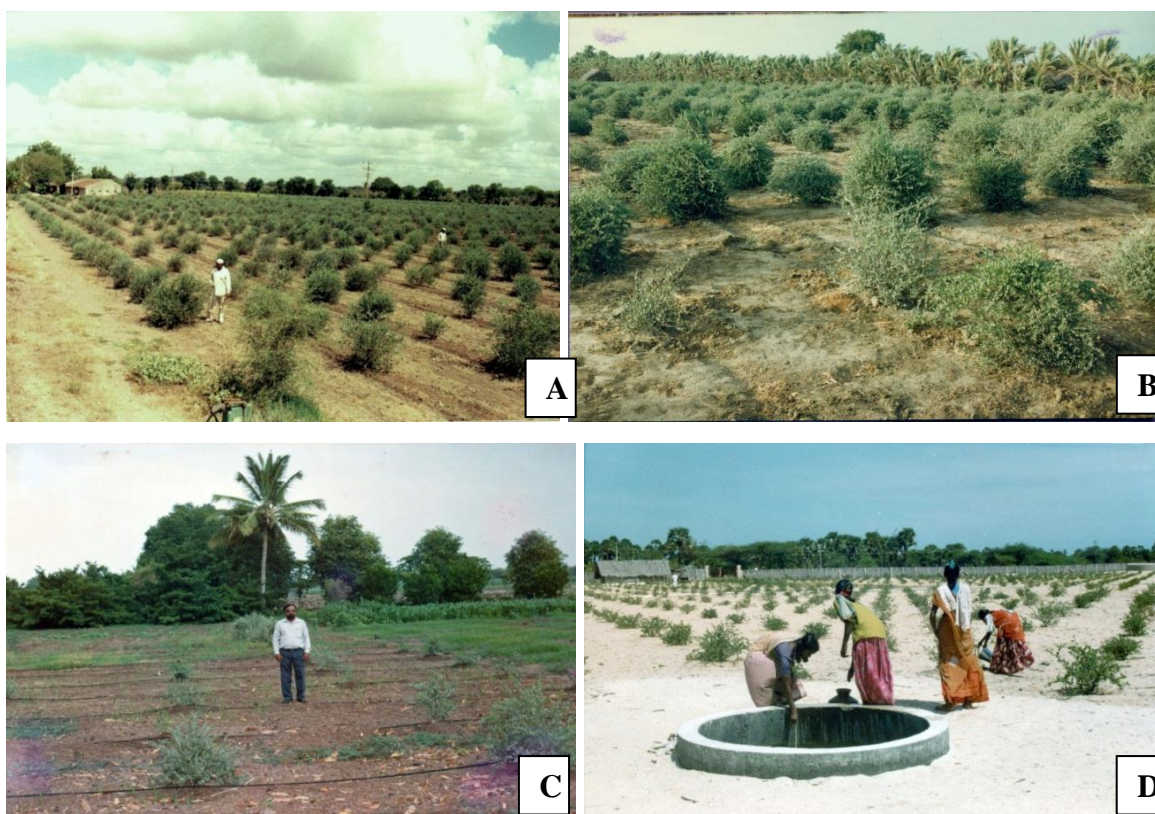
Results indicate that the mixture developed by us is comparable with that of Israel powder. In some genotypes the results of our mixture was better than Israel

hormone. The cuttings were developed on large scale and location trials were initiated with few genotypes in collaboration with AJORP, Jaipur to assess the performance. Stem cuttings were provided to AJORP, Jaipur for root induction at particular temperature and humidity in green house of AJORP at Dhand, Jaipur. The rooting percentage of some of the female and male plants was comparable to the cuttings obtained from other sources. These rooted cuttings were later planted in the field during winter season.

S. No.	Source of cutting material	Date of putting in green house	No. of shoots	Rooted cutting	Rooting duration in days	Rooting percentage
1.	Gupta Farm Achrol, (Jaipur)	22.12.97	646	130	74	20.1%
2.	Raisingh Nagar, 23 P.S.	25.12.97	1710	563	71	32.9%
3.	CSMCRI, Bhavnagar Date 25.12.97	E 21-14	164	89	71	54.3%
	F	E24-8	145	75	71	52.0%
		12-8	150	95	71	63.3%
		16-10	111	88	71	79.3%
	M	E-3-11	38	16	71	42.0%
	<b>Total</b>		<b>608</b>	<b>363</b>		<b>59.70</b>
4.	CAZRI, Jodhpur	28.12.97	1238	510	68	41.2%
5.	Bidasar-Churu	03.01.98	1247	657	62	52.7%

### Extension activities:

Jojoba plantations were also raised at various locations with the help of different collaborating agencies to determine the performance under different climatic and soil conditions. Therefore, seed orchards were developed at village Chotila, Distt. Surendranagar (Gujarat), Rajkot (Gujarat), Kutch (Gujarat), Ahmednagar (Maharashtra) and Rameshwaram (with the help of DRDA, Ramnathpuram).



**A:Rajkot; B: Kutch; C: Rameshwaram; D: Ahmednagar**

Under the **United Nations Development Programme** a project entitled **“UTILIZATION OF WASTELAND WITH NON TRADITIONAL OILSEED PLANT SPECIES “** was taken up to promote cultivation of Jojoba in 500 ha. in five States (Gujarat, Rajasthan, T.N., Maharashtra and A.P.) and Jatropha in 1000 ha. (mainly in Orissa) on marginal or wastelands of the country with the help of farmers and other agencies (both govt. and NGOs).

The project was envisaged as the umbrella programme of GOI-UNDP under the oil seed mission of TMOP (Technology Mission on Oil seeds and Pulses) and a fund of Rs.45.00 lakhs was sanctioned for a period of two years (1996-1998) which was extended till Dec.,1999. The bench mark studies were conducted to assess the potential of cultivation of both the species on marginal lands of the country. A large number of farmers, NGO,s and govt. agencies were contacted in various states. In Gujarat the response was overwhelming from farmers for Jojoba cultivation. They were interested to cultivate even on their cultivable lands due to unavailability of irrigation water for normal crops. Contacts were also established in T. N., Maharashtra, Rajasthan, A.P. and Orissa.



Seedlings of both the species were raised in centralised nurseries at C.S.M.C.R.I. and its field unit at Berhampur (Orissa) with the help of farmers. Seedlings were distributed to interested farmers and method of transplantation in the field was demonstrated. In several cases, seedlings were provided even for gap filling. Seedlings of known sex from selected plants were also multiplied both through stem cuttings and micro propagation. These seedlings were provided to farmers for conducting field trials. AJORP, Rajasthan was also involved in the project and both seedlings and stem cuttings

were provided for taking up seed orchard at their farm. Seeds were also provided to them for gap filling. Initially due to lack of hardening unit there was high mortality of rooted seedlings but after its installation, mortality was reduced considerably.

Seed orchards of Jojoba were raised in a total area of 33.5 ha. while approx.135 ha. area was covered with the help of farmers and various agencies in different states mainly in Gujarat. While in Jatropha, seed orchard in an area of 20 ha.was established in Orissa. Approx. 155 ha.area was covered under Jatropha with the help of tribal farmers.



### **Jojoba seedlings distributed to several farmers under the project**

It was also proposed to provide training and demonstration of agrotechnology of cultivation to 100 farmers and other govt. agencies each for Jojoba and Jatropha. More than 100 farmers besides officials from state agriculture Dept. and other agencies were provided training on agrotechnology of cultivation of both the species. Survey work was also undertaken in Orissa to identify the areas which support the natural growth of *Jatropha curcas*. Altogether 52 farmers in 14 villages in the districts of Ganjam and Khandamal, 14, Govt. agencies and 10 NGO's have shown interest in taking up Jatropha cultivation under this programme. A survey on the social back ground of the beneficiary farmers was carried out. Even tribal farmers in Orissa were encouraged to take up Jatropha cultivation on their marginal lands like hill slopes.



The farmers meet was arranged on 25th November,1997. This was well attended by tribal farmers from hill districts, plains along with persons from NGO's. The meeting was also attended by various State Govt. officials of Soil Conservation and Forest Department. Among the dignitaries, the District Collector, Distt. Ganjam was also present. The farmers were explained various methods of plantation and also demonstrated in our field from seed to seed. More than 100 Farmers were trained including other beneficiaries in four batches.

## *Simarouba glauca*

*Simarouba* belongs to family Simaroubaceae and commonly known as aceituno in Costa Rica and El Salvador. It is an important seed oil tree in Brazil as the kernal



contains 60-70% edible oil. Therefore, the species was introduced in India as a potential source of vegetable oil during late sixties to assess the performance on marginal lands in sub-humid climate of Orissa. A location trial was conducted to investigate the suitability of the species in Kutch (Gujarat) under semiarid conditions. In the field trial, 325 plants were grown on marginal lands with poor irrigation water in Kutch, Gujarat (22° 51'N; 69° 20'E) under semi-arid conditions having average annual rainfall in the range of 200 to 250 mm. The soil is sandy loam and is deficient in total nitrogen (43 kg/ha.) and moderate in available potassium (100 kg/ha.) while available phosphorus (39 kg/ha.) and organic carbon (0.50%) were inadequate. The quality of irrigation water was very poor due to total dissolved salts (TDS) to the extent of 2050 ppm and RSC as high as 2.6 meq/lit. The plants started bearing fruits from fourth year onwards and therefore during the preliminary selection seed samples were collected from 10 different pistillate plants. The seed size and single seed weight was determined and oil content was estimated for above plants through Solvent Extraction Method using n-hexane as solvent.

The species being polygamodioecious in nature shows staminate, pistillate and andromonocious plants among the population. The average seed length and width range from 2.00 - 2.50 cm. and 1.00 - 1.50 cm respectively. Significant variability in seed and kernel size was not observed as earlier reported from El Salvador.

The average single seed weight was estimated as 1.05 gms in ten plants ranging from 0.90 gms - 1.37 gms (Table 2.14). The 15.24% CV indicates some variability in the above character. The kernel percentage showed significant variations and ranged from

34.06- 43.55% kernal percentage was observed 40% in the plants from sub-humid region. This variability can be attributed to the difference in developmental stages of seeds at the time of harvesting.

Seed kernals were reported to contain 55% to 65% fat and in some cases as high as 75%. During the present analysis also oil content ranged from 62.91% in the plant MLS-4 to 69.21% in MLS-6. The oil composition showed sufficient variation in saturated and unsaturated fatty acids (Table 2.14). The palmitic acid ranged from 9.65% in MLS-3 to 17.38% in MLS-1 with an average of 12.17%. The oleic acid which is predominant, ranged from 46.30% in MLS-1 to 60.16% in MLS-3 with an average of 54.78%. Some correlation appears to exist in the palmitic acid and oleic acid content. The stearic acid which ranges 29.62% to 39.58% with an average of 33.06% seems to be independent of other fatty acids. The fatty acid composition of the seeds obtained from sub-humid region reported was as follows: oleic acid (51.1%), stearic acid (30.4%) and palmitic acid (12.6%). It is almost similar to that of seeds analysed during the present studies.

Though, presence of linoleic acid in small quantity and linolenic acid in traces was reported earlier, during the present analysis both the above acids were not observed in any of the ten plants studied. This indicates that the climatic conditions do effect the fatty acid composition of the seed oil as reported by several workers. The deoiled cake which is being utilized as organic fertilizer) contained 64% protein by weight. Samples obtained from sub-humid region of the country found to contain 60-65% protein while during the present analysis, protein in the deoiled cake was little low ranging from 45.63% to 56.75% with an average of 51.81%.

The performance of the plants in the field and results indicate that *Simarouba glaucaca* be exploited for cultivation both in semi arid and arid regions of the country besides sub-humid region on marginal lands without any adverse effects on oil characteristics. The variations observed in seed and oil characteristics can be attributed to the polygamodioecious nature of the species. Although, the oil is reported to be an edible, further investigations are necessary to develop the post harvest technology and several marketable products to make the crop financially viable (**Chikara et.al.1998**).

**Table 2.14: Seed and oil characteristics of ten selected plants of *Simarouba***

Sr. No.	Plant No.	Seed wt (g)	Kernal %	Oil %	Protein % in deoiled Cake	Chemical composition		
						16:0 Palmitic %	18:1 Oleic %	18:0 Stearic %
1	MLS-1	1.00	34.47	65.52	45.63	17.38	46.63	35.99
2	MLS-2	0.92	43.55	65.67	53.81	13.44	50.89	35.67
3	MLS-3	1.21	41.13	62.45	51.25	09.65	60.16	30.19
4.	MLS-4	0.98	40.03	62.93	51.31	10.22	58.68	31.10
<b>5</b>	MLS-5	0.91	42.31	63.24	51.63	09.82	59.42	30.76
<b>6</b>	MLS-6	0.90	41.56	69.21	53.13	10.24	60.14	29.62
<b>7</b>	MLS-7	1.11	41.55	67.66	49.25	10.66	58.47	30.87
<b>8</b>	MLS-8	0.89	42.31	64.86	52.13	12.69	54.96	32.35
<b>9</b>	MLS-9	1.21	34.06	66.46	53.25	13.46	52.12	34.42
<b>10</b>	MLS-10	1.37	39.50	67.33	56.75	14.12	46.30	39.58
<b>Av. Value</b>		<b>1.05</b>	<b>40.05</b>	<b>65.62</b>	<b>51.81</b>	<b>12.17</b>	<b>54.78</b>	<b>33.06</b>
SD(+)		0.16	03.05	02.19	02.82	02.36	05.15	03.01
CV(%)		15.24	07.62	03.34	05.44	19.39	09.40	09.10

## Chapter 3

### Survey and assessment of Halophyte vegetation for genetic improvement

#### Improvement of *Salvadora persica* for yield potential and quantity and quality of seed oil

Survey of wildy growing plants both in saline and non-saline areas in Gujarat was undertaken and selection of plus trees was done. Various characters of these trees are mentioned below in the table on the basis of which selection and identification of plus trees were done. Seed from these trees were collected and variations in fatty acids were determined.

Location	Height (m)	Canopy (m)	Dry seed Yield (kg./plant)	1000seed Weight (g)	Oil content %	Fatty acid composition of oil %	Lauric acid	Myristic acid	Palmitic acid	Oleic acid
<b>Coastal saline soils :</b>										
Dholera	2.19	8.00	3.16	57.35	40.00	27.23	47.86	14.97	9.36	
Sea coast	1.90	7.65	2.94	59.00	39.06	26.36	48.82	14.70	9.75	
	2.50	8.20	3.38	56.84	38.45	25.53	49.30	14.43	10.40	
<b>Inland saline soils:</b>										
Ramtekri	6.02	21.22	11.20	63.26	40.77	33.14	44.67	9.55	11.00	
Aliyasar	5.80	21.36	9.50	64.10	42.00	27.96	46.29	15.48	9.95	
	9.25	19.63	12.05	55.40	39.54	41.00	38.78	7.86	10.06	
<b>Non saline soils :</b>										
Dholka	9.00	30.25	14.00	57.50	38.50	32.25	42.00	10.70	11.15	
Lothal	7.75	25.20	12.50	58.24	40.00	29.85	46.29	11.00	12.22	
Radhu	9.75	35.66	15.00	56.63	38.26	33.07	46.97	10.20	9.25	
Rasikpura	8.36	28.15	13.00	55.74	39.05	40.12	36.26	10.42	12.30	
Salajda	10.00	31.10	13.60	59.18	38.00	31.00	44.60	11.00	13.00	

It was observed that plants which were in inland areas with low salinity were tall like trees while plants in the saline areas near the sea were bushy in nature with large canopy. Characteristics of soil like pH, EC, SAR and chemical analysis of these various locations (as mentioned below) from where plus trees of *Salvadora persica* were selected, were also studied.

Location	Soil Type	Salinity		pH of Soil	In saturation extract meq					SAR
		WHC %	of soils dSm <sup>-1</sup>		Na <sup>+</sup>	k <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Cl <sup>-</sup>	
<b>Coastal saline soils :</b>										
Dholera Sea coast-1	Silty loam	65.90	65.68	7.47	664.89	18.51	3.52	29.55	728.37	40.21
Dholera Sea coast-2	Silty loam	66.00	69.14	7.82	678.15	20.12	3.30	33.64	733.31	36.72
Dholera Sea coast-3	Silty loam	65.50	62.22	7.52	641.26	19.00	4.00	31.24	712.60	36.39
<b>Inland saline soils :</b>										
Ramtekri	Sandy Loam	36.22	34.57	8.02	339.26	13.85	3.49	12.30	395.12	42.40
Aliyasar	Sandy Loam	42.09	27.25	7.83	269.20	15.84	2.60	5.58	310.61	65.56
<b>Non saline soils :</b>										
Dholka	Sandy Loam	44.29	2.08	8.07	10.25	0.96	0.68	1.25	12.07	10.57
Lothal	Silty Loam	48.41	1.79	8.00	5.16	1.47	0.89	0.37	6.97	8.19
Radhu	Sandy Loam	37.03	2.33	7.59	10.14	2.83	0.68	0.37	13.00	19.13
Rasikpura	Silty Loam	45.25	2.71	7.70	15.26	0.97	0.48	0.58	19.02	28.30
Salajda	Silty Loam	46.00	1.18	8.02	7.68	1.00	0.68	0.30	9.36	15.36

Breeding programme was initiated in *Salvadora persica* and crosses were made between plants with white and purple coloured fruits to develop the breeding technique and study the inheritance of the character in the species. The F<sub>1</sub> seeds were planted in the field.

*Suaeda nudiflora*, a halophytic species, grows wild in the highly saline, dry and extreme high tidal belt along the seacoast, mainly in Gujarat. As the seeds contain approx. 30-35% oil, plant has the potential as a future oil seed crop and is highly suitable for producing high protein biomass in saline soils due to C<sub>4</sub> photosynthesis. It is occasionally used as fodder for camels and goats. The tips of the branches in an oily medium are used to cure sores on camel backs. Its aqueous infusion is used as an emetic. Halophytes and mangroves have rarely been attempted for *in vitro* establishment of callus cultures and regeneration. The method describes *in vitro* initiation of callus culture from stem segments and subsequent regeneration of healthy plantlets.



Seeds of *S. nudiflora* were collected from the coastal areas. The young shoots (3-4 cm long) from mature plants raised in the institute's nursery, after washing thoroughly in running tap water, were surface sterilized with 0.1 % mercuric chloride for 5 min and rinsed with sterilized distilled water several times under aseptic conditions. Stem segments (5 mm long) from shoots were aseptically placed horizontally on MS media' (chemicals from Hi-Media, Mumbai). The basal medium (BM 1) was supplemented with BAP (1-benzylaminopurine, Sigma Chemicals, USA) and 2,4-D (2,4-dichloro phenoxyacetic acid, Sigma Chemicals, USA) for callus induction. Subsequently, callus was shifted to media supplemented with various concentrations and combinations of various plant growth hormones (Sigma Chemicals, USA) viz. BAP, NAA (1-naphthaleneacetic acid) and/or KN (kinetin) for regeneration

and proliferation of shoots. Regeneration was obtained within 10-12 days and number of shoot primordia per culture was recorded.

Grown up shoots were harvested and transferred to root inducing media enriched with IAA (indole 3- acetic acid), IPA (indole phenoxyacetic acid) or IBA (indole 3-butyric acid) alone or in combinations with 3% sucrose. To assess the effectiveness of the media, percentage rooting and number of roots per plantlet were recorded. Standard error was calculated to estimate the differences. Media pH was adjusted to 5.7 using 0.1 N HCl or 0.1 N NaOH before autoclaving and gelled with 0.6% (v/v) agar agar. Routinely 20 ml of molten medium was dispensed into culture tubes and steam sterilized at  $1.06 \text{ kg cm}^{-2}$  for 15 min. All the cultures were maintained at  $25 \pm 2^\circ\text{C}$  under white fluorescent light (3000 lux intensity) for 16 hrs photoperiod. However, for induction of callus, cultures were incubated in dark. For each treatment, 20 cultures were raised and experiment was conducted at least two times to confirm the results. The cultures were examined periodically to record morphological changes. Plantlets with well-developed root system were transferred to glass jars for hardening. After 30 days, hardened plants were successfully transferred in the nursery. In *S. nudiflora* callus could be induced from most of the plant parts like leaf, stem, floral stem and flower buds as reported in *Bacopa* species.



During the present study, callus was initiated by placing the stem segments on MS media (BM) supplemented with various concentrations of 2,4-D and BAP. These segments showed callus induction on the lower surface within 10 days in the medium supplemented with BAP (0.5 mg/l) and 2,4-D (1.0 mg/l), Callus was excised and sub-cultured on basal medium enriched with various combinations of BAP and NAA (Table 1).

Though, organogenesis could be achieved on the medium supplemented with BAP (2.00 mg/l) + NAA (1.0 mg/l). Organogenesis in the form well differentiated shoot primordia with leaves were observed in 50-60% cultures after approx. 40 days in the medium supplemented with NAA (1 mg/l) and BAP (3 mg/l). Thus, relatively higher concentration of cytokinin improved regeneration potential of callus as also reported in *Niger*, Elongated shoots were separated and proliferated on media supplemented with BAP (0.5-1.5 mg/l), NAA (0.5-1.0 mg/l) and KN (0.5-1.0 mg/l) alone or in combinations.

No hormone promoted multiple shoot formation individually. Although, BAP (0.5 mg/l) along with NAA (0.5 and 0.6 mg/l) and BAP (1.5 mg/l) with KN (0.5-0.6 mg/l) produced multiple shoots, no further growth was observed and culture dried after 15 days. BAP (1.5 mg/l) with KN (0.8 mg/l) was found to be the most suitable combination for shoot proliferation and growth in the species with approx. 2-8 shoots per culture. This observation indicates specific requirement of growth hormones for enhancement of growth in the present material. Addition of adenine (0.5 mg/l) enhanced the growth of the shoots with dark green leaves. Elongated shoots were later separated individually and transferred to root induction media (MS 1/2) supplemented with IBA+IAA or IBA+IPA individually or in combination. IBA (0.5 mg/l) in combination with IPA (0.1 and 0.5 mg/l) or IAA (0.5 mg/l) failed to induce rooting in the species and induced only callusing at proximal end of the shoots with callus at the base. Addition of NAA (0.1-0.4 mg/l) at the same concentrations developed true roots and simultaneously enhanced the root length.

Among all the combinations, IBA (0.5 mg/l) along with low concentrations of IPA (0.1 mg/l) and NAA (0.2 mg/l) found to be most effective for root induction giving 90% rooting. Well-differentiated fibrous roots (approx.15) with hairs were observed in IBA (0.5 mg/l)+IPA (0.1 mg/l)+NAA (0.1 mg/l). The increase in the concentration of NAA reduced the rooting percentage drastically. Plantlets with well-developed root system were removed from culture tubes, washed thoroughly under tap water so as to remove the excessive media and transferred to glass jars filled with mixture of sterile

soil and farm yard manure (FYM) in equal proportions. Plantlets in the jar were irrigated with MS salts, covered with plastic bag and kept in hardening unit at 30°C with high humidity (60-70%) for 10-15 days. Plantlets were transferred to earthen pots containing soil, sand and FYM (2: 1: 1), irrigated with MS salts (1/2) and kept in green house. After 30 days, hardened plants were transferred in the nursery under natural light. Anatomical studies of leaf and stem also confirmed the gradual hardening of plantlets. All plants established successfully under natural conditions (up to 80%). Earlier, a few reports confirmed somaclonal variants derived from callus cultures in Peach and oil palm. The regenerated plants did not show any detectable variations in the morphological characters as compared to donor plant.

### ***Salicornia brachiata***

Survey and collection of germplasm of *Salicornia* was under taken from the coastal areas near Bhavnagar, different type of morphological variants were identified and cultivated at Hathab field station. Survey and analysis work was done to estimate the biomass production and the salt content in them at five densified sites (Sartanpar, Methla, Nigala, Victor and Diu), albeit without any other management practices (manuring, irrigation etc) followed. It was found that the plant population varied across different samples drawn across different sites from 100000 (Victor port) to 3310000 (Sartanpar) plants/ha with mean value of 8.35 lakhs per plant/ha. The dry biomass also varied among the different samples drawn from different sites and ranged from 1.15 to 4.05 t/ha with an overall average of 2.15 t/ha. Among the sites, the least mean dry biomass was obtained at Sartanpar (1.46 t/ha), while the maximum dry biomass production was favored at Nigala site (3.22t/ha). No significant correlation (-0.04) was observed between plant population and dry biomass production per unit area, while there were indication of negative correlation (correlation coefficient= - 0.53) between plant population and individual plant dry weight.

The percentage amount of seed obtained out of the total dry biomass was upto 7.2 with a mean value of 4.7%, while the seed oil content ranged from 25.2 to 34.6% with a mean of 32.5%. The percentage salt (commercialized as vegetable salt called *Saloni*) obtained from the dry biomass ranged from 9-27% with an average salt content of 16.6%. The Nigala site produced the highest quantity of vegetable salt (829.6 kg/ha). It needs to be further studied how the identified germplasm producing high biomass and giving high salt production at certain sites perform at other sites.

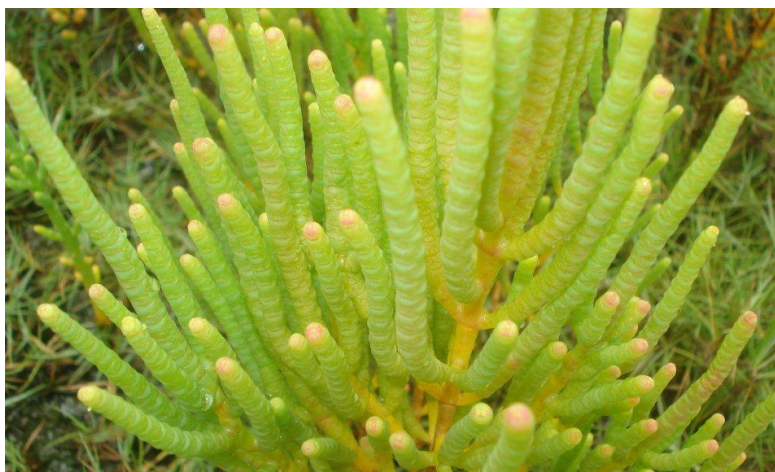


Estimation of different parameters of *Salicornia brachiata* at different coastal salt marshes

<i>Salicornia</i> Parameters	Mean values	Minimum value	Maximum value
Plant population/ha	835600	100000	3310000
Dry biomass (tonnes/ha)	2.15	1.15	4.05
Seed weight (kg/ha) (oven dried)	100.2	35.5	192.4
% seed wt from dry biomass (root+shoot)	4.7	0.08	7.2
1000- seed wight (g)	0.334	0.279	0.408
% oil in seed	30.6	25.2	34.6
Amount of oil (kg/ha)	32.5	14.0	66.5
% salt from biomass (root+shoot-seed)	16.6	8.9	26.6
Amount of salt produced from biomass (root+shoot-seed) (kg/ha)	385.4	127.6	829.6

Soil samples were collected from densified area of salicornia in the areas of Methla, Ningala, Victor and Diu coast. These soils were alkaline in nature (pH2.5:1 varied from 8.5 to 8.75), saline (EC2.5:1 varied from 5.8 to 14.6), low to medium in organic carbon (OC; 0.3 to 0.7%). The texture of soil varied from sandy loam to clay loam. All soils were low in available nitrogen (95 to 140 kg ha<sup>-1</sup>), low to medium in available P (8 to 19kg ha<sup>-1</sup>) and higher in available potassium (1126 to 2645kg ha<sup>-1</sup>). To study diversity in Salicornia population using different molecular markers (RAPD, ISSR and AFLP), samples were collected from different locations (along Gujarat coastal regions) in December 2010. Morphological characters (biomass, shoot height, root length, fresh and dry biomass of shoot and root, spike length and number of branches) for n=30 were recorded. For molecular analysis, PCR usable genomic DNA was extracted. RAPD and ISSR amplifications were performed with 20 RAPD and 15 ISSR primers respectively.

Recently seeds of several halophytic plant species were collected. *S. biglovei* were collected from the site near Porbander during the collection of halophytic germplasm.



### **Survey and assessment of Mangrove population of Gulf of Khambhat**

Gulf of Khambhat (22°30'N to 20°20'N lat. And 71°30'E to 73°10' E long.) is 70 km wide and 130 km long indentation on western shelf of Indian coast between Saurashtra Peninsula and the mainland of Gujarat. In view of the latest Dam Alignment V between Bhavnagar and Aladar, a survey of mangrove vegetation has been conducted to determine its effect on the mangrove vegetation in the gulf, potential areas of future vegetation outside dam alignment and possible remedial measures under this project. Mangroves and other halophytes were surveyed by ground truthing in these districts at all the possible locations up to Diu in Saurashtra region and up to Valsad in South Gujarat region so as to estimate the actual areas.

Ground truthing was carried out for identification of locations where the mangroves are found growing in coastal areas around the Gulf of Khambhat. Geographical Positioning System (GPS) data for all the sites visited is presented separately for each district. The density per unit area was estimated by laying quadrates of 10 x 10 meters randomly both in the moderately dense and sparse/open vegetation at all the sites of study which was later extrapolated on per ha basis by counting number of plants per quadrate.

Initially **41 sites** were surveyed for distribution and to estimate the actual areas of mangroves and other halophytes within the Kalpsar dam enclosure and downstream of Dam alignment V. Later the study area was broadened to find out the potential areas up to down south of Bhavnagar up to Diu and up to Umargam in south Gujarat at 36 locations. The areas were confirmed taking the help of website (Google earth Program)

and depicted in the SOI maps (1:15000) of Kalpsar project area made available by the Kalpsar cell.

As the maps with proper scale were not available beyond Kalpsar Project, the potential areas are indicated as sites in the Google map. Recently, GEC and BISAG had prepared a comprehensive document on mangroves as “**Mangrove’s Atlas of Gujarat**” on the basis of data collected during 2006-2007. The district wise area under mangroves vegetation has been provided and has been compared with the available data from GEC. The increased areas under mangroves reported in Amreli, Ahmedabad, Anand, Bharuch, Surat, Navsari and Valsad during the present study is due to the efforts made by different agencies to protect, conserve and multiply mangroves. During the studies, soil samples were also collected from all the locations surveyed. The samples were air-dried, ground to pass through 2 mm sieve and used for determining the mechanical analysis. Soil pH, EC, Organic carbon percentage and Soil texture was determined. It was observed that predominantly there are deep black and coastal alluvium soils.

Since the upper area of Gulf from (Kalatalav to Aladar) would be go for storage of fresh water from different sources, it is expected that mangroves of Ahmedabad, Anand and partly of Bhavnagar district, falling with in the dam area will not survive over a period of time due to total replacement of sea water with fresh water. Hence it is necessary to develop mangrove vegetation at suitable new locations both on eastern coast and western coast of the gulf outside the Dam area to compensate the above damage. Besides this, densification of thinly growing mangroves by planting germinated seeds at suitable sites would also improve the mangrove plantations. This method is advantageous because it produces the pattern similar to the original forest and cheaper and less disturbing to the habitat.

#### **Bhavnagar, Amreli and Diu:**

With reference to the letter no. PRCH-10-2010-546-Kalpsar dt. 22.06.2010, it is decided not to take up new plantation in areas falling in the fresh water reservoir area and areas above village Gopnath, therefore, areas below this place were considered for new mangrove plantations. It is proposed to take up or densify mud flat having sparse mangroves areas near village Methala and Katpar (665 ha) in Bhavnagar district.

In Amreli district coastal areas near village Patava, Chanch, victor port, Ningala and Dhara bundar are suitable locations to densify the mangroves. The total sparse mangrove area which will be densified is ca. 1976 ha. Since Diu is at the mouth of the

gulf, it is also proposed to densify the existing open/thin mangroves growing on the mud flats. This total potential area in this region is estimated to be ca. 1671 ha.

The potential mangrove areas in the district of Bhavnagar, Amreli and Diu can be rehabilitated with locally available mangrove species i.e. *A.marina*.

**South Gujarat coast:** Similarly, with reference to the letter no. PRCH-10-2010-546-Kalpsar dt. 22.06.2010, it is also decided not to take up new plantation in areas up to Hansot. Hence, it is proposed that on South Gujarat coast at Dahej port, Luhara point, Aliabet near Bharuch, Surat, Navsari (at the mouth of Purna, Ambika and Oranga river) and Valsad districts where lot of mud flats and estuaries are available (Table- 23) can be the would be the suitable areas.

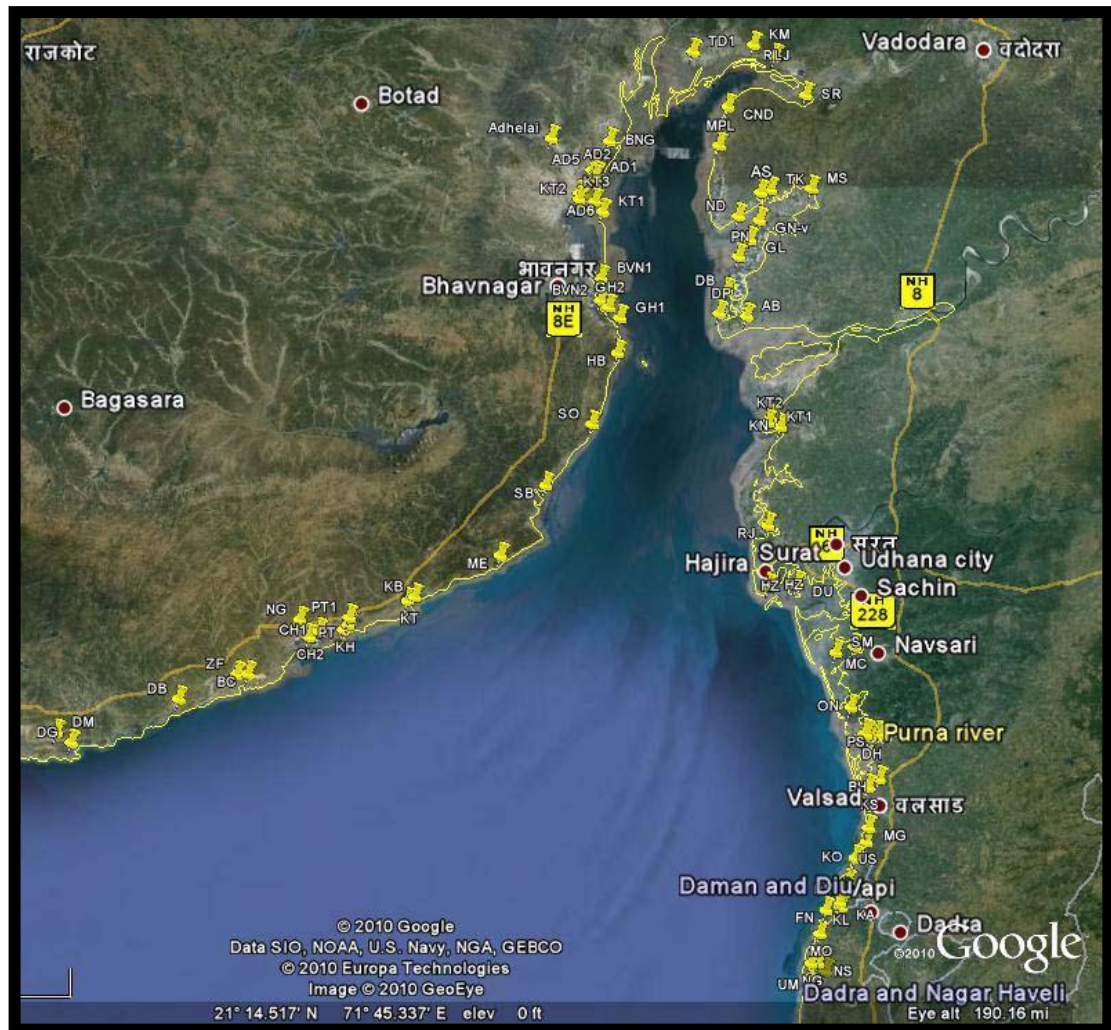


The sites where already scattered or open mangroves are existing; can be densified by rehabilitation of mangroves. Though, large areas in the district viz. Surat, Navsari and Valsad already have mangrove vegetation, still there is scope for some new plantations. Daman (UT) also has quite old vegetation of mangroves along the coast. In Surat approx. 5580 ha mud flats are available for rehabilitation of mangroves. In Navsari district ca. 4400 ha coastal mud flats are found suitable for vegetating mangroves. While in Valsad and Daman ca. 2380 and 75 ha respectively are found suitable for mangrove vegetation.

The open suitable areas in Navsari, Valsad and Daman (UT) districts needs to be densified with mangrove species like, *A. marina*, *Rhizophora* and at certain places *Sonneratia*. While in Surat, *Avicennia* is the predominant species and hence it needs to be densified. The densification by artificial regeneration has its own merits: because the species composition, degraded areas can easily be regenerated; and, plantation can be made according to the objectives of afforestation such as conservation, sustainable yield, and protection of coastal areas. Over all it can be seen that approx. 8847 ha

mangroves in various districts are affected by Dam Alignment V and against that there are adequate potential areas (16759 Sq. km ) for taking up new mangrove vegetation.

### Rehabilitation and cultivation of Mangroves



The flowering in *Avicennia* takes place during May-June and seeds are produced during August-October. Since the seeds are viviparous, they germinate on the plant and later disperse through floating along the water stream. The seeds anchor themselves as soon as get the soft muddy soil. Mangroves particularly *Avicennia* is cultivated either by raising nursery in the raised sea beds or in poly bags. Nursery beds are prepared in the intertidal zone along the tidal current. Care is taken against any infection or algal growth. Direct seeding at the place of plantation can also be done by transporting the germinated seeds in moist gunny bag.

When centralized nursery is raised from the seeds, they are grown for 2-3 months in the bed and later transplanted at the desired location along the channels. Four to five month old saplings are transplanted at a distance of 2x2 M during winters in the mud flats. The areas where few plants are existing can be densified through planting of fresh

saplings and are the most suitable places for rehabilitation of Avicennia plantation. The expenditure for the first year will be ca. Rs. 44000/- but from the second year onwards mainly protection or guarding of plantation is required against grazing and trampling by local animals besides some replacement of seedlings and soil work.

**Suggestion to their rehabilitation and remedial measures to protect mangroves:**

In view of multiple benefits from mangroves to the coastal ecology and the local population, mangrove plantation has to be considered as one of the important components of Integrated Coastal Zone Management. The Coastal States and Union Territories have been requested to identify additional areas suitable for mangrove plantation with people's participation and augment the existing efforts related to education, awareness and alternative/supplementary livelihoods.

During the survey it was observed that mangroves prefer mud and fine-textured substrates. Most of the locations where the mangrove vegetation is available showed loamy to clay type of soils. Some of the areas like Valsad, Diu and Daman, luxuriant growth of mangroves (as compared to other locations under study) was seen during survey, however, there are a number of areas near Navsari, Valsad, Amreli and Bhavnagar where vegetation is very sparse and large tracts of mud flats are available for raising new plantations.

Planting of the seedlings must be done around monsoon time in which the seedlings would grow the most with taking advantage of rain water. It will also help seedlings to withstand higher salinity in the succeeding summers. Even though the mangroves thrives in coastal regions (with regular tidal flushing), still the availability of fresh water will determines the vegetative structure of these valuable ecosystem in some of the areas of low rainfall like Bhavnagar and Amreli. In these areas frequent flow of fresh water as well as sea water must be maintained for better growth of mangroves by creating channels.

**Conservation and Protective measures**

1. The conservation of existing mangroves biodiversity must be undertaken through protection of native species, as well as enrichment of species diversity by introducing new species which are compatible to enhance the productivity of forest ecosystem.
2. Though the mangroves falling within the dam alignment can not be protected but mangroves can be compensated by raising in the same area outside the enclosure at the locations indicated in the table 23.

3. Algal growth and blooms are an important cause for mangrove mortality as they smother the younger saplings. The impact of algae growth must be reduced by building barriers at the mouth of the channels and making water channels
4. The awareness needs to be created about the importance of mangrove and their role in the bio-geosphere to the local community mainly fishers who are exploiting them for fuel and fodder (in drought prone areas like Bhavnagar and Amreli).
5. Provide adequate protection to the saplings and vegetation from garbage accumulation, grazing of sites where the good forest cover exists (as shown in the picture). It would fasten the natural regeneration process. During the studies it was observed that grazing on mangroves by camels drastically affect the vegetation.
6. Soil working must be done wherever mangroves are growing sparsely by creating water channels.

### **Assessment of social, economic and environmental Impacts**

Like most ecosystems, an intricate relationship exists between mankind and mangroves. They have several ecological, socio-economical, and physical functions, which are essential in maintaining biodiversity and protecting human populations from nature calamities.

#### **1. Social and economic Impact**

Most of the coastal residents mainly agarias and fishermen rely on mangroves to sustain their livelihood and traditional cultures. They are the source of timber and firewood and cattle feed. They are excellent nursery grounds for fisheries and in some areas honey bees.

Mangrove's fodder is believed to be as nutritive as cotton seeds and it helps in improving the fat content of milk. Several households in coastal areas survive, particularly in droughts, by cutting mangroves for selling it for fuel and fodder. Secondly, mangroves are the most productive ecosystem in the world that supports a range of creatures for human consumption. Mangroves also support breeding of fish, prawn, shrimp, turtles, crabs and many other sea lives.

The social and economic conditions of the local people mainly fishermen will not be affected to the greater extent. Since, the salinity of the land in the peripheral areas will be reduced gradually over a period of time. It will help in taking up some crops in the command area which will be near to the spill over base line. The studies earlier carried out by GEC also confirm that local poor communities mainly depend upon mangroves for their routine needs.

## **2. Environmental Impact**

An aquatic ecosystem is an ecosystem located in a body of water and communities of organisms that are dependent on each other and on their environment live in aquatic ecosystems. There are two main types of aquatic ecosystems i.e. marine and Aquatic ecosystems and organisms are in general distinctly divided into those species inhabiting fresh water and that inhabiting sea water. Marine ecosystems cover approximately 71% of the Earth's surface and contain approximately 97% of the planet's water. They generate 32% of the world's net primary production. They are distinguished from freshwater ecosystems by the presence of dissolved compounds, especially salts in the water. Approximately 85% of the dissolved materials in seawater are sodium and chlorine. Seawater has an average salinity of 35ppt of water and actual salinity varies among different marine ecosystems. Marine ecosystems can be divided into the following zones: oceanic (the relatively shallow part of the ocean that lies over the continental shelf; profundal (bottom or deep water); benthic (bottom substrates); intertidal (the area between high and low tides); estuaries, salt marshes, coral reefs and hydrothermal vents.

There will be huge impact on the ecology of the region due to conversion of sea water into fresh water system. It is reported that all marine animals of bays and estuaries are practically killed by fresh water. Those which have no method of closing out the fresh water, or which do not live in tubes or burrows are quickly killed by it. Even due the flow of fresh water leading to depletion of nutrients, reduction of organic matter and of mud and silt deposition, affect benthic life on the Continental Shelf and in brackish-water lakes adjoining the sea.

Similarly most of the marine plants are killed due to fresh water as the physiologies of both the plants are different. Mangroves play an important role as protector against erosion and retard excessive siltation in inshore areas. Removal of mangroves and their root system would increase the coastal erosion. These ecosystems functions to improve the environmental health, by controlling the discharges /runoff from domestic, agriculture, and industries. The mangroves are multi-functional systems as they work for harbouring flora and fauna.

The amount of dissolved oxygen in a water body is frequently the key substance in determining the extent and kinds of organic life in the water body. Fish need dissolved oxygen to survive. Conversely, oxygen is fatal to many kinds of anaerobic bacteria. Mangroves are known to remove CO<sub>2</sub> from the atmosphere leading to

reduction in the greenhouse gases and global warming. They fix greater amount of CO<sub>2</sub> per unit area, than what the phytoplankton do in the tropical oceans.

Mangroves significantly reduce the impact of the storm surge and work as bio-shield. Mangroves are more efficient against surface waves and wind as well as providing protection against erosion. Stabilization of mud-flats is a preliminary process during the establishment of mangroves hence it control soil erosion. The roots of these plants help in binding the soil and also help the establishment of micro-organisms which further help in stabilizing the area. Since they are excellent nursery grounds for fisheries removal of mangroves would affect the fish catch.

### **3. Impact of Kalpasar on mangrove vegetation**

The creation of the fresh water reservoir in place of sea water will have significant affect the marine flora as well as vegetation of that particular zone and hence not only algal flora but also mangroves wetlands of Bhavnagar, Ahmedabad, Anand and Bharuch district falling with in the dam area may gradually not survive over a period of time. The ground water in peripheral villages falling within the Dam Alignment V would also gradually become non-saline over a period of time. Mangroves vegetation growing in pockets in these areas will be affected severely (Approx area 8887 ha). However, areas lying along the sea outside the Dam enclosure will not be affected significantly and hence mudflats available near Bhavnagar, Aliabet island, Dahej port, Surat, Valsad will be used for rehabilitation of mangroves.

### **4. Impact of mangrove vegetation on salinity ingress**

The leaves of many mangroves including Avicennia have special salt glands, which are among the most active salt-secreting systems known and remove the salts from the soil. The plants also adjust with soil salinity by concentrating it in the bark or in older leaves which carry it with them when they drop. Since the roots are very good binder they may reduce the salinity ingress by making the soil horizontally impermeable. Mangroves with higher density and good canopy will also protect the air blown salt to adjoining areas and prevent from salt accumulation; furthermore these will reduce the flow of water to ingress in non-saline area there by protect the agricultural land to become saline. Mangroves are shoreline stabilizers as they stabilize sediment that has been deposited largely by geo-morphological processes. Mangroves also protect the coast from erosion and damage. These act as a shelter bed between land and sea and prevent strong winds from damaging vegetation and property on land.

### **Conclusion:**

The Kalpsar project envisages construction of about 30 km long dam across the Gulf of Khambhat between village Kalatalav (Bhavnagar district) on the west bank and village Aladar (Bharuch district) on the east bank to develop a fresh water reservoir, to be used for various activities besides transport agricultural development and ground water quality improvement in command area of Saurashtra region. During the survey, it was observed that on the western coast of Gulf of Khambhat i.e. Bhavnagar, Amreli, Junagadh and Diu, mangroves (*Avicennia marina*) are growing discontinuously in the pockets. While area of mangroves in south Gujarat side is quite large and several mangroves species were observed in Valsad, Navsari district and Daman.

Since, Bhavnagar mangroves will fall both in the Dam area as well as outside the dam area. The total area estimated under mangroves in Bhavnagar district is 1866 ha of which 525 ha will fall within the dam enclosure. The total area in Ahmedabad (Dholera) is 3434 ha and this whole area will also fall in the dam enclosure. Total areas of mangroves in Anand and Bharuch are 1792 ha and 7977 ha respectively of which 1792 ha and 3096 ha come under dam enclosure, respectively. **All together ca. 8847 ha mangroves areas which fall within the enclosure of the dam will be submerged** in the fresh water and may perish over a period of time.

**The total potential area under the study is given in the table 25 which is estimated as 16759 ha** (East coast (4312 ha) and West coast (12447 ha) . These areas include sparse mangroves and available mud flats at different locations. Mainly areas with sparse vegetation in Surat, Navsari, ValsadAmreli can be used for densification and rehabilitation of mangroves. Rehabilitation of mangroves should be done with suitable species for plantation, salinity levels and other geophysical conditions of the region. Rehabilitation of potential areas for mangroves, **GEC has done rehabilitation of mangrove in 4535 ha area of Karanj, Dandi, Nada, Katiyajal, Magdalla, Dholai and Ghogha** (GIDR report.pdf *Source: Gujarat Ecology Commission, March 2010*). Remaining **12224 ha area** to be rehabilitate with mangrove in Bhavnagar, Amreli, Surat, Navsari and Valsad district.

## **Chapter 4**

### **Survey and assessment of *Jatropha curcas* L. for genetic improvement**

India has an advantage of being a tropical and sub-tropical country where several species capable of giving oil-bearing seeds are known to grow. *Jatropha curcas* (Ratanjot, Wild Castor, Jangli Erandi) and *Pongamia pinnata* (Karanj, Honge) are two species, which can thrive on any type of soil, need minimum inputs and management, and have low moisture demand. The propagation is much easier and it starts giving reasonable yields after third year of plantation. Seeds of both the species have high oil content (25-40 %) and the yield is adequate to justify its use for biodiesel production. According to the report of the committee on Development of Biofuel in India set up by the Planning Commission of India (2003), in addition to about 4 million hectares of waste lands, there is potential to cover 13.4 million hectares of other lands with *Jatropha curcas*.

We are principally focusing on fallow lands, excavated land in abandoned mines, protective hedges, land along roads and railway tracks and wastelands which makes available about 10 million hectares of potential area for *Jatropha*. Assuming an average yield of 3.3 tonnes of seed capsule (i.e. 2 tonnes of seed) per hectare in a year, which has been found realistic and achievable based on our primary data, cultivation of *Jatropha* over 10 million hectares of uncultivable lands and wastelands would result in 5.7 million tonnes of neat JME. In addition, 14 million tonnes of organic *Jatropha* cake manure and 13 million tonnes of empty capsule shells – which can substitute for 13 million tonnes of coal – will be obtained. Besides this, c. 0.5 million tonnes of glycerol, 0.3 million tonnes of soap and 0.1 million tonnes of SOP fertilizer would also be obtained. The automotive industry in India is one of the largest in the world and one of the fastest growing globally. India's passenger car and commercial vehicle manufacturing industry is the sixth largest in the world, with an annual production of more than 3.9 million units in 2011. According to recent reports, India overtook Brazil and became the sixth largest passenger car producer in the world, growing 16 to 18 per cent to sell around three million units in the course of 2011-12. Due to the fact there has always been a wide gap between demand and supply of fuel in the country.

The hype that *Jatropha* shrub is a miraculous plant, it would grow on marginal land unfit for agriculture, its water requirement is very low and yields large quantity of seeds prompted us to generate the primary data for greater understanding of the plant.

Gradually as the data was generated, these assumptions proved unrealistic. Though, the plant grows even in harsh conditions, its seed yield is not sufficient unless optimum agronomic conditions are provided. The recent experiment carried out by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) demonstrated that *Jatropha* plantations in Andhra Pradesh wiped out by disease called black rot/ collar rot during summers of 2009 and 2010. The same trend was noted here at one of our plantation sites, where one-fourth of the plantation was affected.

### ***Jatropha curcas***

The genus *Jatropha* belongs to tribe Joannesieae of Crotonoideae in the Euphorbiaceae family and contains approximately 170 known species. Although most of the *Jatropha* species are native to the New World, approximately 66 species are native to the Old World. Dehgan and Webster (1979) offered a key to the intra-generic taxa but this should not be considered as final since information is still lacking on many species. No complete revision of the Old World *Jatropha* exists. Revised 25 Somalian species, all of the subgenus *Jatropha*, and placed them in six sections and five subsections. *Jatropha multifida* L. and *J. Podagrica* Hook. of section *Peltatae*, *J. Integerrima* of section *Polymorphae*, and *J. gossypiifolia* of section *Jatropha* are well known and cultivated throughout the tropics as ornamental plants. Linnaeus (1753) was the first to name the physic nut as *Jatropha curcas* L.

*Jatropha* is a deciduous, small tree or large shrub reaching to a height of up to 5 m. The plant shows articulated growth, with a morphological discontinuity at each increment. Dormancy is induced by fluctuations in rainfall and temperature/light. The plant contains latex. The leaves have 5 to 7 shallow lobes with a length and width of 6 to 15 cm, which are arranged alternately. Inflorescences are formed terminally on branches and are complex, possessing main and co-inflorescences with paracladia. Botanically, it can be described as a cyme. The plant is monoecious and flowers are unisexual; occasionally hermaphrodite flowers were seen. In flower, ten stamens are arranged in two distinct whorls of five each in a single column in the androecium, and in close proximity to each other. In the gynoecium, the three slender styles are connate to about two-thirds of their length, dilating to massive bifurcate stigmata. Pollination of the physic nut is by insects. The rare hermaphrodite flowers can be self-pollinating. After pollination, a trilocular ellipsoidal fruit is formed. The exocarp remains fleshy until the seeds are mature. The seeds are black, 2 cm long and 1 cm thick. The caruncle is rather small.

It is reported to be diploid species with  $2n = 22$  chromosomes. Many parts of the plants are used in traditional medicine. The seeds, however, are toxic to humans and many animals. When the seed germinates, normally, five roots are formed from seedlings, one central and four peripheral. Tap root is not usually formed in vegetatively propagated plants.

The oil obtained from the seeds contains mainly four most important fatty acids: pamic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acid (C18:2). The average saturated fatty acid content of the seed samples is low i.e. 15.38% for pamic (C16:0) and 6.24% for stearic acid (C18:0). The average content of the unsaturated fatty acids, oleic (C18:1) and linoleic acid (C18:2) is considerably higher at 40.23 and 36.32%. The toxicity of the seeds is mainly due to the seed components like: a toxic protein (curcin) and diterpene esters. Curcin is similar to ricin, the toxic protein of the castor bean (*Ricinus communis*). *Jatropha* is a drought-resistant species and due to the fact it is widely cultivated in India as a living fence. The oil has traditionally been used as lamp oil and in soap manufacture. *Jatropha* has the potential to improve soil properties and can help in carbon sequestration because of huge biomass production.

#### **Environmental and edaphic conditions:**

Whilst *Jatropha* grows well in low rainfall conditions (requiring only about 200 mm of rain to survive) it can also respond to higher rainfall (up to 1200 mm) particularly in hot climatic conditions. In Nicaragua for example, *Jatropha* grows very well in the country's hot climate with rainfall of 1000 mm or more. However, it does not thrive in wetland conditions. In equatorial regions where moisture is not a limiting factor (i.e. continuously wet tropics or under irrigation), *Jatropha* can bloom and produce fruit all-round the year. A drier climate has been found to improve the oil yields of the seeds, though to withstand times of extreme drought, *Jatropha* plant will shed leaves in an attempt to conserve moisture which results in poor growth. Although *Jatropha* is adapted to low fertility sites, better yields are obtained on poor quality soils if fertilizers are used. Mycorrhizal associations have been observed with *Jatropha* and are known to aid the plant's growth under conditions where phosphate is limiting.

R & D activities on *Jatropha* cultivation were initiated way back during 1996-1999 under the project sponsored UNDP-NOVOD board to promote *Jatropha* cultivation on marginal lands involving tribal and marginal farmers in Orissa. Later, collaborative project was initiated by Industries Commissionerate, Industries and Mines Department, Government of Gujarat. Another project entitled '**biofuels from eroded soils in India**' was taken up in collaboration with Daimler-Chrysler AG, Germany, DC

India, University of Hohenheim, Germany, to work on determining the specific agro-climatic requirements, identifying superior provenances, proper space management, nutrient management, critical moisture regime for flower induction and fruiting, enhancing the seed yield and calculation of cost-benefit analysis etc in two phases.



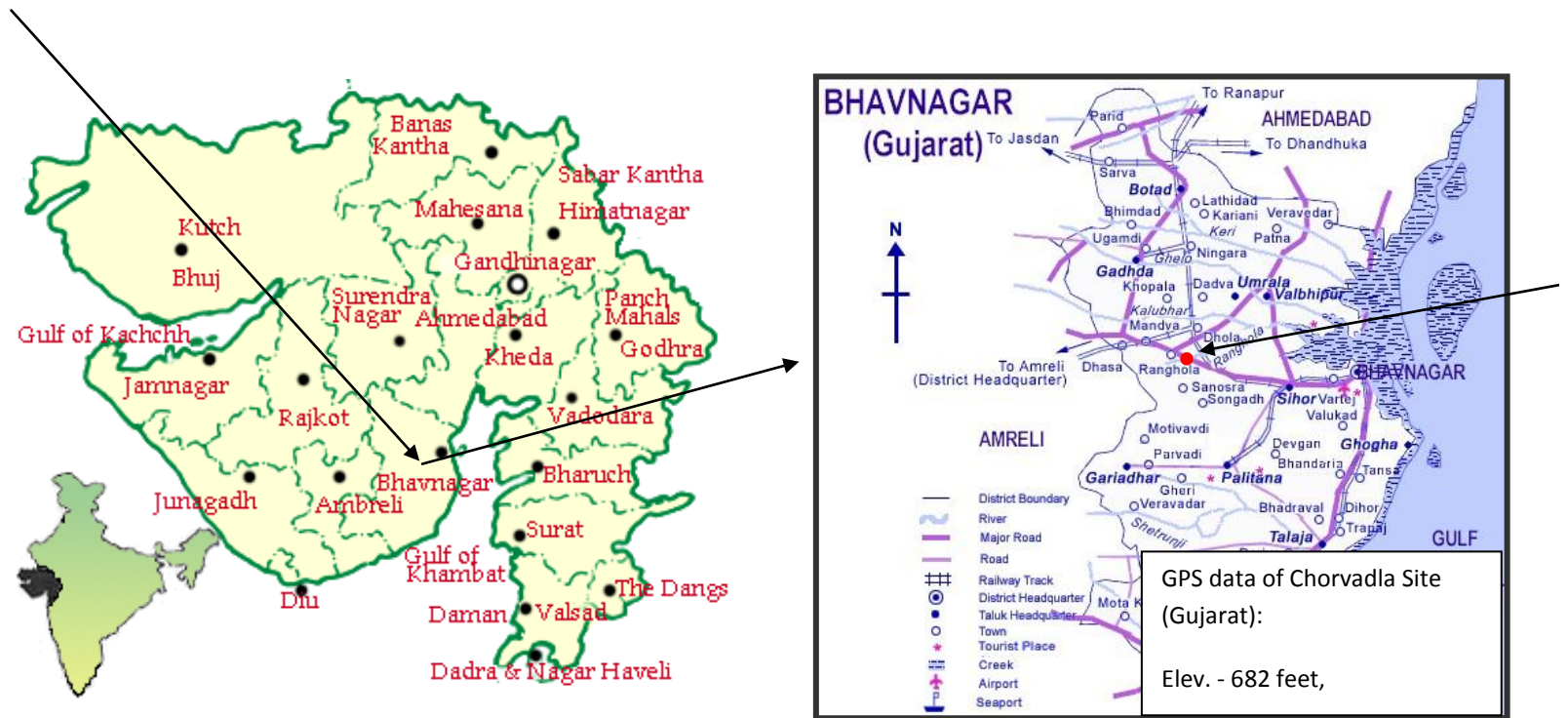
To initiate the experiments, survey of Jatropha growing areas of Gujarat and Rajasthan was conducted to identify the plus trees. During the process a number of plus trees were selected on the basis of robustness, seed yield and oil content etc. Both seeds as well as cuttings were collected from these plants and seedlings and cuttings were raised in nursery and used in the field trials.

**Table 4.1:Details of locations in Gujarat from where germplasm of Jatropha was collected after survey**

<b>S.No.</b>	<b>Location/Genotype</b>	<b>Approx. Age of the plant (in Yrs)</b>	<b>Elevation Latitude/longitude</b>
1.	Ranpur, Gujarat	20-25	Elevation: 891 ft Latitude: N 24°17.935' Longitude: E 72° 55.810'
2.	Chikhli, Gujarat	10-12	Elevation: 1305 ft Latitude: N 24° 19.846' Longitude: E 72° 53.212'
3.	Meen, Gujarat	10-12	Elevation: 1630 ft Latitude: N 24° 22.651' Longitude: E 72° 53.570'
4.	Rintoda, Gujarat	10-12	Elevation: 767 ft Latitude: N 23° 44.57' Longitude: E 73° 16.632'
5.	Buret, Gujarat	15-20	Elevation: 684 ft Latitude: N 23° 45.275' Longitude: E 73° 15.787'
6.	Shyamlaji, Gujarat	10-12	Elevation: 664 ft Latitude: N 23° 41.275' Longitude: E 73° 23.091'
7.	Kangaroo, Gujarat	10-12	Elevation: 1625 ft Latitude: N 24° 17.53' Longitude: E 72° 44.963'
8.	Amblimal -1, Gujarat	15-20	Elevation: 1165 ft Latitude: N 24° 21.403' Longitude: E 72° 49.322'
9.	Amblimal-2, Gujarat	10	Elevation: 1165 ft Latitude: N 24° 21.403' Longitude: E 72° 49.322'
	Amblimal-3, Gujarat	10	Elevation: 1165 ft Latitude: N 24° 21.403' Longitude: E 72° 49.322'
9.	PCM-1(ZOJ), Gujarat	5	Elevation: 842 ft Latitude: N 22°26.381' Longitude: E 073° 57.996'
10.	PCM-2, Gujarat	7	Elevation: 742 ft Latitude: N 23° 07.939' Longitude: E 074° 04.856'
11.	PCM-3, Gujarat	3-4	Elevation: 770 ft Latitude: N 23° 07.940' Longitude: E 073° 04.885'

12.	PCM-4, Gujarat	10	Elevation: 605 ft. Latitude: N 23°11.835' Longitude: E 073° 52.708'
13.	PCM-5, Gujarat	10	Elevation: 605 ft. Latitude: N 23°11.835' Longitude: E 073° 52.708'
14.	PCM-6, Gujarat	8	Elevation: 605 ft. Latitude: N 23°11.835' Longitude: E 073° 52.708'
15.	Shaktipeth-1, Gujarat	12	-
16.	Shaktipeth- 2, Gujarat	9-10	Latitude: N 24°18.94' Longitude: E 72° 46.32'
17.	Shaktipeth-3, Gujarat	8	-
18.	Rajasthan-6 (Vill. Losing)	8	Elevation: 2635ft Latitude: N 24° 48.503' Longitude: E 073°37.532'
19.	Rajasthan-8 (vill. Naliawada)	7	Elevation: 1349 ft Latitude: N 24° 12.635' Longitude: E 073° 42.007'

**Fig 4.1. Location for Jatropha experimental Plantation at Chorvadla, Bhavnagar**



Approx. 32 ha. of wasteland was acquired in village Chorvadla, Tal. Sihor, Dist. Bhavnagar (N 21° 40.856 E 071° 47.107). According to capability class the soil belong to category VIes with low fertility. Similarly 20 ha at village Nesvad, Tal. Talaja and vill. Bhadraval in Bhavnagar district was taken. The land at all the places was cleared and temporary fencing was done with thorny bushes so as to avoid biotic interference.

Village Chorvadla is situated in a typical semi-arid eco system where the annual rainfall (400-700 mm) is 2-3 times lesser than the evapo-transpiration. The regional rainfall (south west monsoon) commences after the middle of June and extends till end September/ beginning of October. More than 90 per cent of the total rainfall is received during the above 14-15 weeks period, while the rest 38 weeks of the year remains dry. Even during these 14 weeks, the number of rainy days hardly exceeds 30.

<b>District</b>	<b>Village /Survey no.</b>	<b>Area covered</b>	<b>Type of Soil</b>
Chorvadla/ Bhavnagar/ Gujarat	Chorvadla/Panchayat land	10 ha.	Wasteland
	Chorvadla/ Government land	17 ha	Wasteland
	Chorvadla/ Forest department land	5 ha	Wasteland
Nesvad/ Bhavnagar	Government land	20 ha	Wasteland
Bhadraval/ Bhavnagar	Panchayat land/ sandy loam and stony	20 ha	Wasteland
Kanode/ Panchmahal	Panchayat land/ black heavy soil with water stagnation	25 ha	Wasteland
	<b>Total</b>	<b>97 ha</b>	

Land was identified at village Kanode for raising another 25 ha of new plantation using the best identified germplasm and agro-technology. Observations on the plant populations were recorded using eight quadrates of size 20m x 20m at each of the sites. Thereafter, the vegetation were removed along with roots, species-wise, to assess the initial biomass to be replaced by Jatropha plantation. Fresh and dry biomass was collected to determine dry matter production and carbon status. The soils at Kanod were found to be heavier in texture with mostly sandy clay loam to clay upto 60 cm of solumand higher salt concentration at lower soil layers. The pH of the soil varied narrowly from 7.30 to 7.54 across the different layers.

### Soil conditions of plantation sites:

According to capability class the soil belongs at Chorvadla belongs to category VIes with low fertility. It belongs to order Entisols in subgroup Lithic Ustorthents, family Coarse loamy, mixed, iso-hyperthermic of Amergadh soil series. This series comprises of very shallow to shallow soils, strong brown to dark brown in colour, non-calcareous, loamy skeletal soils derived from basalt. The solum is 10 to 22 cm thick, gravelly sandy clay loam, mildly alkaline and excessively drained. The water holding capacity is poor due to low solum thickness (10-20 cm) and excessive drainage. The soil productivity is poor. These soils are suitable only for pasture development and afforestation and not for growing agricultural crops.

The physical and chemical properties of soil including macro-nutrient composition of newly established different Jatropha sites were analyzed and the results are depicted below in Table 4.2 and 4.3.

**Table 4.2: Physical characterization of soil**

S. No.	Location of the sites	Range of various parameters of soil physical properties						
		Percent concretion (in0-40 cm)	Sand (%)	Silt (%)	Clay (%)	Texture	EC (ds/m)	pH
1	Nesvad	16.3-20.9	71.9-80.3	11.7-15.8	7.2-12.3	Sandy loam	0.09-0.13	7.0-7.6
2	Bhadrawal	19.1-22.3	68.5-76.3	16.7-21.6	7.0-9.9	Sandy loam to Sandy clay loam	0.18-0.34	7.3-8.1
3	Kanod	2.1-4.8	25.8-29.9	11.9-16.3	57.9-58.1	Sandy clay loam	0.07-0.44	6.8-7.3
4	Chorvadla	2.2-55	57.5-76.2	14.7-30.0	9.0-17.8	Sandy loam to sandy clay loam	0.11-0.29	7.1-7.9

**Table 4.3: Chemical characterization of soil**

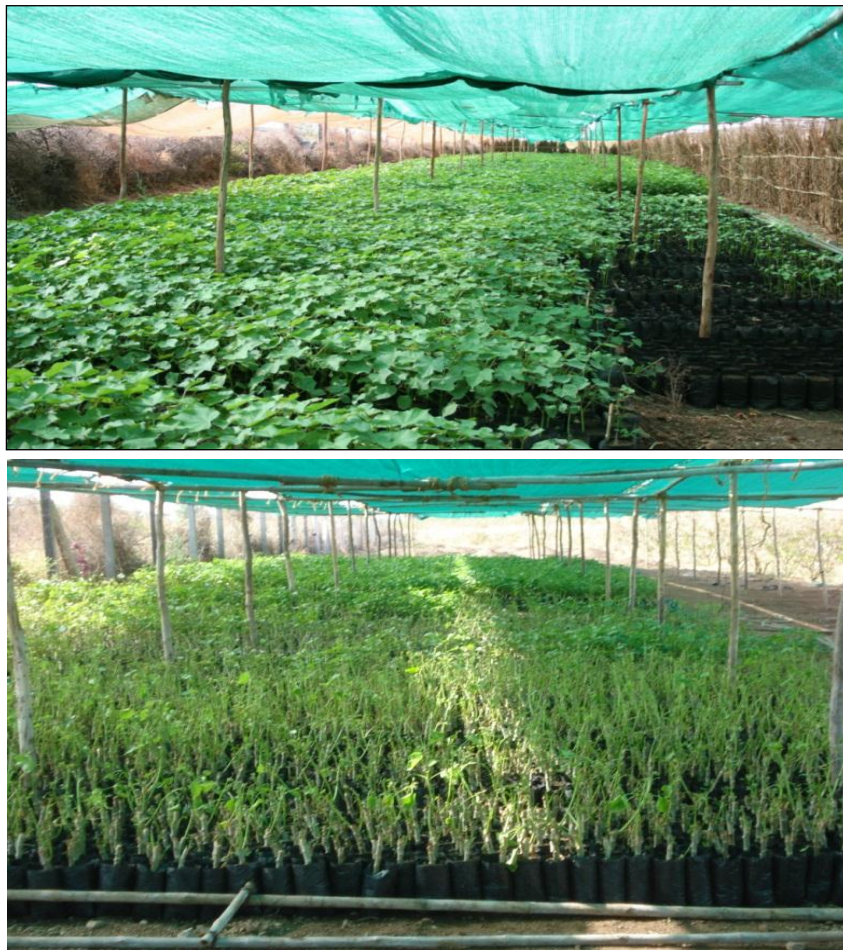
S. No.	Location of the sites	Range of various parameters of soil chemical properties						
		%Org. Carbon	CaCO <sub>3</sub> (%)	CEC (meq/100g)	Total N (%)	Avail N (kg ha <sup>-1</sup> )	Avail P (kg ha <sup>-1</sup> )	Avail K (kg ha <sup>-1</sup> )
1	Nesvad	0.22-0.36	4.0-5.8	30.5-38.9	0.014-0.031	143.1-185.0	1.24-6.64	146.7-281.3
2	Bhadraval	0.38-0.47	14.8-9.8	28.1-43.8	0.038-0.045	142.0-200.7	1.94-3.73	269.9-325.9
3	Kanod	0.55-0.81	2.8-3.8	37.7-49.3	0.05-0.069	103.5-251.1	2.9-14.5	185.9-201.6
4	Chorvadla	0.45-0.89	4.0-24.0	25.9-38.0	0.035-0.104	159.9-238.3	0.97-5.21	161.3-346.1

The primary limitation at Kanod site had been that parts of the land remained water-logged for a considerable length of time during the rainy season due to the low permeable heavy-textured soil coupled with site situation which does not allow proper drainage of water. As a result of which annual arable crops cannot attain good growth and yield. Therefore, before planting, soil amendment was done to allow proper penetration-cum- aeration of the roots. Suitable drainage provision was established to divert substantial run-off from the root zone of *Jatropha*.

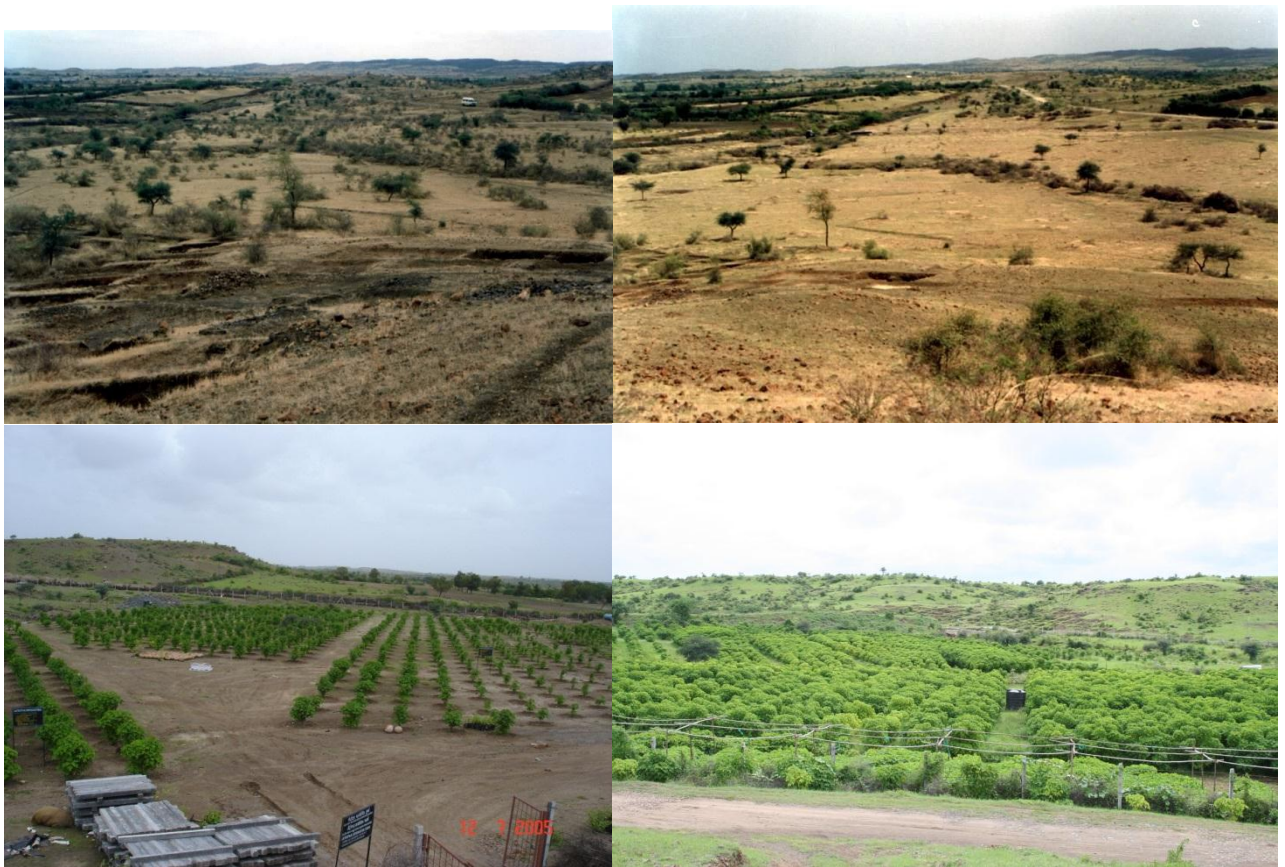
### **Raising of nurseries of *Jatropha* in Orissa and Gujarat from elite germplasm**

Nursery of 23 selected genotypes was raised in nursery during April-June 2004. Seeds were soaked in water for 6-8 hrs and later sown in nursery bags of approx.10 x 23 cm size, filled with nursery mixture containing soil and Farm Yard Manure (3:1). Germination in fresh seeds is very quick. Similarly, cuttings were also raised from selected provenances.

Later, a nursery of ca. 75,000 stem cuttings from elite germplasm of *Jatropha* was raised at Orissa and the rooted plantlets were transported to Kanod experimental Farm at Panchmahal district of Gujarat and transplanted during the period during July to September 2010 and 2011. Similarly, nursery of 27000 cuttings of elite *Jatropha* germplasm was also established at Nesvad village of Gujarat and cuttings were transplanted at various experimental units in Gujarat.



In the second phase of *Jatropha* research, CSMCRI entered into a joint collaborative project for three years (started in Jan, 2010) with General Motors Corporation, US and US Department of Energy, with an aim to demonstrate 50 ha plantation of *Jatropha curcas* on non-arable marginal lands with cuttings and micro-propagated elite germplasm. It was also envisaged to improve the best practices developed by the institute so far to produce significant quantities of *Jatropha* oil for conversion to biodiesel. A full life cycle analysis of *Jatropha* system was also proposed. In 2010-11, new sites were identified and acquired for plantation at village Bhadraval (Bhavnagar) (N 21° 29' 53.0''; E 072° 00' 16.7''), Kanod (Panchmahal) (N 22° 41' 37.6'' E 073° 29' 64.9'') and Nesvad (Bhavnagar) (N 21° 30' 49.4'' E 072° 02' 18.5'') and subsequently, plantations were raised.



**Development of *Jatropha* experimental site (Before and after)**

## **A. Selection and improvement:**

To initiate breeding programme in any plant species, it is essential to determine existing variability before embarking upon selection of superior germplasm. In our study we observed considerable variation in flowering and fruiting pattern in all the 23 accessions. In literature most of the information available on seed production is from the plants which have not yet reached maturity (<5 years old). Earlier Aker (1997) conducted an exploratory study to detect patterns of variation in flower, fruit and seed production in one-year-old plants of *Jatropha curcas* (Euphorbiaceae) in response to variation in environmental conditions in Nicaragua.

Long back, Matsuno *et al.* (1984) and Foidl *et al.* (1996) presented yield data for mature plantations and projected seed yields of 4 and 5 t/ha, respectively. Heller (1992) reported seed yields between 0.1 to 8.0 T/ha. The details of planting material and other experimental details under which this yields were achieved were not clear. Later, Openshaw (2000) reported the seed yield between 0.4-12 t/ha. The realistic figures on seed yield are still not available in the literature but may come in next few years, as recently established plantations have begun to reach maturity. Recently Brittain and Litaladio (2010) compiled the seed yields mentioned by various workers in one of the publications in the series of Integrated Crop Management published by Food and Agriculture Organisation of the United Nations. It indicates that there is significant variability in seed yield because of locations, soil and environmental conditions.

Indian Council of Agricultural Research has identified first ever *Jatropha* variety, SDAUJ-1 (Chatrapati) for commercial cultivation in India because of high yields and oil content. Though, significant amount of work has been done on selection of superior genotypes of *Jatropha*, still lot of work needed to be undertaken so that the plant can be grown as captive plantations for biodiesel production on commercial scale. It has been demonstrated that plant can also be grown under low saline/alkaline conditions with amendments in soil conditions (Kumar *et al.* 2010a & b) hence large tracts of wastelands with low salinity/ alkalinity levels can also be exploited for production of biofuels.

## Provenance trial

In a provenance trial, 23 genotypes were randomly planted at a distance of 3x2 m with three replications of 10 plants each. The trial included the genotypes collected by collected CSMCRI, S.K. Nagar University Dantiwada and Forest Department, Gandhinagar. The same genotypes were also provided to SD Agriculture University Dantiwada and Gujarat Forest Department for assessing the performance.

As a basal dose of 50 g Single Super Phosphate (SSP) and 45 g Ammonium Sulphate (AS), 2 kg FYM and 1 kg Jatropha cake was applied per pit (size 45x45x45 cm) at the time of transplantation. The transplantation of the seedlings was carried out during August taking the advantage of rains. Lifesaving irrigation was provided @ 15 l/plant once in a month. Top dressing with 40 gm of DAP per plant was also applied once.



Out of ten plants, five from each replication were randomly selected for recording observations on phenological and yield contributing characters like height, canopy, girth at the base, flowering period, male-female flowers ratio, total number of fruits/plant, total seed yield/plant, 100 seed weight etc.

## Floral biology

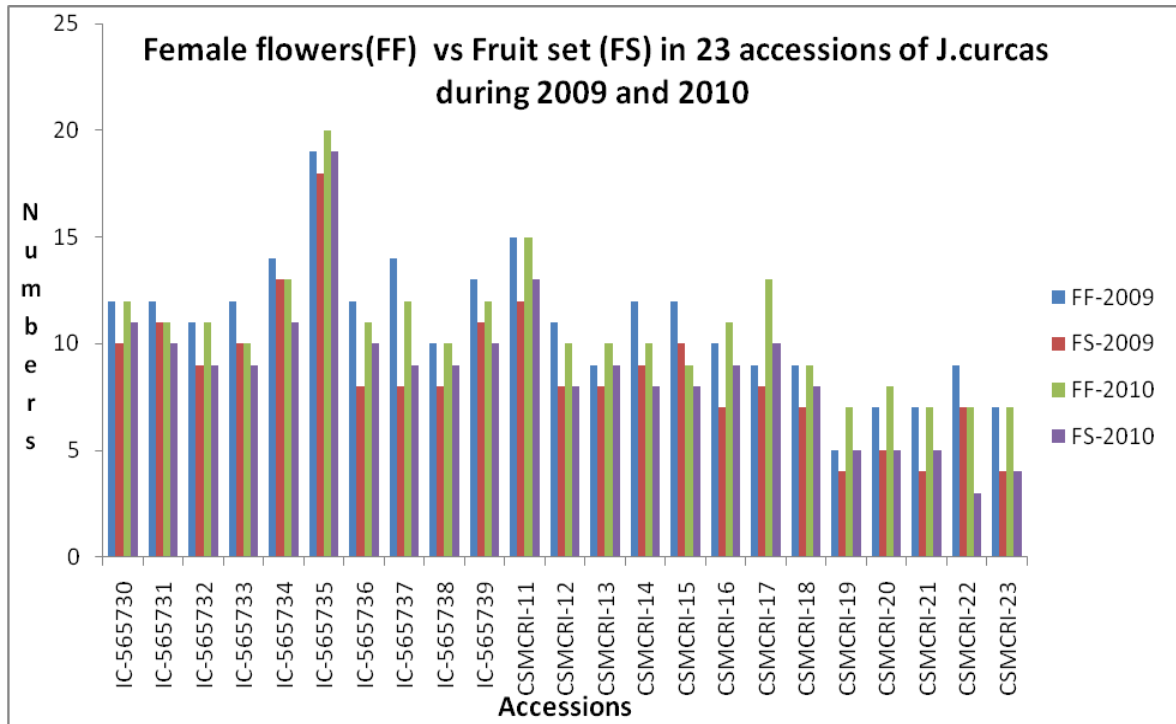
Different morphological characters were studied in all the provenances during 2005-06 while yield contributing characters were continuously observed during 2004-05 and 2005-06.

The size of inflorescence and the proportion of male to female flower is quite variable in this species. The ratio of both male and female flowers was recorded for two consecutive years in the provenances during the first episode. During first year, it ranged from 18:1 to 31:1 with mean value of 25:1 whereas, it reduced to almost half ranging from 10:1 to 16:1 with a mean value of 13:1 in different provenances. This indicates that in the second year the size of inflorescence increased leading to increase in more number of female flowers as compared to male flowers per inflorescence.

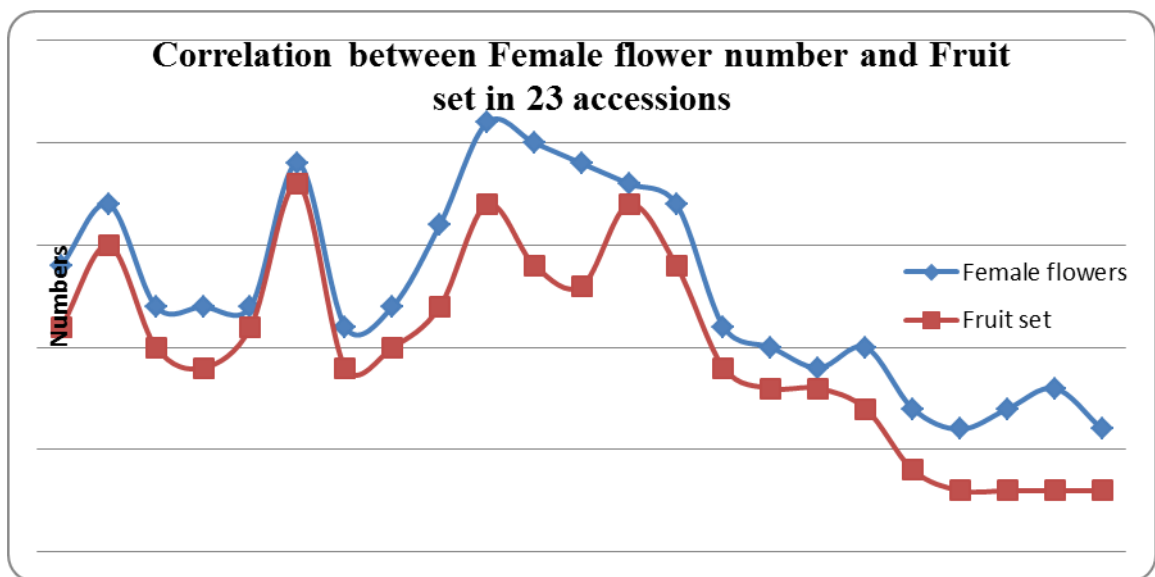
The reproductive biology of flowering plants is important for determining barriers to seed and fruit set, pollination and breeding systems that regulate the genetic structure of populations. Floral biology has great significance as intra varietal and intra specific hybridization which offer possibilities in transfer of useful genes from one genotype to another. Information on male to female ratio is a pre-requisite to select suitable genotype from the existing population in jatropha. Normally, in the inflorescence, a dichasialcyme, produces a central female flower surrounded by a group of male flowers which are much more numerous and occupy subordinate positions within the inflorescences. Inflorescences are axillary, formed terminally on the new shoots and are complex, possessing main and *co-florescences*. We observed that the ratio of male and female flowers in all the 23 accessions in our progeny trial fluctuated tremendously when studied for early four consecutive years (Bhuva et al. 2007) and affected the fruit set and seed yield. It was concluded that apart from M/F ratio, number of female flowers converting into true fruits is more important to select superior genotypes from the existing population (Fig.4.2). Previously Raju and Ezradanam (2002) studied the pollen ecology and fruiting behaviour in *J.curcas* and found male to female ratio as 29:1. He also reported that both type of florets open synchronously and sexual system facilitates geitonogamy and xenogamy.

According to Kumar et al. (2008) the number of female flowers per inflorescence showed maximum variation while the difference for the number of male flowers/inflorescence was narrow. This variation is reported to be due to fluctuating

environmental conditions. Looking at the wide variations in male to female flower ratio, it is suggested that number of female flowers in an inflorescence appears to be more consistent and influencing the seed yield instead ratio of male and female flowers. Our studies have confirmed the direct correlation between number of female flowers per inflorescence and fruit set. (Fig. 4.3)

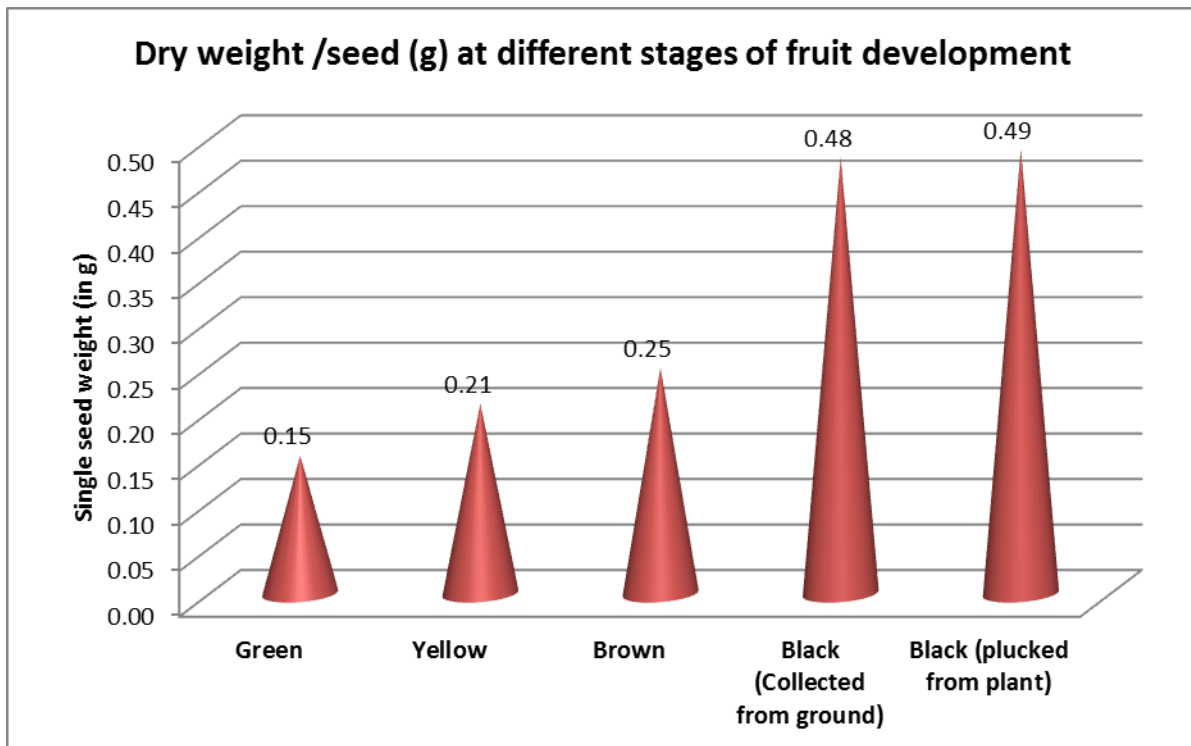


**Fig.4.2. Variation in number of female flowers converting into fruits during last two years.**



**Fig.4.3. Correlation between female flower number and number of fruit set.**

Attempts were also made to study the growth pattern during the fruit development (depicted in graph below). As expected there was fast increase in fruit weight during initial phase and later weight gain was found to be slow. Similarly pattern in the oil content was also noticed.



The fatty acid profile was determined in seed oil of all the selected accessions through GC-MS (Shimadzu, QP-2010 make). It can be seen from the table (Table 3.2.8) that Oleic acid and Linoleic acid are the major fatty acid in the oil followed by Palmitic acid (little higher than the report of Gubitz 1999) and stearic acid. Small quantity of other three fatty acids like Arachidic acid, Palmitiolic and Elaidic acid were also present.

S. No.	Accessions	Palmitoleic acid	Palmitic Acid	Linoleic Acid	Oleic acid	Elaidic Acid	Stearic Acid
1	CSMCRI-GUJ-Banas-1205-C1	0.21	16.18	26.11	47.08	0.85	9.56
2	CRIDA-MP-Jhabua-02-03-JJ-06	0.80	18.30	25.41	46.06	0.78	8.45
3	NBPGR-GUJ-SKN-0605-Hans	0.19	18.50	27.11	44.08	0.76	9.42
4	AFRI-TN-Coimb-0206-C-6	0.21	18.00	29.65	42.97	0.55	8.70
5	AFRI-KER-Palak-0206-C-1	0.20	16.10	26.78	47.26	0.63	9.29
6	CRIDA-MP-Dhar-1105-C-4	0.17	18.16	28.68	43.23	0.61	9.21
7	CSMCRI-GUJ-Panch-0106-C3	0.19	17.60	25.47	44.75	0.87	10.15
8	FRI-HP-Shim-0306C-13	0.18	17.20	26.03	47.25	0.88	8.53
9	FRI-HAR-Jhajj-1105-C-24	0.21	17.38	26.09	45.20	0.92	10.27
10	NBPGR-RJ-Rajsam-0905-C-15	0.20	18.30	25.82	46.58	0.77	8.28
11	NBPGR-UA-Paur-0306-C-9	0.18	17.80	30.32	41.75	0.63	9.40
12	NBPGR-CHH-Bilas-0306-C-30	0.18	17.20	28.06	44.55	0.64	9.36
13	RRL-AP-Papum-1105-C-1	0.19	16.95	26.00	47.44	0.70	8.72
14	RRL-MNP-Imph-1005-C-1	0.19	18.20	23.06	50.14	0.63	7.76

### **Yield parameters**

The plants started bearing fruits from December 2005 onwards i.e. 6 months after planting. During 2004-05, though the fruiting was observed in most of the provenances, the yield was very low. The seed yield per plant was highest in SKNJ-7 (15.34 g) followed by SKNJ-1 (15.33 g) and CSMCRI-2 (9.93 g). There was not much difference among the provenances for test weight. However, highest 100-seedweight was observed in CSMCRI-6 (59.78 g) followed by CSMCRI-5 (59.77 g) and CSMCRI-2 (59.75 g). During 2005-06 also yield and yield-attributing characters significantly differed in different provenances of *Jatropha* (Table 4.4 & 4.5). It was observed from the data that highest seed yield (223.56 g/plant) was recorded in the provenance CSMCRI-2 followed by CSMCRI-6 (189.76 g/plant) while lowest was recorded in Guj F-5 (49.21 g/plant). The 100-seed weight was also marginally higher in these provenances. From these figures, it is expected that yield would increase gradually.

During third year i.e. 2006-07, it was observed that productivity was low in all the provenances as compared to last year. However, the trend remained almost the same. The data for yield potential of different provenances for last three has been given below (Table2). CSMCRI-1 and CSMCRI-2 have performed better than others followed by SKNJ-7. The oil content significantly varied among the provenances and during different years. The oil content varied from 26.30 % to 35.00% in different provenances. However, it also varies during different years indicating the environment influence over the character. Based on the results for three years, it can be concluded that few of the provenances like CSMCRI-2, SKNJ-7, CSMCRI-5, CSMCRI-6 and CSMCRI-1 proved to be superior among all the provenances. At this stage, these provenances have been multiplied for further extension of plantation. However, results need to be confirmed for longer period of years under the same climatic conditions to determine the yield potential of different provenances on degraded land and select the potential ones for further multiplication and use in the extension programme.

**Table 4.4: Yield Attributing Characters of Jatropha Provenance Trial (2005-06)**

Sr. No.	Name of Provenance	No.of Capsules/ plant	No.of seeds/ plant	Seed Yield/ plant (g)	100 seeds Wt. (g)	Oil Content (%)
1	CSMCRI-1	97.37	247.07	147.27	59.59	33.14
2	CSMCRI-2	130.21	347.91	223.33	64.01	32.84
3	CSMCRI-3	93.66	236.20	145.62	61.69	28.54
4	CSMCRI-4	79.73	208.47	122.25	58.00	31.73
5	CSMCRI-5	97.92	258.55	163.45	63.36	30.84
6	CSMCRI-6	113.21	296.27	186.29	63.04	31.43
7	CSMCRI-7	68.49	166.77	100.48	60.47	29.50
8	CSMCRI-8	61.84	151.61	89.31	58.93	31.11
9	CSMCRI-9	88.04	222.61	118.03	53.75	31.39
10	CSMCRI-10	85.61	220.72	121.92	55.18	29.84
11	SKNJ-1	83.31	218.30	130.51	59.84	31.32
12	SKNJ-2	85.74	218.56	132.21	60.00	32.06
13	SKNJ-7	93.41	248.91	154.11	61.19	34.90
14	SKNJ-8	82.61	212.77	127.57	60.56	34.09
15	SKNJ-9	67.34	171.14	103.44	61.04	34.73
16	SKNJ-10	43.13	110.16	63.47	58.62	34.34
17	SKNJ-11	70.66	176.12	106.63	59.24	32.27
18	SKNJ-12	46.77	122.14	63.84	54.06	26.30
19	Koteshwar-F	67.59	170.05	95.18	55.73	34.47
20	Ambaji-F	57.76	147.26	85.18	57.60	26.73
21	Panchha-F	53.16	134.27	76.84	57.32	32.68
22	Ranpur-F	60.12	147.17	84.20	57.19	32.56
23	Kumbharia-F	32.71	82.33	45.26	54.72	-
<b>C.D.</b>		<b>26.46</b>	<b>66.74</b>	<b>42.70</b>	<b>NS</b>	<b>-</b>
<b>C.V. %</b>		<b>20.99</b>	<b>20.65</b>	<b>22.20</b>	<b>7.73</b>	<b>-</b>

Provenance trial of 23 provenances planted during 2004 was continued to determine the long term performance of various accessions. The average seed yield obtained from different accessions during 2009-10 varied from 756 g/plant to 1190g/plant (N=9/accession) at Chorvadla, Gujarat. It indicates that the best germplasm (IC-565736) has given almost 2t/ha of seeds. The experiment has been continued further to determine the difference in yield pattern between seed raised plants and plants raised from cuttings of superior germplasm.

**Table 4.5: Yield behaviour of provenances from 2006-2008**

Sr. No.	Name of Provenance	Seed yield / plant (g) (2006-07)	Seed yield/ plant(g) (2007-08)	Seed yield/ plant (g) (2008-09)	Seed yield/ plant (g) (2009-10)
1	IC 565730	73.1	240.4	895.2	1042
2	IC 565731	43.0	251.3	841.5	962
3	IC 565732	15.7	133.8	873.5	1031
4	IC 565733	5.3	152.0	843.6	841
5	IC 565734	32.7	276.9	783.2	914
6	IC 565735	22.1	302.2	854.2	1091
7	IC 565736	21.3	251.3	929.9	1190
8	IC 565737	0.0	301.8	845.4	1091
9	IC 565738	16.1	187.4	638.1	970
10	IC 565739	9.2	78.8	547.5	1001
11	CSM-11	6.4	98.3	396.2	800
12	CSM-12	13.6	186.0	637.9	1093
13	CSM-13	40.0	244.5	976.7	878
14	CSM-14	31.3	301.5	723.9	984
15	CSM-15	14.4	289.3	650.7	804
16	CSM-16	10.6	178.3	534.3	864
17	CSM-17	27.1	247.4	644.2	980
18	CSM-18	15.3	191.3	566.9	931
19	CSM-19	16.0	103.9	405.4	759
20	CSM-20	10.7	154.6	440.5	798
21	CSM-21	7.4	122.4	409.9	883
22	CSM-22	5.8	142.7	415.3	899
23	CSM-23	4.8	88.0	451.7	943

In this experiment highest seed yield of only 302.2 g per plant was recorded after 3 years from the accession IC 565735, while other accession IC 565737 and SKNJ 8 were also statistically at par to it. The low seed yields in this experiment were mainly due to poor soil conditions of the plot. The higher yield corresponded to higher percentage of success in fruit set (71-81%) in these accessions. While in 2008-09, high seed yield per plant (over 900 g/plant with corresponding yield of 1.6 t/ha) was obtained from two provenances SKNJ-7 and IC 565736 (CP-13). Other promising accessions were IC

565730, IC 565732 and IC 565735 yielding in excess of 850 g/plant. In the sixth year of the plantation at Bhavnagar, Gujarat, the highest seed yield of 1190 g per plant was recorded from the accession IC 565736, while IC 565735, IC 565737 and SK-2(n=9) were also statistically at par to it. These yield correspond to 1.82 to 1.98 t/ha (~ 2t/ha).

The accession IC 565739 (planted in 1999), which has proved high yielding under sand dunes of Gujarat yielded 5.2 kg/plant in 2008-09, while in 2009-10, it yielded 5.55 kg/plant.

In few provenances, oil content was determined during different flushes to study the effect of harvesting time on oil yield. It was interesting to observe that oil content greatly reduced during the last flush of the season in most of the provenances. This may be due the nutritional deficiency in the plants at the last phase of fruiting.

Sr. No.	Provenance	Oil content (%)	
		Sept. 2007	Dec. 2007
1	CSMCRI - 6	26.4	25.9
2	CSMCRI - 7	30.9	23
3	CSMCRI - 8	30.6	24.4
4	SKNJ-8	20.9	15
5	SKNJ-10	25.8	20.1
6	SKNJ-11	16.6	14.1
7	SKNJ- 12	22.9	18.8
8	Kumbharia - F	15.3	20.1

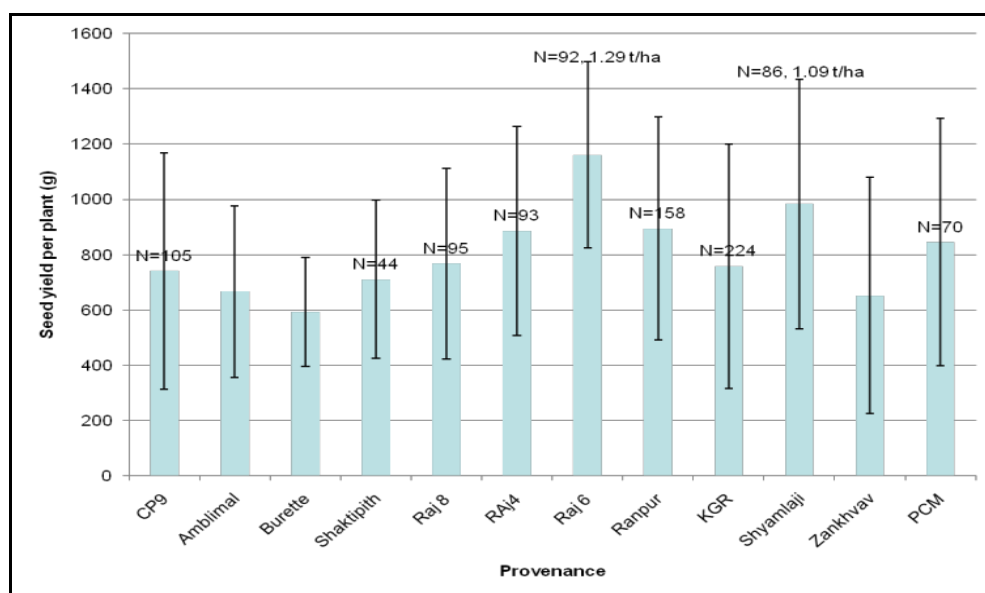
Similarly yield performance of best performing accession CP-9 (this accession was registered with NBPGR and provided the accession number IC 565735), along with other elites planted in Jan 2003 was recorded in Orissa. The 100 seed weight and oil content recorded for elite genotypes at Berhampur, Orissa at different location during October 2007 has been provided in the table below. It can be seen that the 100 seed weight was as high as 77g and oil content was 40 %.

Plant no.	Location	100 seed weight (g)	Oil content %
CP-9	Mohuda Govt. site	77.1	40
CP-9	Mohuda Govt. site	76.5	37
CP-13	Mohuda Govt. site	68.7	30
CP-13	Gopalpur	65.2	35



CP-17	Mohuda Govt. site	67.6	30
VP-2	Gopalpur	49.4	36

### Establishment of new experimental *Jatropha* plantations and assessment of yield

Based on our earlier experiments conducted at Chorvadla (Gujarat) regarding fertilizer requirements spacing, irrigation, mulching, intercropping etc, new plantations were established at different sites at Chorvadla during 2006 and Neswad during 2007 to test long term performance of plants that were raised through cuttings of elite germplasm. Best agro-technological knowledge based on results of the various experiments were employed in the Neswad plantation and average yield as high as 830g/plant (N=100) was obtained in 2008 itself. Seed oil content varied from 29-35.5%. These plantations were later used for investigation in-depth as part of the proposed project with General Motors.



In sub humid conditions of Orissa, we have obtained, for the progeny line (CP-9) of *Jatropha curcas*, an average seed yield of 1.4 kg per plant after 4.5 years and 2.0 kg per plant after 5.5 years (Table). The yield should rise further as the plants mature and unequivocally it can be stated that getting 2 tonnes of seed per hectare, on average, is an achievable target under wasteland conditions. One plant, VP-2 (Date of planting- August, 1999) has yielded 5.41 kg of seed in sand dune of Gopalpur, Orissa, while another accession IC 565739 has proved high yielding under similar conditions of Gujarat.

	
Site: Chorvadla, Gujarat Date of planting: Aug, 2004 Spacing: 3m x 2m Irrigation: 80 L/plant/month during dry months	Site: Mohuda, Orissa Date of planting: January 2003 Spacing: 4m x 3m Irrigation: 30 L/plant/month during dry months

#### **Performance of cuttings of accessions CP-9 at Mohuda, Orissa**

At Orissa, the performance evaluation of the accession IC 565735 was continued and presented below. However, the seed yield dipped in 2012-12 due to lower rainfall and higher heat

Mean seed yield in kg/plant (N=10)							
Year	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12
Seed yield	0.30	0.70±0.2	1.40±0.8	2.00±0.8	2.04±0.6	2.2±0.5	1.3±0.4

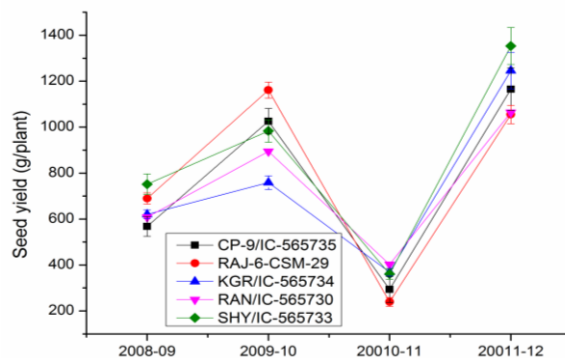
In Gujarat, the accession IC 565739 (planted in 1999), which proved high yielding under sand dunes of Gujarat yielded 5.2 kg/plant in 2008-09, while in 2009-10, it yielded 5.55 kg/plant however, during 2010-11 yield declined to 3.74 kg/ plant (Jatropha yields for the year 2010-11 were uniformly low across all places in Gujarat). In 2011-2012 the plants were affected by white ant attack leading to poor yield. However, control measures have been implemented.

Year	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12
Seed yield (kg/plant;(average of two plants)	1.49	4.14	5.20	5.55	3.74	2.01*

\* Due to white ant attack, the yield for the plants were drastically reduced; control measures were put in place

### Performances of 2007 planted selected plants at Nesvad during 2008-09 to 2011-12.

It was found that the seed yield in terms of hectare (converted from average yield per sample number of plants) obtained from few selected accession reached 1.5 to 1.6 tonnes per ha in the fourth harvest. Jatropha cake applied back into the plantation gave good response.



The superior seed yield of the selected accessions were witnessed by independent experts at the behest of MNRE (Ministry of New and Renewable Energy) during the fruiting season of 2011 (Figure below) whose comments are highlighted below



#### Highlights of Report of MNRE Expert after visit to CSMCRI's Nesvad Jatropha Plantation

##### Point #1:

*"The very fact that the drooping branches cultivar had high fruit output makes it a desirable phenotype. It is strongly recommended that CSMCRI develops it as a distinct cultivar as this is distinct, uniform and has high yield.*

*The phenotypic trait of the other erect type was not distinct but was also relatively uniform. Since it has been found to be resistant to local conditions (reportedly also to sites at Berhampur, Orissa), it is also a good cultivar and should be clonally multiplied for plantation."*

##### Point #9:

*"The plots were healthy and as informed are minimally irrigated and only limited fertilization is done. Overall, it was a very successful trial and a good demonstration."*

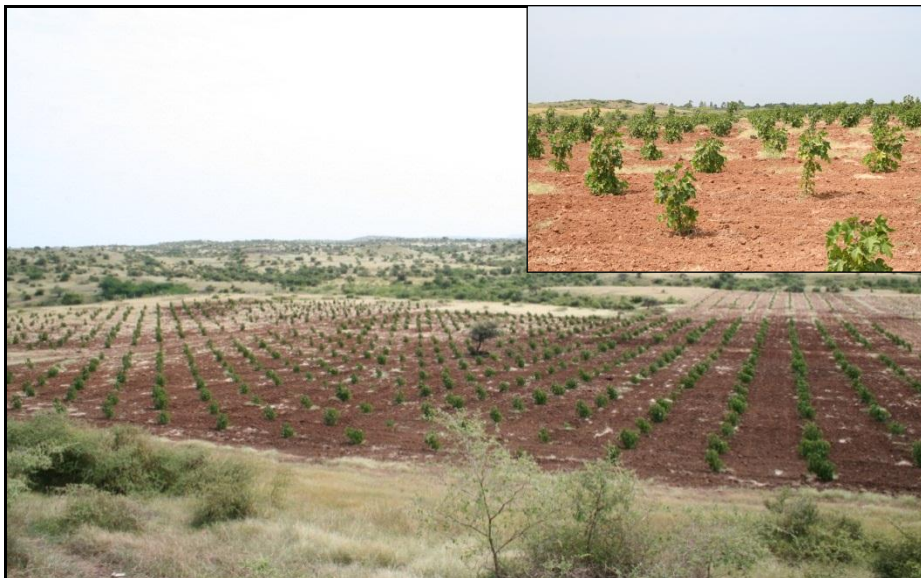
*Dr.HM Behl  
September 30, 2011*

**Multiplication of superior provenances for extension of area of cultivation:**

Seedlings and cuttings to the extent of 20,000 from selected provenances were raised. Since pruning has been done in the plantation, cuttings (approx. 6000) obtained thereby were kept for rooting in the field itself. Approx. 90 % rooting was observed in the cuttings under field conditions.



The extension of Jatropha plantation was carried out on Government site in village Chorvadla with the selected provenances and approx. 5 ha area was covered. Irrigation facilities were created along with storage room for keeping the field implements. Various experiments like fertilizer trial, spacing trial and management trial were conducted at the location.



Since the site was rocky most of the plants were blown away due to heavy winds and experiments could not be continued for a long time. Therefore soil amendments were carried out for better anchorage of plants.

**NMITLI project entitled “Genetic Improvement of *Jatropha curcas* for adaptability and oil yield**

Looking at the strategic importance of *Jatropha*, a NMITLI project was formulated by CSIR, New Delhi, on development of rapid screening methods, provenance selection based on multiplication field trials, multiplication of elite clones, genetic transformation and cloning of relevant genes. Due to long generation time and cross pollinated nature of the species, genetic enhancement through breeding methods based on the development of pure lines, hybridization and progeny testing is desirable, though it needs a longer time period. In view of increasing destabilization of agriculture due to water scarcity and need to gainfully use saline and sodic lands, the present project was focused on developing clones of *Jatropha curcas* and agrotechnologies for such soils.

Survey was conducted and a number of plants were identified from natural stands of *Jatropha* from different parts of the country for undertaking multilocational trial involving seven institutes and identify location specific accessions. The trees were identified based on the age of the tree, number of branches, inflorescences, seeds per capsule, seed size and seed weight, oil content etc. per plant. Initially cuttings were obtained from 185 (24 elite + 161 native accessions) naturally growing *J. curcas* plants. However, passport data including habitat, type of soil, topography of the land, latitude and longitude was collected for 17 elites and 61 native accessions and submitted to NBPGR, New Delhi for assigning the accession numbers. After assessing the growth and yield performance of all these accessions, following 14 *J. curcas* accessions were short listed which performed better at most of the locations (Table 4.6). Various parameters of these 14 accessions were analysed statistically.

**Table 4.6: 14 common accessions across the locations**

1	CSMCRI-GUJ-Banas-1205-C-1	8	FRI-HP-Shim-0306C-13
2	CRIDA-MP-Jhabua-02-03-JJ-06	9	FRI-HAR-Jhajj-1105-C-24
3	NBPGR-GUJ-SKN-0605-Hans	10	NBPGR-RJ-Rajsam-0905-C-15
4	AFRI-TN-Coimb-0206-C-6	11	NBPGR-UA-Paur-0306-C-9
5	AFRI-KER-Palak-0206-C-1	12	NBPGR-CHH-Bilas-0306-C-30
6	CRIDA-MP-Dhar-1105-C-4	13	RRL-AP-Papum-1105--C-1
7	CSMCRI-GUJ-Panchm-0106-C-7	14	RRL-MNP-Imph-1005C-1

It was observed that all the accessions performed more or less uniformly for test weight and oil content. Variation was very high in seed yield as compared to other characters. Variance components for various traits presented in Table 4.7 revealed that magnitude of phenotypic covariance was higher than the corresponding genotypic and environmental ones for all characters. Seed yield showed highest GCV (49.69%) and PCV (49.91%). Heritability estimates in broad sense ranged from 0.5 to 1.0 which reflected moderate to high predominance of heritable variation for all the traits. The highest heritability was recorded for seed oil content and very closely followed by seed yield, seed coat and kernel weight with the same value i.e. 0.99. The genetic advance ranged as low as 0.24 for seed yield to 3.44 as highest for seed oil content. CV values were low (2.66-14.36) for all the characters for pooled performance of 14 accessions.

**Table 4.7: Various yield parameters in the short listed 14 accessions growing at CSIR-CSMCRI, Bhavnagar.**

	<b>seed yield (g/plant)</b>	<b>Test wt (g)</b>	<b>seed coat wt (g)</b>	<b>kernel wt (g)</b>	<b>seed oil (%)</b>
Mean	419.10**	50.33**	39.59**	46.13**	28.96**
Maximum	391.33	55.21	52.82	47.18	32.17
Minimum	643.00	52.79	44.00	56.00	33.27
Standard Deviation	202.80	21.04	16.78	19.39	12.00
Genotypic Covariance	43366.43	458.93	295.09	395.65	152.47
Phenotypic Covariance	43752.38	468.65	298.75	399.32	153.06
Environmental Covariance	385.95	9.73	3.66	3.66	0.59
Genotypic Coefficient of Variation	49.69	42.56	43.39	43.12	42.63
Phenotypic Coefficient of Variation	49.91	43.01	43.66	43.32	42.72
Heritability (Broad Sense)	0.99	0.98	0.99	0.99	1.00
Genetic Advance	0.24	1.95	2.49	2.15	3.44

Genotypic ( $r_g$ ), Phenotypic ( $r_p$ ) and environmental ( $r_e$ ) correlation coefficient among different traits in 14 accessions was worked out. In general, the genotypic correlation coefficient values were higher than corresponding phenotypic and environmental correlation coefficient. Highly significant values for genotypic and phenotypic correlation coefficients among various traits indicated high degree of association of traits at both these levels. All the environmental correlation coefficients were non-significant and in some cases, values were negative indicating low influence of environment among various traits.

### **Breeding between diversified accessions**

Twenty crosses were made at CSMCRI from the list of combinations. The number of female flowers ranged from 2-25, while M/F flower ratio in these inflorescences ranged from 7.91 to 28.31. Maximum fruit wt. (28.71g) and seed wt. (18.66g) was recorded in fruits developed from the cross made between NBRI-J-18 (elite) and CSMCRI-OR-GUJ-1205-C-1.



**A view of hybrid plants growing in field**

Total 471 seeds were obtained from all these crosses. Out of this 182 seeds were used for oil analysis and remaining 289 to raise F1s. Maximum oil (40.56%) was observed in the seeds obtained from RRL-AP-Papum-1105-C-1X CSMCRI-OR-Ganj-1205-C-5 cross combination. Plant wise raw data collected for all the F1s along with their respective parental plants were sent to AAU for heterosis analysis.

Relative heterosis and Heterobeltiosis were worked out in all the 20 cross combination for growth characters viz. Plant height, Number of branches, Canopy diameter and Stem girth. Maximum relative heterosis for plant height (21.51%) was exhibited by the cross RRL-AP-Papum-1105-C-1XRRL-NL-MON-1205-C-1 and for number of branches (50.00%) by RRL-ASM-Kurbi-1205-C-6 X CSMCRI-OR-Ganj-1205-C-5. Maximum relative heterosis for Canopy diameter (8.79%) was recorded in cross RRL-AP-Papum-1105-C-1 X RRL-ASM -Golag-1105-C-1and for Stem girth (21.09%) by NBRI-J-18 (Elite) X CSMCRI-OR-Guj- Banas-1205-C-1.

Cross RRL - ASM - Golag -1105-C -1 X CSMCRI -OR-Ganj -1205-C-5 showed maximum heterobeltiosis (7.79%) for plant height, whereas, for number of branches, it was by the cross RRL-ASM-Kurbi - 1205-C-6 X CSMCRI-OR-Ganj-1205-C-5 (39.02%). Cross NBRI-J-18 (Elite) X CSMCRI-OR-Guj-Banas -1205-C-1 recorded maximum heterobeltiosis (15.80%) for stem girth. All the crosses showed negative values for Canopy diameter.

### **Growth and yield performance of hybrid lines derived from F1 cuttings**

In one of the breeding programme, hybrid lines of *Jatropha curcas* were derived from identified *Jatropha* accessions and their performance was observed for two successive

generations. Promising hybrid lines were multiplied through stem cuttings and transplanted in an isolated plot at Bhadraval during Monsoon. Early seedling growth was assessed on approx. 1500 stem cuttings raised from various promising hybrid lines in nursery. Out of eight cross combinations, cuttings raised from hybrids lines namely IC565733×IC565734 and IC565735× IC565739 showed better growth and more branches as compared to other six combinations. Around 74% F1s showed increase in fruit set over their respective F1s. Cross IC565735 × IC565739 showed the maximum fruits (21 numbers per inflorescence) and 180g seed yield per plant. As it was the first year of plants performing in the field, most of the plants could yield between 40 g to 90g of seeds. Few F1s ranged between 108.92-178.47g whereas for parents it was between 89.56-104.75g.

## **B. Agronomic trials**

### **Agronomic trial on Fertilizer requirement:**

A single genotype was used for this trial. The experimental design was RBD with 3 replications and 10 plants per replication with a spacing of 2x2 m. The experiment tested the response of *Jatropha curcas* plants grown on wasteland with different levels of nitrogen (N) and phosphorus (P). A factorial experiment was carried out with four levels of nitrogen(0,30,45,60 kg/ha) and four levels of phosphorus(0,10,20,30 kg/ha). Treatments were applied at planting and one year after planting. Life support irrigation was given as 25 litres per plant whenever required. In the initial phase of experiment various phenological characters like plant height, canopy, stem girth etc. were studied.



It was seen from the Table 4.8 that the 60 kg N ha<sup>-1</sup> applied at planting and one year thereafter, increased height 23%, plant canopy 31 while 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased the

respective parameters by 17 and 24% after 2 years. Nitrogen treatments significantly increased total dry matter accumulation with increasing level of N up to 45 kg N ha<sup>-1</sup> level only while the increase by P fertilizer with increasing level of P was only up to 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The seed yield of *Jatropha* was significantly influenced by application of nitrogenous and phosphate fertilizers.

**Table 4.8: Effect of different doses of Nitrogen and Phosphorus on growth Characters**

Treatments	Plant height (cm)		Plant canopy (cm)		Total dry matter accumulation (kg/ha)	Seed yield (kg ha <sup>-1</sup> )		Oil yield (kg ha <sup>-1</sup> )
	2004-05	2005-06	2004-05	2005-06	2005-06	2004-05	2005-06	2005-06
<b>Nitrogen</b>								
N <sub>0</sub> (0 kg)	87.5b	163.7c	151.3b	352.3c	7740.3c	22.3b	254.4c	80.32c
N <sub>30</sub> (30 kg)	98.0a	184.6b	162.1ab	406.8b	8960.2b	32.2a	332.4b	109.97b
N <sub>45</sub> (45 kg)	104.9a	196.8ab	170.6a	436.8ab	9589.4ab	35.6a	436.9a	141.73a
N <sub>60</sub> (60 kg)	107.1a	201.9a	176.2a	461.8a	10226.4a	34.6a	467.2a	150.91a
<b>Phosphorus</b>								
P <sub>0</sub> (0 kg)	90.9b	169.4b	152.4b	360.3b	8251.3c	23.9b	305.6c	94.61c
P <sub>10</sub> (10 kg)	97.3ab	186.6a	163.3ab	418.4a	8928.3b	31.2a	354.3b	115.20b
P <sub>20</sub> (20 kg)	102.8a	192.7a	169.3a	431.2a	9379.6ab	35.0a	403.7a	131.51a
P <sub>30</sub> (30 kg)	106.5a	198.2a	175.1a	447.8a	9957.0a	34.6a	427.3a	141.62a
S.Em (±)*	2.8	3.8	4.9	10.6	223.2	1.5	15.5	5.19

The treatment combination with combined application of 60 kg N ha<sup>-1</sup> and 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (N<sub>60</sub>P<sub>30</sub>) recorded the highest seed yield of 518.5 kg ha<sup>-1</sup> which was 163.5% higher over absolute control treatment (N<sub>0</sub>P<sub>0</sub>). However, the treatments 30 kg N ha<sup>-1</sup> and 10 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> proved optimum during 2004-05 while corresponding optimums for subsequent year were 45 kg N ha<sup>-1</sup> and 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> treatments.

The oil content of Jatropha seeds ranged from 31.53 to 32.99 depending upon different treatments (data not shown). The oil yield thus obtained differed significantly with varying N and P fertilizer levels. Highest oil yields of 150.91 kg ha<sup>-1</sup> and 141.62 kg ha<sup>-1</sup> were obtained under N<sub>60</sub> and P<sub>30</sub> levels, respectively during 2005-06. But these highest level treatments were on par with corresponding next lower levels i.e. N<sub>45</sub> and P<sub>20</sub> with respect to oil yield realized.

The results of the experiment have shown that fertilization in jatropha was very effective in promoting growth and yield and not fertilizing it at all was detrimental. Nitrogen level of 30 kg ha<sup>-1</sup> and phosphorus level of 10 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> during the first year and 45 kg N ha<sup>-1</sup> and 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> during the subsequent year proved optimum for seed and oil production under semi-arid wasteland of Gujarat. It was observed that the optimum level of fertilizer requirement is dynamic and changes with the age of plantation. It is expected that as the plant grows in future, it being a perennial species, the fertilizer requirement will change necessitating long term fertilizer trial study under different agro-ecological regions.

This experiment on fertilizer trial started in Aug, 2004 was continued with modified treatments. [Fertilizer combinations: N (4 levels, 0, 1, 2 and 3 signifying 0, 40, 80, 120 kg/ha):P<sub>2</sub>O<sub>5</sub> (4 levels 0,1,2,3,4 signifying 0, 15, 30, 45 kg/ha) and blanket application of K<sub>2</sub>O at 20 kg/ha. Cake was applied only in 2008-09 and 2009-10 after 2004-05@ 1 kg/plant = 2.5 t/ha]. In 2008-09, the combination of 40 kg N and 15 kg P in conjugation with 1 kg of Jatropha cake per plant was the optimum dose that gave significantly higher yield of ca. 1.6 t ha<sup>-1</sup> in 2008-09, while the combination of 40 kg N and 0 kg P in conjugation with 1 kg of Jatropha cake per plant was the optimum dose that gave significantly higher yield of ca. 1.7 t ha<sup>-1</sup> in 2009-10. It was apparent that with accumulation of phosphorus in soils upon application of P fertilizers over the period of years, yield response to P decreased and finally no response to phosphatic fertilizer was observed in 2009-10.

### **Agronomic trial on Spacing**

Detailed studies were carried out to determine the effect of spacing on morphological parameters. The data pertaining to changes in height, canopy circumference, canopy depth, basal girth, branching angle, number of primary, secondary and tertiary branches and mean branch length due to different spacing was recorded and are being statistically analysed.

A single genotype was used for this trial. As a basal dose 50 gm Single Super Phosphate (SSP) and 21 gm Urea, 2 kg FYM and 1 kg Jatropa cake was applied per pit (size 45x45x45 cm) at the time of transplantation. Different Spacing viz. 1 x 1 m (S1), 1.5 x 1.5 m (S2), 2 x 1 m (S3), 2 x 2 m (S4) and 3x2m (S5) were taken as treatments. The experimental Design followed was RBD with 3 replications. Life support irrigation was given as 25 litres per plant once in a month.



Effect of various spacing on seed yield and its attributing characters during 2005-06 has been given in the (Table 4.9). It is seen that with increase in spacing, number of capsules per plant, number of seeds per plant and seed yield/plant increased significantly. However, when seen on per unit area basis, the seed yield per hectare decreased with increase in spacing. The spacing did not affect the 100- seed weight significantly. However, it needs to be observed over the coming years whether the same trend is maintained.

**Table 4.9: Effect of various spacing on seed yield and its attributing characters during 2005-06**

Treatment No.	Spacing	No.of Capsules/ plant	No.of Seeds/ Capsule	Seeds Yield/ plant (g)	Seeds Yield (Kg/ha)	100 seeds wt (g)	No.of Seeds/ plant
1	1m x 1m	23.60	2.48	31.87	318.70	53.47	58.49
2	2m x 1m	36.00	2.50	49.68	248.38	54.33	90.28
3	1.5mx1.5m	39.32	2.46	52.43	233.04	54.25	96.57
4	2m x 2m	58.40	2.50	82.26	205.64	56.00	145.81
5	3m x 2m	67.56	2.53	94.23	157.06	58.76	170.74
<b>C.D.</b>		13.74	NS	17.63	72.46	NS	32.72
<b>C.V. %</b>		16.22	1.56	15.08	16.55	4.49	15.46

During 2006-07, though the growth was good but seed yield was very poor and hence yield data are not been given. To ascertain the reasons nutrient analysis both of soil and leaves was undertaken (Table 4.10). It was observed that as plant population increased the N and P contents of foliage decreased, while other nutrients did not follow a consistent pattern. Furthermore, no significant variation in foliage elemental concentrations in response to spacing of *Jatropha* plants was noticed. Analysis of soil samples indicated that as plant spacing increase, the soil N availability also increased significantly. The low density stand (3mX2m) showed 18 per cent more soil N than the densest stand (1X1) due to more utilization of N by plant in higher population. Analysis of soil samples indicated that as plant spacing increase, the soil N availability also increased significantly. The plants with closer spacing i.e. 1m x 1m became very close and faced competition among them and as a result grew tall and did not yield at all.

Treatment	No. of plants/ha	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
		(% )						(mg kg <sup>-1</sup> )			
<i>1m x 1m</i>	10000	1.83	0.18	0.87	3.52	2.03	0.12	5.57	247.67	42.27	10.47
<i>2m x 1m</i>	5000	1.85	0.18	0.85	3.31	2.05	0.11	4.67	242.80	34.40	9.13
<i>1.5m x 1.5m</i>	4444	1.64	0.20	1.00	3.95	2.10	0.12	5.47	281.40	36.40	8.20
<i>2m x 2m</i>	2500	1.93	0.21	0.86	3.65	1.86	0.12	3.80	172.87	39.67	7.60
<i>3m x 2m</i>	1666	1.90	0.22	0.90	3.89	2.07	0.11	3.90	184.93	44.73	7.67
S.E.		0.27	0.02	0.06	0.53	0.16	0.01	0.76	50.20	6.59	1.22
		NS*	NS	NS	NS	NS	NS	NS	NS	NS	NS

**Table 4.10: Nutrient content in plant leaves in spacing experiment.**

Later, a new experiment was designed during 2006 with wider spacing and provision to uproot plants to increase spacing in order to understand the interaction between plant density and seed yield. During 2008-09, maximum yield was obtained in 4x4 m spacing. It is established from the experiment that wider spacing of 4x4 m is suitable for getting higher yields. It is because of very high the demand of sunlight of the plant.

Treatments	Seed yield (g/plant) in 2008-09 (i.e., after 2 years)
2x2	113
3x2	109
3x3	118
4x3	148
4x4	262
S.Em (±)	23
CD (p=0.05)	75
CV%	26.6

### **Utilization of deoiled cake as manure:**

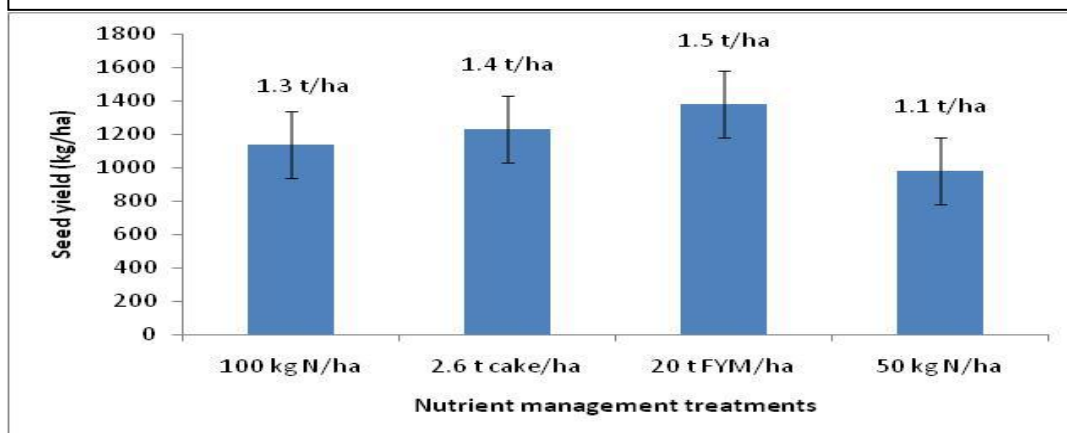
For every metric ton of biodiesel produced, roughly thrice the amount of solid *Jatropha* cake is produced as one of the main by-products. Presence of toxins and anti-nutrients make it unfit for use as animal feed despite that it has high protein content, making its disposal a concern. Different environmentally sustainable ways to effectively utilize *Jatropha* cake are discussed in chapter. *Jatropha* cake is a rich source of plant macro- and micronutrients and was found to effectively substitute for inorganic mineral fertilizers. *Jatropha* plant itself significantly responded well to *Jatropha* cake and was demonstrated to have better fertilizer effect as compared to other cakes (neem, mahua, karanj) on selected vegetable crops.

Thus efforts were made to study the response of *Jatropha* to *Jatropha* cake applied as manure in plantation. The use of *Jatropha* cake @ 4t/ha brought about an increase in yield (865g/plant, N=54) of 30% over the use of sole inorganic fertilizers after 2 years of planting at Nesvad, Gujarat under wasteland condition. In another experiment at Chorvadla, Gujarat, wherein cake and sulphur were applied at different combination and quantities, it was observed that oil content of seeds was significantly increased by S and cake application. Highest oil content (34.88 %) was observed at application of 40 kg S ha<sup>-1</sup>. De-oiled seed analysis showed that N, S, Fe and Zn content were significantly increased by integrated application of S and cake. Height N (3.63%), Fe (45.33 mg kg<sup>-1</sup>) and Zn (32.60 mg kg<sup>-1</sup>) were observed at treatment received 40 kg S and 3.3 t cake ha<sup>-1</sup>, whereas, highest S (0.17%) was observed at application of 40 kg S and 6.6 t cake ha<sup>-1</sup>.

In an another experiment at Gujarat, the application of *Jatropha* cake resulted in ca. 21% improvement in the seed yield against the treatment where equivalent amount of nitrogen (140 kg N/ha) contained in *Jatropha* cake was supplied through inorganic fertilizer in 2010-11. In 2011-12, this experiment comprising application of 50% recommended dose of fertilizer (RDF) or 100% Nitrogen nutrient to *Jatropha* through different sources (urea, cake and Farm yard manure) on equivalent substitution basis was continued, albeit with a lower dose of N (100 kg/ha). The application of nutrients through *Jatropha* cake or Farmyard manure was found at par and slightly better than the treatment applying nutrients solely through inorganic fertilizer (Figure 4.4). The highest seed yield of 1.5 t/ha was recorded in treatment receiving nutrients through Farmyard manure, closely followed by

that (1.4t/ha) found by application of *Jatropha* cake. Soil enzymatic activity was found much better in organic amended soils as compared to urea fertilized soils.

**Fig.4.4:** Effect of different sources of nutrients on *Jatropha* seed productivity



Experiments carried out by CSMCRI during 2005-06, wherein, *Jatropha* cake was applied as basal dose to pearl millet, sesame and cotton @ 1.5, 1.0 and 2.0 t/ha, respectively revealed that there was increment of 11.3%, 14.3% and 15.20% in pearl millet grain-, sesame seed- and seed cotton- yield, respectively over their controls (i.e. without application of cake).

Crop	Application (t/ha)	Yield/ha	
		Control	Treated
Pearl millet	1.5	1.90	2.15
Sesame	1.0	0.56	0.64
Cotton	2.0	1.65	1.90

#### **Changes in soil properties of degraded Entisol with *Jatropha curcas* cultivation:**

Top soil (0-10 cm) mean weight diameter, aggregate stability, bulk density, total and particulate organic matter associated carbon and nitrogen were assessed as soil quality indicators under a 30 months old *Jatropha* plantation in Chorvadla village. The assessment was carried out between December 2006 and January 2007 on an Entisol under rehabilitation with *Jatropha*. Selected treatments were, *Jatropha curcas* with and without mineral fertilizer amendment (both treatment received organic amendment in form of *Jatropha* cake) and, an adjacent native vegetation of *Zizyphus mauritiana* was included as

reference for comparison. Under *Jatropha curcas*, improvement in soil structure was recorded, with 15% increase in mean weight diameter and 6-30% increase in macro aggregate stability. Soil bulk density was reduced by 20% while, fine particulate organic matter associated (POM) carbon and nitrogen increased by an average of 33%. Aggregated silt and clay, which is an index of micro aggregate stability, was however lower in soil under *Jatropha curcas* than the natural vegetation (Table 4.11 & 4.12). Although, there were marked variability (CV of 18-25%) in values of soil quality indicators under *Jatropha* cultivation, there exist a potential for soil quality recovery (particularly, soil structural improvement) under *Jatropha* plantation within a short period of cultivation.

**Table 4.11: Effect of *Jatropha curcas* L. plantation on soil aggregate stability in a degraded Indian Entisol**

Treatments/Quality indices	Mean weight diameter (mm)	Fraction of water-stable macro aggregate	Aggregated silt and clay	Soil bulk density (Mg/m <sup>3</sup> )
Natural vegetation	0.68b	0.52ab	3.91a	1.10
<i>Jatropha</i> (zero fertilizer amendment)	0.84a	0.68a	3.30b	0.81
<i>Jatropha</i> (with nitrogen fertilizer)	0.79ab	0.55ab	3.72ab	0.99
LSD (0.05)	0.103	0.166	0.152	*NS

\*NS- Not significant

**Table 4.12: Effect of *Jatropha curcas* L plantation on soil organic matter dynamics (g kg<sup>-1</sup>) in a degraded land**

Treatments /parameters	Fine POM-C (0.25-0.053)	Total soil nitrogen	Medium POM-N	Fine POM-N
Natural vegetation	4.92b	1.17ab	1.02a	0.45b
<i>Jatropha</i> (zero fertilizer amendment)	9.18a	1.56a	1.19a	1.24a
<i>Jatropha</i> (with Nitrogen fertilizer)	4.66b	0.70b	0.73ab	0.39b
LSD (0.05)	1.68	0.29	0.343	0.463

To ascertain the relation between plant nutrition and plant population, nutrient analysis of both soil and leaves was undertaken. It was observed that as plant population increased the N and P contents of foliage decreased, while other nutrients did not follow a consistent pattern. Furthermore, no significant variations in foliage elemental concentrations in response to spacing of *Jatropha* plants were noticed. Analysis of soil

samples indicated that as plant spacing increase, the soil N availability also increased significantly. The plants with closer spacing i.e. 1m x 1m became very close and faced competition among them and as a result grew tall and did not yield at all.

### **FAME profiling of microbial community structure during *Jatropha curcas* L. residue decomposition**

The amended soil microcosms (amended with jatropha residues: leaves, cake, fruit shell or control) were incubated in the laboratory and destructively samples at 3, 10, 20, 40 or 60 days. At each sampling, the soil microbial communities were profiled by fatty acid methyl ester (FAME) analysis and analysed for the activity of enzymes ( $\beta$  glucosidase, cellulase and urease). The microbial biomass (FAME<sub>tot</sub>), bacterial and fungal FAMEs were significantly higher in cake amended soil compared to other treatments. Averaged across all treatments, FAME<sub>tot</sub>, and fungal and bacterial FAMEs were highest on day 3 and subsequently decreased significantly. Significantly higher fungal to bacterial FAME ratios were found in residue amended soils over control, further significantly highest ratio was in fruit shell amended soil (except day 3). A significant decrease in the stress indicator (saturated to monounsaturated ratio) was found in cake amended soil. Fungal, bacterial and actinomycetes biomarker were significantly correlated with  $\beta$  glucosidase and cellulase activities. The study shows the potential of FAME analysis for profiling of microbial communities and their stress status in soil.

### **C. Life cycle assessment**

The land at all the sites typically had sparse vegetation and the different species of plants found growing were identified, enumerated and removed. The above ground and below ground biomass was determined for carrying out carbon balance and life cycle assessment.

Studies towards life cycle assessment to determine carbon and energy balances were initiated. The species-wise dry biomass (Fig 2) and total carbon (Fig3) displaced on account of uprooting the initial biomass of native vegetation and the *Jatropha* dry biomass accumulation as well as carbon sequestration of *Jatropha* planted after 1 year was determined by destructive sampling and chemical analysis thereof.

At Bhadrawal site, the total carbon in the native biomass amounted to ca. 3.05 t/ha of which 2.33 t/ha was from the above-ground vegetation. The growth of *Jatropha* in Bhadrawal (Fig 4) after one year was significantly higher than the other plantations and the

cumulative carbon sequestered by above and below ground parts of Jatropha was ca. 0.6 t/ha, thus being successful in mopping up significant carbon from atmosphere after one year.

At Kanod, the total carbon in the perennial native biomass displaced amounted to ca. 1.62 t/ha of which 0.77 t/ha was from the above-ground vegetation, while the remaining 0.86 t/ha was attributable to below-ground biomass. Optimally grown Jatropha plants on the same piece of land after 1 year accumulated 0.80 t/ha of dry biomass and sequestered 0.3 t/ha of carbon.

At Neswad site, it is apparent that not much loss in terms of total carbon (0.3t/ha) occurred as there was sparse native vegetation. Jatropha grown in the same piece of land sequestered 0.03t/ha of C in its biomass in the first year. The studies would be continued over the years.

During October 25<sup>th</sup>, 2010, US Biofuels Mission comprising of Scientists/Experts from US Department of Energy (DOE) and various other US National Laboratories like Argonne National laboratory, US, Oak Ridge National laboratory, US, NREL, USA, Yale University, US visited CSIR-CSMCRI, Bhavnagar. The Mission was headed by Mr. Glenn Sonntag, Lead, US-India Biofuels Team, Office of the Biomass Program, United States Department of Energy. Dr. Candace Wheeler, Principal Investigator (General Motors) also accompanied the team. The members of the mission visited various institutional facilities following which a trip to the jatropha plantations sites located at Bhadraval and Nesvad was made. The members of the delegation transplanted planted micro-propagated elite Jatropha saplings at the newly established plantation site of Bhadraval.



## **Improving the productivity of unproductive *Jatropha* plants**

Although process for making EN 14214 grade *Jatropha* methyl ester (biodiesel) capable of running unmodified diesel engines in neat form has been demonstrated, getting high seed yield from *Jatropha* shrub in wastelands is critical to success of *Jatropha* biodiesel. But, low productivity is inherent in many *Jatropha curcas* germplasm and raising large scale plantations using such untested planting material can lead to wasteful expenditure. Unreliable and, poor flowering and fruiting are important factors responsible for low productivity in this species. Though much is known about growth retardants applied to field and horticultural crops, their role in improving seed productivity of *Jatropha* have never been explored. Here we report for the first time that paclobutrazol could be an extremely useful chemical, whose dose and time of application if optimized can significantly reduce unwanted vegetative growth, with concomitant improvement in yield and seed oil content of *Jatropha*. In the year following application of paclobutrazol, unexpected increase in seed yield, as high as 1127% relative to control, was obtained from one such unproductive *Jatropha* germplasm. We hypothesize that low seed production in this species is resultant of excess vegetative growth brought about by unfavourable endogenous hormonal configuration which competes with growth and development of flower, fruit or seed. This undesired physiological state can be reversed by paclobutrazol application to achieve maximum oil yield from this energy shrub that holds great promise in future (*J Plant Growth Regul* 2010).



**Infructescence structure of a representative *Jatropha curcas* at 375 days after treatment showing (left) less fruits in control shrubs and (right) showing compact bunch of more fruits in shrubs treated with paclobutrazol**

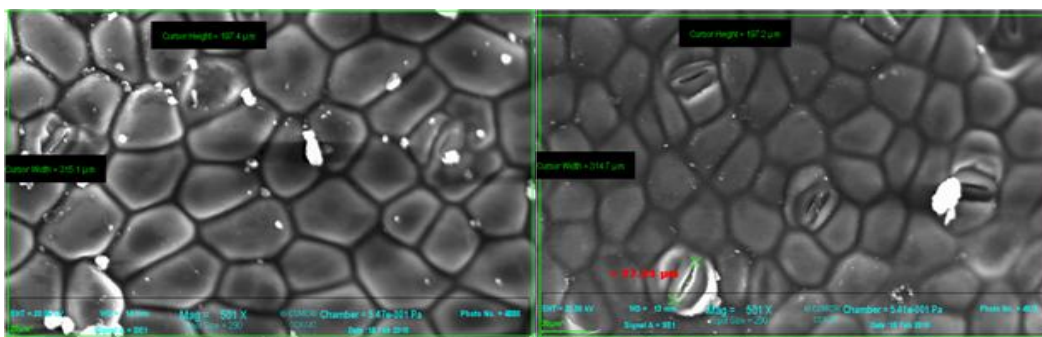
## Improving the productivity of *Jatropha* plants after pruning by use of growth regulators

As reported earlier that seed yield of *Jatropha* drastically decreases upon pruning; therefore, efforts were made to offset yield reduction due to pruning of *Jatropha* as noticed from the results of previous experiments. It was found that paclobutrazol (PBZ) application induced flowering and fruiting in pruned shrubs and partially made up for the yield loss, besides keeping a lower plant height. With PBZ application, the yield reduction compared to non-pruned shrubs was limited to 67%, while 98% reduction in capsule yield was observed in untreated pruned shrubs. Paclobutrazol application along with pruning altered the morphology and vegetative growth of *Jatropha*, producing shrubs with smaller leaves with enhanced chlorophyll and nitrogen content and increased photosynthetic rate. It was found that though more secondary branching is induced in pruned plants, lesser tertiary branches on them results in no additional advantage with respect to total branches. However, the important objective of keeping the branches nearer to the ground is met. The seed oil content due to paclobutrazol applications also differed significantly (*Biomass and Bioenergy 2010*)

**Table 4.13:** Height, leaf chlorophyll index, leaf nitrogen content infructescence size, capsule yield and seed oil content of *Jatropha curcas* in different experimental treatments. (Values represented are mean  $\pm$  standard deviation of 10 replicates).

Treatments	Height (cm)	Chlorophyll Index	Leaf Nitrogen content (%)	No. of fruits set per infructescence in last flush	Total Capsule harvest (kg/shrub)	Seed oil content (%)
No pruning	339.0 <sup>a</sup> $\pm$ 19.1	17.7 <sup>b</sup> $\pm$ 3.3	3.1 <sup>b</sup> $\pm$ 0.4	3.5 <sup>b</sup> $\pm$ 0.7	1.79 <sup>a</sup> $\pm$ 0.30	31.0 <sup>b</sup> $\pm$ 0.4
Pruning + PBZ	258.5 <sup>c</sup> $\pm$ 18.1	29.8 <sup>a</sup> $\pm$ 8.5	3.6 <sup>a</sup> $\pm$ 0.1	9.1 <sup>a</sup> $\pm$ 2.9	0.59 <sup>b</sup> $\pm$ 0.17	33.3 <sup>a</sup> $\pm$ 1.6
Pruning	290.5 <sup>b</sup> $\pm$ 18.5	25.4 <sup>a</sup> $\pm$ 4.5	3.6 <sup>a</sup> $\pm$ 0.2	2.7 <sup>b</sup> $\pm$ 0.5	0.04 <sup>c</sup> $\pm$ 0.02	30.8 <sup>b</sup> $\pm$ 0.9
S.E <sub>mean</sub> ( $\pm$ )	5.9	1.9	0.1	0.5 $\pm$	0.06	0.3
LSD <sub>0.05</sub>	17.0	5.4	0.2	1.6	0.19	1.0

Different letters indicate the values significantly different at the P < 0.05 level;



**Fig 4.5:** Scanning Electron Microscope image showing difference in epidermal cell size, number and stomatal density of abaxial surface of leaf of pruned plants without PBZ treatment (left) pruned plants with PBZ treatment (right)

#### **D. Micro propagation of *J. curcas***

*Jatropha* being wild in nature needs selection of plus trees before embarking upon raising of productive plantations. Rapid production of desirable plus trees with attractive traits are the urgent need in *J. curcas* improvement program. Hence, *in vitro* propagation techniques are of immense importance in this context. At present, there are several protocols and even patent are available for *in vitro* propagation of *J. curcas*, however, benefits of an efficient protocol for commercial scale production are still to be realised. Moreover, being low value crop, the challenges that are faced today by the tissue culture industry are cost competence. Therefore, it is necessary to bring about further improvements in the existing protocols through cost reduction in the inputs, *ex vitro* rooting that would minimize the cost of rooted plantlets and ultimately lead to commercialization of technology.

*In vitro* propagation of *J. curcas* via somatic embryos offers a powerful tool for rapid propagation and needs great attention due certain drawbacks in clonal propagation. *Jatropha* under field trials is found to be prone to a number of diseases and pests particularly viral diseases. Micropropagation technique can be effectively utilized to generate disease free plants. Direct regeneration protocols using leaf explants are being used in generating plants for abiotic and biotic stress through genetic transformation. Now that CSMCRI has several proven germplasm for which data exists on field performance both of mother plants and progenies, the requirement should be to propagate these high quality germplasm in large numbers. Though, the species can be multiplied

through stem cuttings, the process has its own limitations. The development of techniques for in vitro multiplication will be the best procedure for large scale propagation of true-to-type elite plants. Micropropagation of *J.curcas* is performed in following steps (1) Generations of aseptic cultures (2) multiple shoot induction (3) shoot elongation and proliferation (4) rooting and hardening.

Micropropagation protocol of the species was developed using nodal and shoot tips explants. Intensive work is in progress to improve the multiplication rate and rooting percentage from the previously obtained results. The first lot of hardened plants transplanted in 2006 established well on wasteland of Chorvadla and later at Bhadraval which are doing well. The yield data of these plants were very encouraging. The variations recorded in the seed yield of seed raised seedlings can be seen very high as compared to cuttings and tissue culture raised plants.

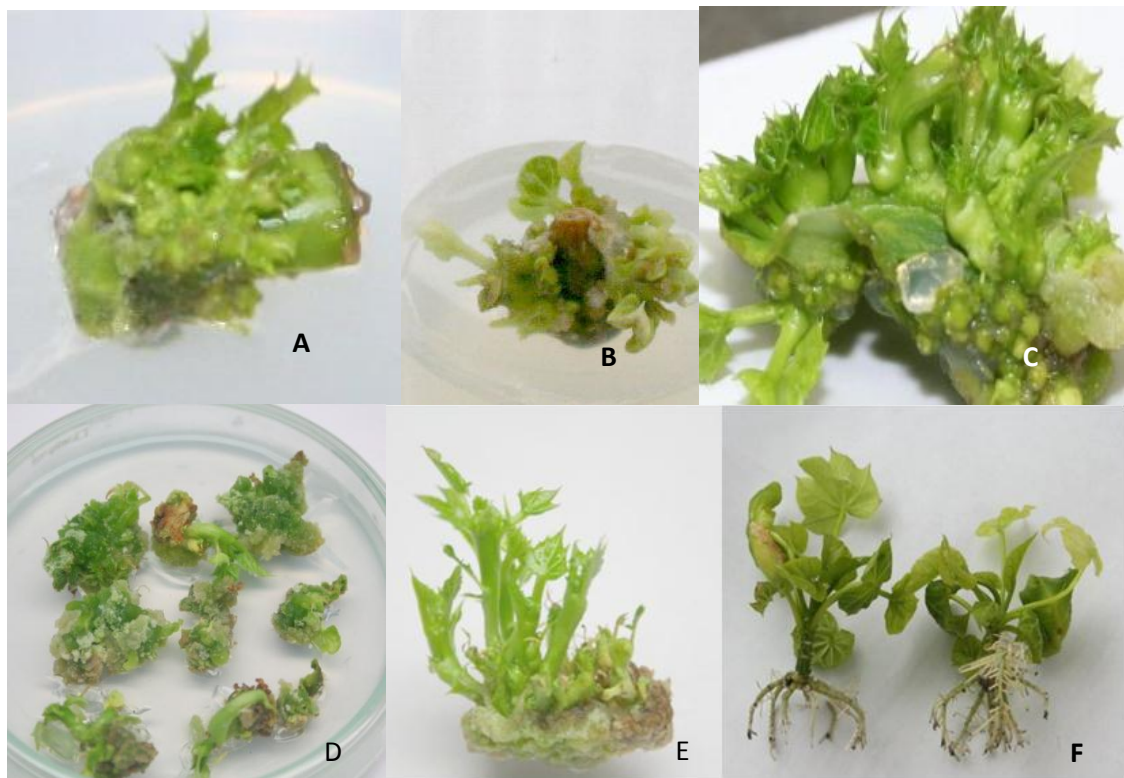
**Table 4.14: Variability of tissue culture/cutting/seedling raised plants**

Accession	Seed yield (g) Average of 20 plants (2007)	CV %	Seed yield (g) Average of 20 plants (2008)	CV %	Seed yield(g)Average of 10 plants (2009)	CV %
Micropropagation	127.1	42.1	266.4	31.2	178.4	31.0
Cuttings	122.7	42.1	190.2	26.1	147.7	31.8
Seedling	72.6	59.9	177.5	31.8	80.0	91.5



Micropropagated plants showed better growth (plant height, number of branches and canopy) and seed yield as compared to seed raised plants and cuttings. It can be seen from the table that variation was maximum in seed raised seedlings.

The high cost is one of the limitations on the practical application of tissue culture methods and research on reducing the production cost has been our primary focus. Ex vitro rooting is a promising method, skipping the phase of in vitro rooting, shortens the micropropagation cycle by 2–3 weeks and reduces cost. Micropropagation protocol of *J. curcas* was developed using nodal and shoot tips explants with 6-12 shoots per culture showed no decline even after a number of sub cultures. Elongated shoots were harvested and placed in sterile sand for ex vitro rooting and 40 % ex vitro rooting was achieved. Work is in the progress to improve ex vitro rooting percentage. Regeneration was successfully achieved from stem, cotyledonary leaf, petiole, callus of leaf and petiole. Regenerated plantlets were successfully established in nursery.



**Figure:** Plant regeneration from (A) petiole (B) stem (C) cotyledonary leaf (D) regenerative callus from petiole (E) elongated multiple shoots (F) in vitro rooted shoots.

## **Studies on micropropagated plants to ascertain their clonal fidelity using molecular markers of *Jatropha curcas* provenances.**

Somaclonal variations are potential drawback when the primary objective is to maintain the advantageous character of the donor plant. Thus it is required that cultures are timely analysed for their clonal fidelity. Microshoots obtained by axillary shoot bud proliferation were used to analyze somaclonal variation. No visible morphological differences were revealed among regenerated microshoots of their respective mother plant. In first experiment, clones obtained from three different generations (culture cycles) of the genotype, CSMCRI-JC2, were screened for presence or absence of any somaclonal variation. The selected 21 primers yielded total of 177 bands in the 2nd subculture with average of 8.43 bands per primer and 174 scorable bands with an average of 8.28 bands per primer in 8th and 16th subculture, respectively. In the 2nd subculture 4 out of 177 bands were observed to be polymorphic. IDT-05, OPN-15 and OPQ-07 produced 1, 1 and 2 polymorphic band, respectively. Among two bands found to be polymorphic in DNA profile obtained by OPQ-07, 1 was characterized by presence of band and the other by absence of band. No polymorphic bands were observed in the 8th and 16th generation. The three primers that revealed polymorphism in the 2nd generation failed to identify any polymorphism in 8th and 16th subculture samples.

### **AFLP analysis**

AFLP method was applied to investigate possible genomic changes in the clones that failed to give any variation in RAPD analysis. AFLP allows, by combination of restriction and PCR amplification, detection of both point mutations at restriction sites and insertions/deletions. AFLP analysis of controls and in vitro sample genomes indicated some sequence polymorphism among samples. Out of 64 combinations of EcoR1/Mse1 primers screened in the present investigation, 15 combinations having reproducible banding pattern in all the genotypes were selected for further analysis. Only two combinations (E-ACT/ M-CAG, E-ACT/ M-CTA) gave 4 polymorphic bands out 631 bands and were observed only in the 2nd generation. No polymorphic bands were observed in the 8th and 16th generation.

The axillary shoot bud proliferation can safely be used as an efficient micropropagation method for long term conservation and mass propagation of selected elite adult tree of *J. curcas*. As there is an increasing interest in *J. curcas* as a source of

bio-fuels, tissue culture of the plant through axillary shoot bud proliferation can play significant role in the endeavor as it allow propagating true to type plants of elite trees for uniform production of bio-fuel and also selecting lines resistant to the various stresses that lower crop productivity.

The effect of various heavy metals on growth and antioxidant enzyme response was evaluated at cellular level to assess whether the plant can survive under metal stress conditions. The metals used in the study were like Cadmium, Copper, Aluminium, Chromium and Nickel. Among the metals tested at 30 ppm, Cadmium was the least toxic whereas nickel was more toxic to the plant at cellular level. Experiment was carried out to assess the effect of nickel on leaf regeneration. Studies showed that the percent regeneration increased at lower concentration (0.01 mM nickel) as compared to control. Concentrations more than 0.01 mM decreased regeneration and complete arrest of regeneration was observed at 1 mM Nickel. It was also shown that nickel toxicity might be either partly or completely due to the alterations in plant DNA sequence which is caused due to presence of higher concentrations and established its mutagenic behaviour in plants. (First report on nickel induced DNA damage in plants- *Biometals* 2010, 23:1149-1158).

### **Protocol for micropropagation of *Jatropha curcas* L. using nodal shoot segments as explant**

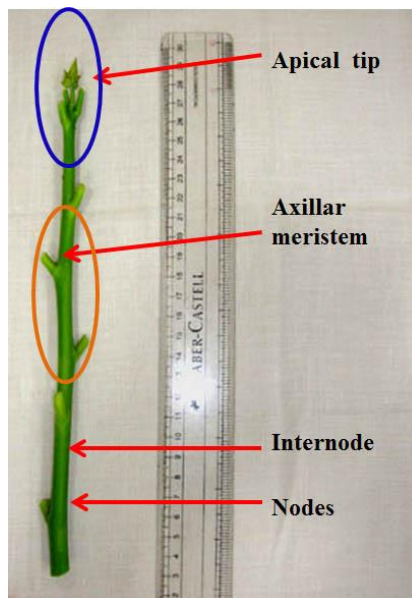
A protocol for micropropagation was developed to generate true to type elite clones of *Jatropha*. The plant being perennial in nature it has its own limitations even in tissue culture like low culture establishment, slow growth, high rate of infection and low rooting percentage. These problems are still being addressed to improve and make the protocol more efficient.

#### **Collection of plant material, preparation and surface sterilization of explants**

- Take the fresh nodal shoot segments from selected plant/genotype and bring to the laboratory. The size of shoots can be up 08-15 inches depending upon the conditions of mother plant and availability of material. Avoid harder portion of stem segment.



- Dress the shoot segments by removing leaves and leaving a small portion of petiole intact; care should be taken of injuries to the stem segments during dressing of materials. Also during leaf removal axillary bud/meristem should not be damaged.



- Dissect/cut the shoot segment with sterile blade in nodal segments of suitable size (approximately one-two nodes per explant).

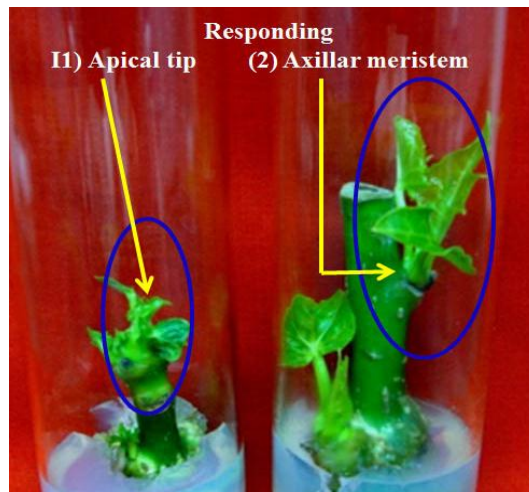


- Wash the explants extensively with running tap water and finally with sterile water to get rid of dust particles or washable microbial contaminants.
- Surface sterilize the explants with aqueous solution of 0.1% HgCl<sub>2</sub> (w/v) for 10-15 minutes. The time duration can be increased or decreased depending upon thickness/hardness/juvenility of explants. Wash the mercuric chloride treated explants 3-4 times with sterile water for 3-5 minutes. Finally allow the surface sterilized explants to air dry under aseptic conditions.
- Cut approximately 0.25 cm portion of explant from top and bottom positions to avoid toxic effect of HgCl<sub>2</sub>. If petiole segments (small portions of petiole) are left with nodal segments before sterilization then cut these (0.2-0.3 cm) portions (without damaging the axillary meristem) using sterile blade before inoculation onto the culture initiation medium. Else explants can be inoculated or cultured directly.



#### **Inoculation of explants to establish multiple shoot cultures**

- Culture the explants vertically maintaining the polarity onto the 30 mL of 0.75-0.8% agar-gelled Murashige and Skoog's basal medium (**culture initiation medium**) supplemented with BAP, IAA and Kinin 35x200 mm culture tubes plugged with non-absorbent cotton (PGR formulation for **culture initiation medium**: 2.0- 3.0 mgL<sup>-1</sup> BAP + 2.0-3.0 mgL<sup>-1</sup> IAA+ 1.0-2.0 mgL<sup>-1</sup> Kin)
- Within 10-15 days 60-80% of explants show bud break.



- After 4-6 weeks; transfer/subculture the cultures onto the fresh MS medium supplied with BAP,IAA. During transfer, the old, dead mother tissues/explant portion are/is cut/removed and the newly differentiated shoot bud/shoots along 0.5-0.75 cm mother explant is transferred onto fresh medium (i.e. repeated transfer). (PGR formulation: 1.0-2.0 mgL<sup>-1</sup> BAP + 0.1-0.2 mgL<sup>-1</sup> IAA).
- Sometimes (30-50% cases) there is differentiation of 3-5 shoot buds per explant; which needs separation of shoot buds during transfer or else they get damaged and die due to overcrowding and unwanted pressure during subculture.



### **Multiplication and maintenance of multiple shoot bud cultures**

- After 3<sup>rd</sup> - 4<sup>th</sup> passages of transfer (4-6 months) of a culture on medium with BAP and IAA (**culture/shoot multiplication medium**); when complete mother tissues are get

detached from newly regenerated shoots; establishment of an axenic culture should be considered (as during these transfers there is loss of cultures due to drying of cultures and contaminations).



- Subculture these cultures regularly after every 4-6 weeks depending upon growth and vigor of cultures on **shoot multiplication medium**. A shoot after 3<sup>rd</sup> passage on same medium attains 5-6 cm height or a culture produces 3-5 shoot buds; which during this period (3 passages of subculture or 4 months) gives multiplication ratio of 1:3.
- During subculture cut 1.0-1.5 cm portion of the elongated shoots (4-5 cm) from bottom position and after removing callus from the stem segment; this along with shoot (upper portion) is put on fresh medium for subsequent growth. The shoot will grow and stem segment will produce 3-4 shoot buds further.
- The cycle is repeated



### ***Ex vitro* root regeneration and Acclimatization**

- The elongated, hardy and mature shoots were put on sterile soil (sand) in 10x15 cm transparent polythene bag and moisten these with Hogland Salt solution and fungicide (Bavistin).
- Cover the polythene bag containing shoots kept for *ex vitro* root regeneration with same size of inverted polythene bag to maintain high humidity around the developing plantlets.



**(These plantlets are incubate in culture room conditions)**

- After 10-15 d; check these plantlets for fungal growth/drying of tissue etc. and if required supply nutrient solutions/water. After 20-30 d make pin holes in the inverted polythene bag to make gaseous exchange with external environment. Subsequently increase number of holes.
- And finally remove the inverted polythene bags and after sufficient growth of plantlets, transfer these plantlets into larger black polythene bags.



**Medium details:** Throughout the protocol, Murashige and Skoog's (MS; Murashige and Skoog, 1962) basal medium was used. Medium was supplemented with 3.0% (w/v) sucrose (SRL) and gelled with 0.75% agar (S.D. Fine). The pH of the medium was adjusted to  $5.7\pm 0.1$  and sterilized for 15 min at  $121^{\circ}\text{C}$  and 15 psi.

**Culture conditions:** The temperature was maintained at  $24\pm 2^{\circ}\text{C}$  in culture room and light was provided for 16 hrs using white cool fluorescent tubes (Philips India Ltd). Starting from inoculation to *ex vitro* root regeneration, cultures were maintained in culture room. Once root regeneration was confirmed these were shifted slightly at higher temperature outside culture room.

## **E. Molecular biology studies**

### **Genetic diversity studies among *Jatropha curcas* germplasm in Neswad field using RAPD**

Seventeen different germplasm of *J. curcas* namely Shyamlaji, Ranpur, Raj-6, KGR, non -toxic, Raj-4, Raj-8, Amblimal, Shakthipith, PCM, CP-9, Buret, Zanjmer, CP13, Chikhli, Zankhvav, Akola and Nesvad were used for the analysis of genetic relatedness among themselves. Among these, overall percentage of Polymorphism was found to be 69% and genetic similarity was 0.6. The highest polymorphism was found between KGR and non- toxic (48.7%).

### **Studies of environmental influence on epigenetic changes among elite germplasm of *J. curcas* using methyl sensitive AFLP**

It is conceivable that epigenetic alleles (epi-alleles) in the form of altered DNA methylation patterns are important in genome evolution. Studies were carried out to assess the methylation polymorphism of the germplasm cultivated in geographically different locations i.e. different states. Three different germplasm (CP-9, CP-13 and CP-17) of *J. curcas* cultivated in duplicates both at Gujarat (Chorvadla) and Orissa (Berhampur) were checked for the epigenetic changes. The percentage of methylation among CP9, CP13 and CP17 were found to be 36%, 40% and 70% respectively. The percentages of methylation polymorphism were found to be 69% (CP-9), 66% (CP-13) and 96% (CP-17). CP-13 was found to have less percentage of methylation polymorphism (66%). This can be helpful in generating the stable population which can be cultivated in multiple geographical locations without affecting the yield attributes.

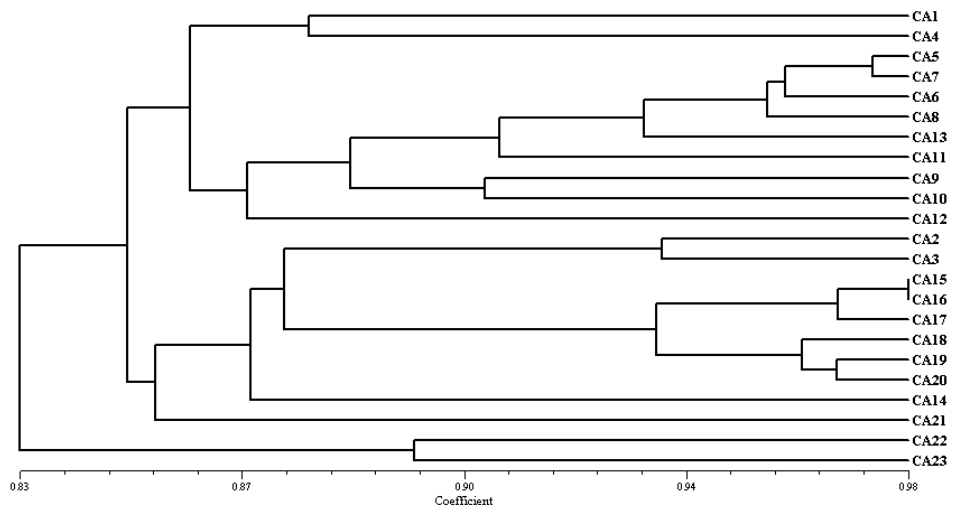
### **Molecular characterization of CSMCRI accessions of *Jatropha curcas* through RAPD and SSR markers.**

The PCR based DNA fingerprinting namely Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) analysis was carried to analyze the molecular relatedness among 23 high yielding plants of selected cultivars collected from distinct geographical area whose performance was evaluated in the field. In present study overall PP was found to be 54.11% through RAPD analysis; whereas, mean PP given by primers studied was observed to be 52.55%. The mean GS among accessions was found to be 0.911 through RAPD which was supported by mean genetic similarity obtained through

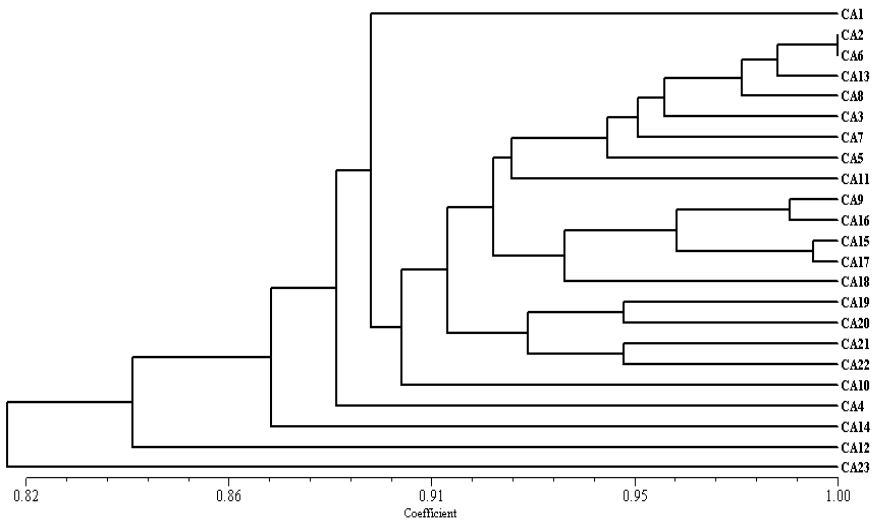
SSR markers (0.90). CA15 has shown maximum similarity to other cultivars both through RAPD and SSR analysis. Both RAPD and SSR dendrograms generated using UPGMA for 23 cultivars/accessions has shown good correlation to each other. CA23 got separated from other cultivars in the cluster analysis showing its distinctness with other cultivars through RAPD analysis. Similar result was obtained through cluster analysis of the data generated by SSRs. Both in RAPD and SSR dendrograms cultivars CA6, CA7, CA8, CA11 and CA13 got clustered together in major cluster showing close relationship with each other, whereas CA5 got separated from the cluster showing its distinctness from rest of the 5 cultivars. In minor clusters CA15, CA16, CA17 got grouped together in one cluster. CA19 and CA20 got grouped in another minor cluster both in RAPD and SSR dendrograms showing closeness to each other (Fig 4.6 and Fig 4.7). The present study of genotypic analysis among various cultivars through RAPD and SSR markers have shown the diverged ones which can be useful in further breeding programs for generation of hybrids and in maintenance of elite genetic stocks. CA23 which was found to be most diverge from the other cultivars can be used for further breeding programs to produce hybrids with diverge characters. The cultivars present in different cluster can be used for the generation of hybrids with efficient characters, for creation of mapping population and linkage analysis and QTL analysis for improvement of various yield attributing characters of *Jatropha*.

**Table 4.15: Accession numbers of *J. curcas* germplasm studied for molecular characterization**

S.NO	ACCESSION NAME / NUMBER	ABBREVIATIONS USED	S.NO	ACCESSION NAME / NUMBER	ABBREVIATIONS USED
1	IC565730	CA1	13	SKNJ-7	CA13
2	IC565731	CA2	14	SKNJ-8	CA14
3	IC565732	CA3	15	SKNJ-9	CA15
4	IC565733	CA4	16	SKNJ-10	CA16
5	IC565734	CA5	17	SKNJ-11	CA17
6	IC565735	CA6	18	SKNJ-12	CA18
7	IC565736	CA7	19	KOTESHWAR	CA19
8	IC565737	CA8	20	AMBAJI	CA20
9	IC565738	CA9	21	PANCHMAHAL	CA21
10	IC565739	CA10	22	RANPUR-F	CA22
11	SKNJ-1	CA11	23	KUMBHARIA	CA23
12	SKNJ-2	CA12			



**Fig 4.6:** Dendrogram showing the genetic relatedness among the *J. curcas* accessions using RAPD.



**Fig 4.7:** Dendrogram showing the genetic relatedness among the *J. curcas* accessions using SSR.

## Novel marker Isolation and genetic diversity studies

The earlier studies on inter- and intraspecific genetic diversity of *Jatropha* found low levels of variation based on surveys of random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers.

Thus, Isolation of SSR markers was undertaken from six different species of *Jatropha* and 12 microsatellite (SSR) markers (Table 9) isolated from *Jatropha curcas* were checked for cross species (*J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, *J. podagrica* and *J. tanzorensis*) amplification. A minimum of eight and a maximum of 11 loci were successfully amplified from each species demonstrating narrow genetic diversity among the species. Within *J. curcas*, observed and expected heterozygosity ranged from 0.94 to 0.54 and from 0.95 to 0.56, respectively. Of the 12 loci, five showed significant deviation from Hardy–Weinberg equilibrium. There was no significant linkage disequilibrium detected between any of the loci.

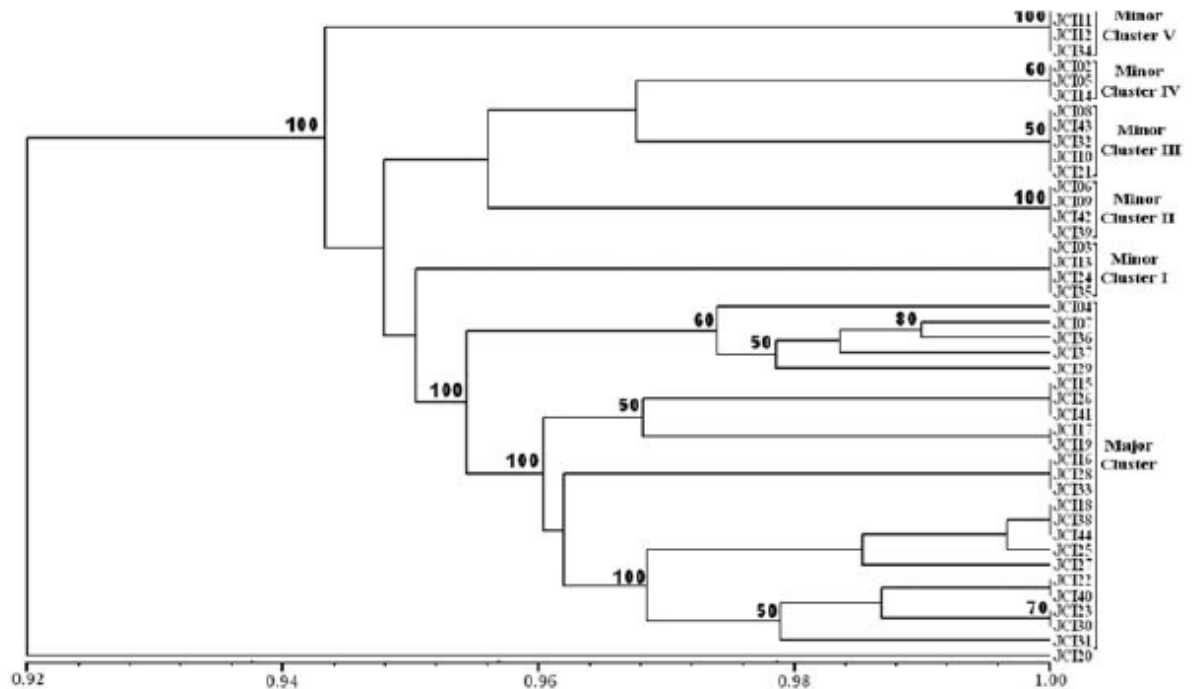


These loci are potentially useful for investigating population processes, genetic structure, population genetic studies across this set of six species of *Jatropha*, and may also be useful in other *Jatropha* species.

Novel microsatellites were isolated using FIASCO by dual probe enrichment technique from *J. curcas* L. and genetic equilibrium and diversity of 44 germplasm collected from distinct geographical areas in India was checked using isolated microsatellites (Fig 10). The overall efficiency of the enrichment of microsatellite by dual probe in the present study found to be 54% and among the sequences obtained the percentage of sequences having suitable flanking regions for the primer designing was found to be 89.58%. The mean co-efficient of genetic similarity (CGS) was found to be 0.97. The overall diversity obtained by microsatellites was found to be low in comparison with the diversity reported by multilocus markers systems; however, good allele polymorphism was observed. The overall dendrogram of microsatellite analysis resulted in

random clustering of germplasm and not in accordance to geographical area of collection (Fig 4.8).

**Allele polymorphism among 44 accessions by isolated SSR JCPS20**

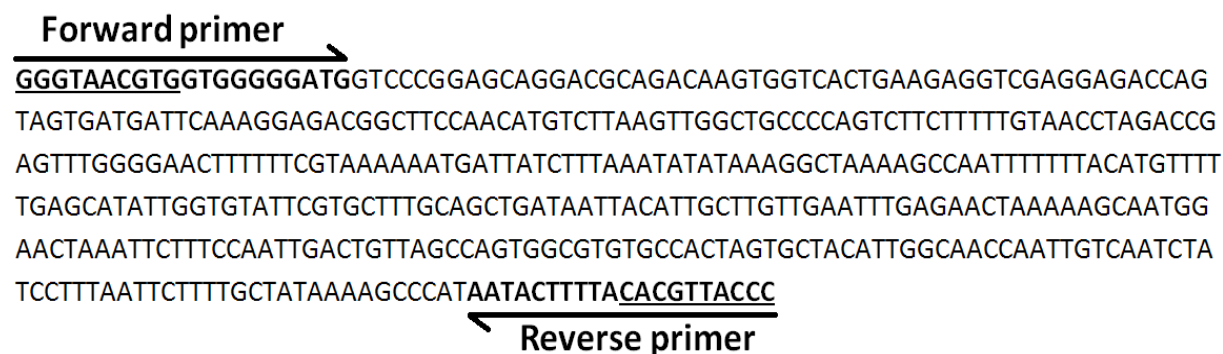


**Fig 4.8: Dendrogram generated using UPGMA for 44 germplasm of *J. curcas* based on allele polymorphism obtained by 49 novel microsatellite loci.**

**Development of SCAR Marker Specific to Non-Toxic *Jatropha curcas* L. and designing a Novel Multiplexing PCR along with nrDNA ITS Primers to circumvent the false Negative detection**

Development of molecular marker will enable to differentiate non-toxic from toxic variety of *J. curcas* in a mixed population. In the present study, the efforts were made to generate the specific SCAR marker for toxic and/or non-toxic *J. curcas* from RAPD markers. Among the markers specific for toxic and non-toxic varieties, four were selected, purified, cloned, sequenced, and designed primers out of which one set of primers NT-JC/SCAR I/OPQ15-F and R was able to distinguish the non-toxic with toxic *Jatropha* by giving expected 430 bp size amplification. Furthermore, novel multiplex PCR was designed using the nrDNAITS primers to overcome the false identification. Present work also demonstrates utility of the conserved regions of nrDNA coding genes in ruling out the

artifacts in PCR-like false identification frequently occur in SCAR due to various reasons. The specific SCAR markers generated have applications in quality control for selective cultivation of non-toxic variety and will also assist in breeding and molecular mapping studies.



*Nucleotide sequence of RAPD amplicon specific to non-toxic J. curcas (NT-JC/SCAR I/OPQ15); arrows represent forward primer (NT-JC/SCAR I/OPL15-F) and reverse primer NT-JC/SCAR I/OPQ15-R). the underlined sequence belongs to the oliadecamer primer*

**Figure 22:** Sequence of non-toxic specific SCAR marker.

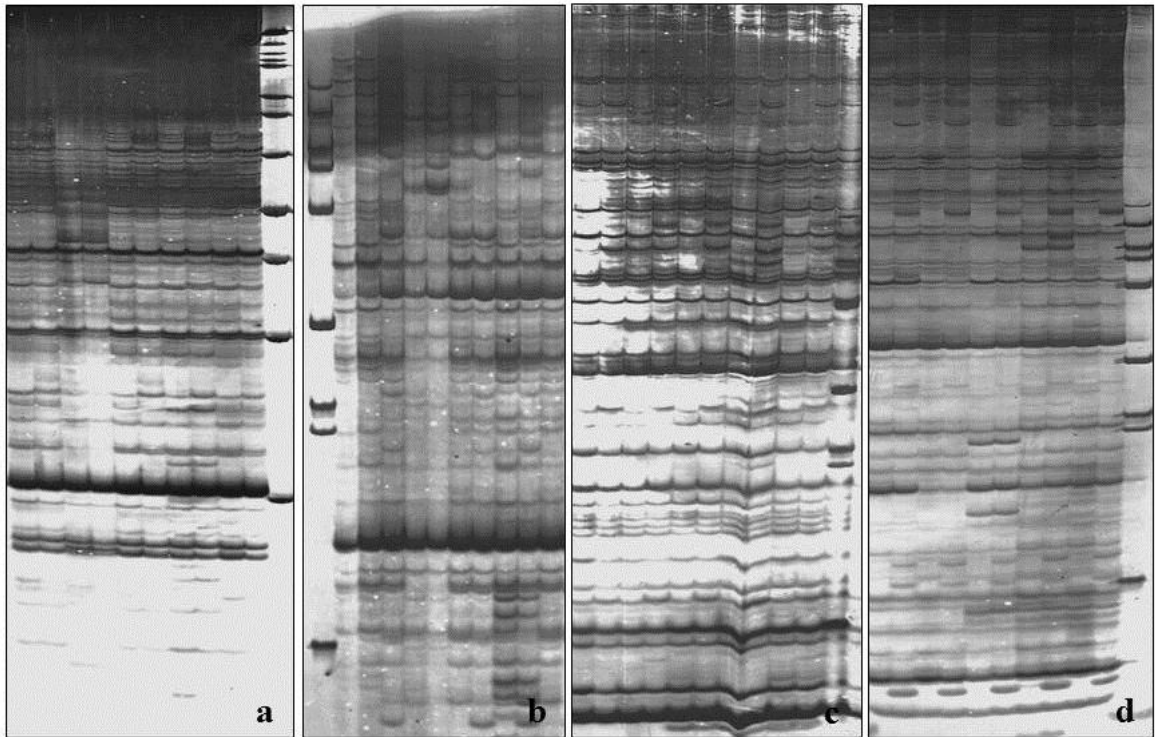
### **Molecular Characterization and Comparative Diversity Analysis using Dominant and Codominant Markers among Elite Germplasm of *Jatropha curcas* L.**

The PCR based DNA fingerprinting namely Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) analysis was carried to analyze the molecular relatedness among 23 high yielding plants of selected cultivars collected from distinct geographical area whose performance was evaluated in the field. In present study overall PP was found to be 54.11% through RAPD analysis; whereas, mean PP given by primers studied was observed to be 52.55%. The mean GS among accessions was found to be 0.911 through RAPD which was supported by means genetic similarity obtained through SSR markers (0.90). SKNJ9 has shown maximum similarity to other cultivars both through RAPD and SSR analysis. Both RAPD and SSR dendrograms generated using UPGMA for 23 cultivars/accessions has shown good correlation to each other. KUMBHARIA got separated from other cultivars in the cluster analysis showing its distinctness with other cultivars through RAPD analysis. Similar result was obtained through cluster analysis of the data generated by SSRs. Both in RAPD and SSR dendrograms cultivars CP9, CP13, CP17, SKNJ1 and SKNJ7 got clustered together in major cluster showing close relationship with each other, whereas KGR got separated from

the cluster showing its distinctness from rest of the 5 cultivars. In minor clusters SKNJ9, SKNJ10 and SKNJ11 got grouped together in one cluster. KOTESHWAR and AMBAJI got grouped in another minor cluster both in RAPD and SSR dendrograms showing closeness to each other. The present study of genotypic analysis among various cultivars through RAPD and SSR markers has shown the diverged ones those can be useful in further breeding programs for generation of hybrids and in maintenance of elite genetic stocks. KUMBHARIA which was found to be most diverged from the other cultivars can be used for further breeding programs to produce hybrids with diverge characters. The cultivars present in different cluster can be used for the generation of hybrids with efficient characters, for creation of mapping population and linkage analysis and QTL analysis for improvement of various yield attributing characters of *Jatropha*.

#### **Physio-biochemical and molecular changes in *Jatropha* associated with salinity:**

To study the physiological and biochemical/molecular aspects in *J. curcas* associated with salinity stress, seedlings of elite accession were generated in root trainers using perlite; and basal salt mixture (Hogaland Salt Mixture). Attempts were made to study the methylation of DNA in whole genome and polymorphism in methylated DNA in root and leaf tissues of *Jatropha curcas* L. seedlings subjected to different levels of salinity stress for 24 hrs. The genomic DNA extracted from sample tissues were subjected to methylation sensitive amplified polymorphism (MSAP) study using 10 selective primers (Fig 4.9). In root and leaf tissues 70.17% and 58.63% methylation was observed respectively; while 99.44% and 90.86% polymorphism was observed in methylated bands in root and leaf tissues respectively. The percentage methylation and methylation polymorphism in roots of plants treated with different salinity was found in order of 50 mM < 100 mM < 75 mM < 25 mM and 25 mM < 75 mM < 100 mM < 50 mM respectively when compared with control. Similarly percentage methylation and methylation polymorphism in leaves of plants treated with different salinity was found in order of 50 mM < 75 mM < 100 mM < 25 mM and 100 mM < 25 mM < 75 mM < 50 mM respectively when compared with control. Regarding physiological aspects related to salinity stress in the species, relative growth of plants, relative water content, relative growth in mass, chlorophyll index, net photosynthesis rate, internal carbon dioxide concentrations, leaf conductance to water vapor, transpiration and water use efficiency were also determined/studies.



**Fig.4.9:** MSAP fingerprints of root (a, b) and leaf (c, d) tissues of *J. curcas* L. seedlings subjected to different levels of salinity with different MSAP primers.

### Enhancing stress tolerance in *Jatropha*

Collar Rot caused by *Macrophomina* causes heavy loss in *Jatropha*. *Macrophomina* is soil borne, anamorphic, ascomycetes fungi. Microsclerotia in soil, seed or host tissue serve as inoculums and plants die/ decay due to production of fungal toxin phaseolinone and production of fungal tissue that plug the host xylem vessels. With an aim at enhancing abiotic and biotic stress tolerance in *Jatropha*, a method for isolation of good quality RNA from *Jatropha* leaves was developed and full length PR genes and WRKY TFs was isolated for enhancing disease tolerance by genetic transformation.

## Genetic Transformation

The study was initiated with an objective of developing a reproducible and efficient protocol for *Agrobacterium* mediated transformation and molecular characterization of transformed plants.

The shoot bud induction directly from *in vitro* and *in vivo* leaf and petiole explant of toxic and non-toxic *J. curcas* could be achieved on MS basal medium supplemented with TDZ or BAP individually and in combination with IBA. It was observed that TDZ was more effective than BAP and the response of explant forming shoot bud decreased with the addition of IBA to TDZ or BAP containing medium due to formation of callus. Regeneration efficiency was also affected by source of explant. The frequency of adventitious shoot regeneration or shoot morphogenesis differed depending on the type of explant. The regeneration efficiency and number of shoot buds were higher in cotyledonary leaf and cotyledonary petiole as compared to leaf and petiole from established shoot culture in both toxic and a non-toxic cultivar of *J. curcas*. The regeneration efficiency and number of shoot buds were higher in leaf than petiole irrespective of nature of explant source. Genotype/cultivar is one of the most important factors affecting regeneration. The regeneration efficiency and number of shoot buds were higher in toxic cultivar as compared to non-toxic cultivar.

MS Medium containing 2-4 mg/L Kn, 1-2 mg/L BAP and 1-2 mg/L NAA was found suitable for shoot proliferation and upon transfer to another medium desired elongation could be achieved. The maximum elongation was obtained in BAP and IAA combinations as compared to BAP and IBA, and BAP and NAA combinations. Significant differences were observed between toxic and non-toxic cultivar however, the differences were not significant with in genotypes of toxic *J. curcas*.

For optimization of transformation protocol, a range of parameters were evaluated. These parameters included the length of the pre-culture period, bacterial growth phase, bacterial cell density, method of wounding of leaf explant, infection time, length of co-cultivation period, pH of the co-cultivation medium and acetosyringone concentration.

Leaf explants pre-cultured for 4 days had the highest transformation efficiency. At the late-log phase, corresponding to  $OD_{600} = 0.6$ , we obtained the maximum rate of

transformation efficiency. The highest transformation efficiency was scored when intact leaf explant was used as compared to injured leaf explant. Optimum results were obtained with a density of  $10^9$  cells/ml. With increasing durations of incubation period as tested for explant infection with diluted *Agrobacterium* culture, the maximum transformation frequency was recorded with 20 min long treatment at a bacterial cell density of  $10^9$  cells/ml after 4 days of co-cultivation. Co-cultivation medium pH of ca. 5.6 was found to be the best with respect to transformation efficiency. At 100  $\mu$ M of acetosyringone maximum rate of transformation was obtained. Gene integration was confirmed by PCR, Dot Blot and Southern hybridization while the expression of gene was confirmed by GUS assay. After transformation, regenerated putative transformed buds could be proliferated on M.S. Medium containing Kn, BAP and NAA and upon transfer to elongation medium desired shoot was obtained. About 40% rooting was observed on half strength MS medium with activated charcoal when shoots were pulse treated with MS media supplemented with 2% sucrose and IBA, IAA, NAA and 0.25 mg/L. After 6-8 weeks, 50-60% of plants survived.

The possibility of *Agrobacterium*-mediated genetic transformation using leaf explants of *J. curcas* has already been demonstrated. The genes NTF2 and Profilin were obtained from ICGEB, New Delhi and In vitro leaf was co-cultivated with *Agrobacterium* strain LBA 4404 having gene NTF2 and Prof1 for different days (1-5 days), than they were shifted on regeneration MS solid medium supplemented with cytokinin, 3% sucrose, 0.8% agar. Intact leaf explant was inoculated horizontally on medium surface in culture vessel. After 45 days leaf was shifted on shoot elongation medium supplemented with cytokinin in combination with auxin.

Shoot elongation was better in culture with gene Prof1as compared to NTF2. Elongated shoots were transferred on selection medium supplemented with hygromycin (5-7mg/L). Shoots in control dried within 10-20 days. After 30 days leaves from selection medium were used for histo-chemical GUS assay. GUS expression was scored in the leaf cocultivated in *Agrobacterium* strain LBA 4404 having gene Prof.1

## Extension and Training:

Two workshops were arranged on 30<sup>th</sup> March 2006 and 18<sup>th</sup> March 2007 at plantation site in village Chorvadla, Gujarat to provide the training to the local farmers regarding cultivation of Jatropha. Nearly 50-60 farmers were invited from the neighbouring villages for the purpose. Farmers were provided guidance regarding raising nursery, its maintenance, transplantation of seedlings in the field, maintenance of plantation, pruning and harvesting etc.



Farmers were explained the importance of Jatropha and its cultivation in their fields and neighborhood. They were also explained the importance and use of by-products and demonstrated use of biodiesel for running the tractor which is being used at the farm. A write up giving all the details of Jatropha was also provided to among them for their future use.

Based on the results of the experiments, cuttings of elite germplasm, identified by CSMCRI, were provided to ICRISAT, Hyderabad which were subsequently raised in Andhra Pradesh (Figures).



**Cuttings of elite germplasm being provided to ICRISAT by CSMCRI (left) Jatropha plantation raised at ICRISAT, Hyderabad (A.P.) with elite material given by CSMCRI (right)**

Our studies since 2001 have enabled us to identify a few good accessions. Based on the multi-locational trials the relatively better initial performance has been validated and one accession (CP-9/IC 565735) has been identified by MNRE for cultivation in Rajasthan. Accordingly this accession was mass propagated and its cuttings were sent to Rajasthan for large scale plantation from CSMCRI field unit at Berhampur, Orissa.



**Rooted cuttings of CP-9/IC 565735 being sent for cultivation in Rajasthan**

During late 2008, seeds of our elite accession (IC565735) were provided to Dr. Ahmed Al-Busaidi, College of Agricultural & Marine Sciences, Sultan Qaboos University, Oman to evaluate the ability of Jatropha to grow under adverse conditions of Oman. The experiments were initiated during summer 2009 to assess the performance and survival under saline irrigation (3 & 6 dS/m) and heat stress condition. The plants survived even when irrigated with saline water up to 6 dS/m and there was accumulation of salts on the oil surface. The plants planted out door in the field also started bearing fruits from subsequent year.

S. No.	Funding agency	Project Title	Year of starting/ ending	Total amount sanctioned (lakhs)
1.	UNDP-TMOP	UNDP umbrella project of GOI on NTO plant species suitable for wasteland ( Jojoba and Jatropha )	1996-1999	52.00
2.	GSFC Foundation, Vadodara	Studies on Halophytes and their Improvement.	2001-2003	50.00
3.	Govt. of Gujarat, Gandhinagar	Development of Biodiesel /Biomass from <i>Jatropha curcas</i> .	2004-2008	15.00
4.	CSIR, New Delhi (NMITLI)	Genetic Improvement of <i>Jatropha curcas</i> for adaptability and oil yield.	2005-sept.2012	127.00
5.	Daimler Chrysler	Biofuels from Eroded Soils in India Phase I using <i>Jatropha</i> plants. Phase II	2003-2005 2005-2007	\$ 402230
6.	General Motors	Determination of the economic viability & technical feasibility of commercial <i>Jatropha curcas</i> production for generation of <i>Jatropha</i> oil as bio-fuels feedstock from wastelands.	2010-2014	\$ 939408 (Total) \$ 626353 (Up to 2012)
7.	MNRE	Large scale micro propagation of elite genotypes of <i>J. curcas</i> .	2010-2013	181.70
8	Kalpasar Deptt., Govt of Gujarat, Gandhinagar	Survey and assessment of Mangrove population of Gulf of Khambhat.	2009- cont.	9.38
9	CSIR LSF project	Multi-institutional demonstration trials showing efficacy of liquid seaweed sap from <i>Kappaphycus alvarezii</i> and <i>Gracilaria edulis</i> on different crops	20011-2013	1161
10	GM India	Developing 2 acre <i>Jatropha</i> plantation termination of the economic viability & technical feasibility of commercial <i>Jatropha curcas</i> production for generation of <i>Jatropha</i> oil as bio- fuels feedstock from wastelands	2010-2013	Ca.25.00

**Projects in which involved as Principal Investigator or as team member**

## **Consultancies in which involved as a team member**

- a. Mr M.K. Kotecha, Kotecha Charitable trust, Rajkot, Gujarat
- b. M/S Kamdar cement Company, Veraval, Gujarat
- c. Mr. Narendra Punetar, Sanjay Baug, Vill. Dhandhalpur, Dist. Surendranagar, Gujarat
- d. Mr. Vasanjibhai Furia, Vill. Mota Laiza, Mandvi, Kutch, Gujarat
- e. Mr.M.K.Mahajan, Vill.& Post Hingone, Yawal, Dist.Jalgaon, Maharashtra
- f. M/S Somalinga Jojoba Estate Pvt.Ltd, 1009, RangaiGowder Street, Coimbatore
- g. M/S Pilu Produce Company N.C.kelkar Road, Dadar, Mumbai
- h. Governmant of Tamil Nadu, DPAP project, Ramnathpuram
- i. M/S Gujarat Science foundation GSFC, Vadodara
- j. ILFS, New Delhi
- k. IOC, Paradip, Orissa
- l. Daimler-Chrysler,Germany

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