The present study was undertaken to increase the knowledge and compare the agronomy and physiology of plants as well the biochemical oil characteristics of 24 jatropha accessions collected from various localities of North-East India in the light to contribute for future selection of ideotypes with low undesirable free fatty acids content and potential valorization of secondary oil products (PEs, tocopherols) to enhancing economic viability and sustainability of the jatropha oil-based biodiesel chain. The experiments for this study were conducted at CSIR-North East Institute of Science & Technology (NEIST), Jorhat. For this study 24 accession sources of *J. curcas* L. were used from different locations of North–Eastern states of India.

4.1 Collection and Plantation of *J. curcas* L. accessions

The collected samples of stem cuttings were planted in three replications in the experimental field of NEIST. The detail procedure of plantation was described in the Chapter 3 and shown in the Fig. 3.1. After attaining 1 meter of height by the accession sources in the field the other experiments were carried out periodically after every one year for 3 years and results are presented in tabular and as well as in graphical format separately for each and every parameters studied in this chapter.

4.2 Study of growth, morphological, physiological and flowering parameters

It is obvious that the magnitude of growth and adaptability of all the accessions must not be same as they were collected from different geographical locations. There must be some extent of genetic variation will be present among them. To understand the magnitude of variation, the parameters like height, stem girth, primary branch number and canopy size were studied and the corresponding mean performance were presented in the Table 4.1 and graphical presentation was done in Fig. 4.1 and 4.2 for height and primary branch number respectively. The highest plant height found in the accession *Jc-10* which was of 157 cm and shortest was only of 50cm (*Jc-03*) in the first

year. But till the end of third year scenario became changed and maximum height showed by accession *Jc-16* (203.67 cm) which was followed by *Jc-14* with 198.67cm of height. In the first year highest stem girth was measured for accession no. *Jc-14* i.e. of 13.55cm and it remain the leading accession with highest measure till third year which is of 23.00 cm, whereas the lowest was of 15.38cm found in *Jc-02* in third year. Average highest primary branch no. was found in *Jc-14*, *Jc-18* & *Jc-21* with the measure of 8.67 during first year, but in the third year only *Jc-14* showed the highest primary branch no. which was of 12.67. The lowest no. of primary branch was calculated for the accession nos. *Jc-10* & *Jc-24 i.e.* of only 8.00. Till third year the largest canopy size was found in *Jc-09* with the measure of 187 cm.

In case of flowering parameters the data were recorded for total no. of inflorescence, total no. of male flower, total no. of female flower per accession and ratio between male and female flower per inflorescence. There also significant variation in the data found for all the parameters studied. The corresponding mean values were presented in the Table 4.2 and graphical presentation was done for total no. of inflorescence per accession and total no. of female flower per accession in Fig. 4.3 and 4.4 respectively. In the 1st year the highest no. of total inflorescences was 19 in *Jc-20* & *Jc-04*, whereas in the 3rd year in accession no. *Jc-14* showed highest for the same with 144 no. which was followed by *Jc-03* with 112 no. of total inflorescences. The highest number of male flowers per accession raised from 983 (*Jc-16*) in 1st year to 7482 (*Jc-19*) during 3rd year with CV% 35.68. On the other hand the average of 13.13 in total no. of female flower in the first year became 140.13 till the third year, where the highest was shown by accession no. *Jc-14* with 179.00 and lowest was shown by accession no. *Jc-04* with 67.00. The ratio between the male flower and female flower was ranged from 22.94:1 to 100.87:1 with CV% 37.18 in the first year of observation. But in the third year this ratio was significantly reduced to the range of 7.34:1 to 51.70:1.

Table 4.1. Year wise morphological data (Mean data):

Sl. No.	Accession Code	Height (cm)			Stem Girth (cm)			Branch No.			Canopy Size (cm)		
		1st year	2nd year	3rd year	1st year	2nd year	3rd year	1st year	2nd year	3rd year	1st year	2nd year	3rd year
1.	***Jc-01***	96.05	132.05	176.00	12.46	17.00	20.00	06.00	07.00	11.33	90.00	110.33	149.67
2.	***Jc-02***	96.06	109.67	133.33	8.83	12.98	15.38	08.00	09.75	12.00	102.63	154.00	176.67
3.	***Jc-03***	50.03	98.25	121.00	12.47	17.59	19.57	05.67	07.33	08.33	119.67	142.00	172.50
4.	***Jc-04***	90.00	110.00	151.00	12.00	14.56	16.67	06.00	08.67	10.00	87.00	93.67	127.00
5.	***Jc-05***	84.00	116.75	159.25	11.74	16.63	19.63	08.33	09.00	10.50	97.00	102.00	125.00
6.	***Jc-06***	110.50	132.00	166.00	10.65	18.11	21.23	06.00	07.25	09.50	110.50	134.50	161.83
7.	***Jc-07***	130.00	120.00	169.00	10.82	15.63	17.67	07.50	08.00	10.50	106.55	135.33	172.67
8.	***Jc-08***	102.67	158.33	194.33	13.61	17.07	21.00	04.50	06.93	10.33	122.50	146.33	180.67
9.	***Jc-09***	94.5	119.05	139.00	10.47	13.00	16.50	08.33	09.33	11.00	134.03	161.67	187.00
10.	***Jc-10***	157.00	126.00	179.50	10.53	14.43	17.57	06.33	07.00	08.00	124.05	153.67	183.50
11.	***Jc-11***	96.45	134.50	170.00	8.50	13.35	16.78	07.00	08.00	09.00	103.00	122.00	167.67
12.	***Jc-12***	88.00	123.50	166.00	13.00	17.47	21.47	06.33	08.75	10.67	109.07	120.00	157.83
13.	***Jc-13***	101.67	139.00	176.33	8.80	12.45	17.93	06.00	08.67	11.33	106.00	125.00	143.67
14.	***Jc-14***	114.00	155.50	198.67	13.55	18.37	23.00	08.67	10.63	12.67	126.33	163.75	183.33
15.	***Jc-15***	122.50	133.33	177.00	11.17	14.80	20.83	07.67	10.00	11.50	120.00	145.50	171.83
16.	***Jc-16***	124.00	165.80	203.67	8.50	12.45	18.84	07.00	08.33	10.67	121.00	161.00	178.17
17.	***Jc-17***	115.30	145.05	162.33	11.00	13.25	18.65	07.33	07.75	09.78	57.56	63.67	108.00
18.	***Jc-18***	78.00	128.33	144.33	13.72	14.16	21.16	08.67	09.00	10.67	118.00	131.50	164.33
19.	***Jc-19***	93.00	130.33	149.05	11.35	13.33	17.98	06.67	07.33	09.33	100.50	104.00	129.50
20.	***Jc-20***	110.50	130.75	168.00	11.25	15.10	19.15	06.00	07.75	09.00	102.00	120.00	142.50
21.	***Jc-21***	106.50	114.87	143.33	9.67	13.96	20.56	08.67	09.00	10.75	82.50	95.00	149.67
22.	***Jc-22***	83.03	98.00	127.50	10.56	12.64	16.67	07.67	08.33	09.73	76.50	92.00	132.67
23.	***Jc-23***	87.00	109.25	124.50	12.33	15.16	20.35	07.00	08.55	10.50	96.00	104.00	128.33
24.	***Jc-24***	91.50	104.33	135.06	10.67	13.75	18.00	05.33	06.87	08.00	69.67	98.00	123.67
Mean		103.24	126.44	159.76	11.15	14.89	19.02	06.94	08.30	10.21	103.42	124.12	154.90
SD		20.45	24.65	22.35	01.87	01.88	02.18	01.15	01.21	01.17	23.37	21.18	24.53
CV%		19.80	19.49	13.99	16.77	12.63	11.46	16.57	14.58	11.46	22.60	17.06	15.84
± SEM		04.17	05.03	04.56	00.38	00.38	00.44	00.24	00.25	00.24	04.77	04.32	05.00

SD= Standard deviation; CV= Coefficient of variance; SEM= Standard error of mean.

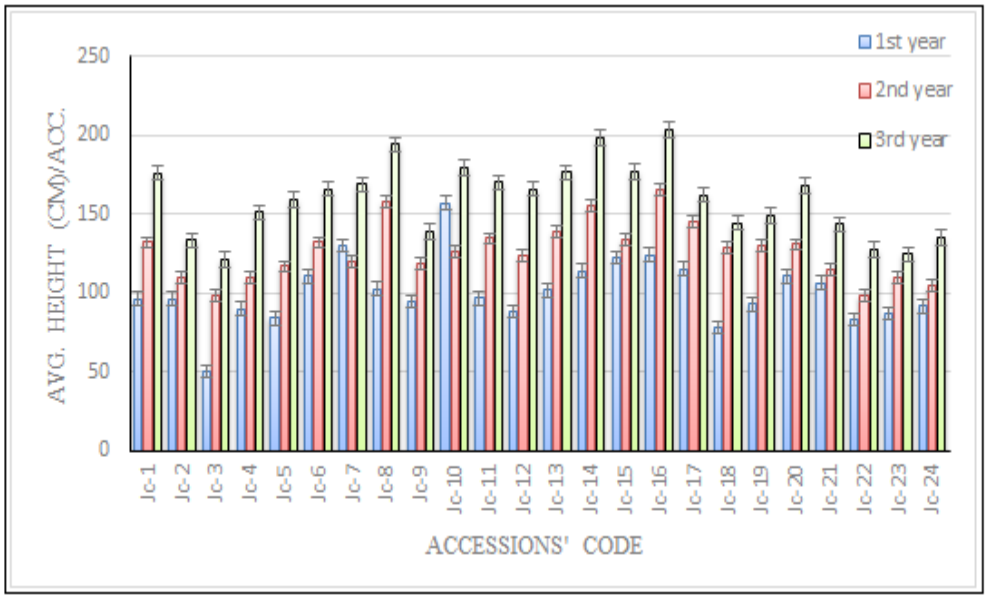

Fig. 4.1: Graph representing the variation of height among the 24 *J. curcas* accessions in three years study.

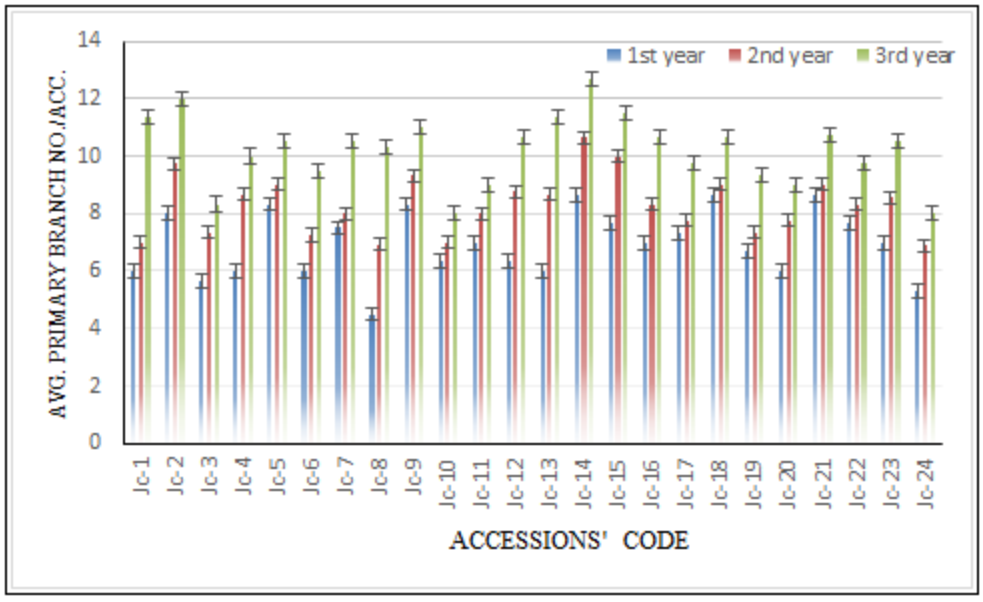

Fig. 4.2: Graph representing the variation in primary branch number among the 24 *J. curcas* accessions in three years study

Table 4.2. Year wise Flowering data:

Sl. No.	Accession Code	Total Inflorescences/Accession			Total ♂ Flower/Accession			Total ♀ Flower/Accession			Average ♂:♀/Inflorescence		
		1st year	2nd year	3rd year	1st year	2nd year	3rd year	1st year	2nd year	3rd year	1st year	2nd year	3rd year
1.	***Jc-01***	10.00	30.00	102.00	353.00	1131.00	3875.00	13.00	39.00	124.00	27.15:1	29.00:1	31.25:1
2.	***Jc-02***	15.00	42.00	73.00	619.00	1579.00	2838.00	14.00	47.00	86.00	44.21:1	33.60:1	33.00:1
3.	***Jc-03***	16.00	32.00	112.00	559.00	1947.00	3339.00	13.00	63.00	131.00	43.00:1	30.90:1	25.48:1
4.	***Jc-04***	19.00	39.00	70.00	483.00	1219.00	2741.00	13.00	35.00	67.00	37.15:1	34.83:1	40.91:1
5.	***Jc-05***	18.00	37.00	86.00	788.00	2270.00	3260.00	15.00	78.00	177.00	52.53:1	29.21:1	18.41:1
6.	***Jc-06***	18.00	37.00	87.00	950.00	2239.00	3039.00	17.00	71.00	151.00	55.88:1	31.68:1	20.12:1
7.	***Jc-07***	17.00	36.00	84.00	350.00	3260.00	2270.00	13.00	77.00	172.00	26.92:1	42.33:1	13.20:1
8.	***Jc-08***	10.00	30.00	87.00	614.00	945.00	2768.00	12.00	35.00	103.00	51.17:1	27.00:1	26.87:1
9.	***Jc-09***	10.00	27.00	97.00	549.00	876.00	3239.00	10.00	28.00	141.00	54.90:1	31.20:1	22.97:1
10.	***Jc-10***	16.00	36.00	95.00	480.00	3437.00	4298.00	18.00	29.00	170.00	26.67:1	118.52:1	25.28:1
11.	***Jc-11***	13.00	48.00	64.00	875.00	2319.00	2270.00	13.00	57.00	173.00	67.31::1	40.60:1	13.12:1
12.	***Jc-12***	14.00	38.00	54.00	413.00	2218.00	3800.00	18.00	44.00	131.00	22.94:1	50.75:1	29.01:1
13.	***Jc-13***	17.00	43.00	107.00	576.00	1570.00	5039.00	12.00	47.00	141.00	48.00:1	33.50:1	35.74:1
14.	***Jc-14***	16.00	45.00	144.00	557.00	2578.00	4996.00	16.00	79.00	179.00	34.81:1	32.55:1	27.91:1
15.	***Jc-15***	15.00	29.00	87.00	516.00	2270.00	3270.00	14.00	68.00	176.00	36.86:1	33.45:1	18.58:1
16.	***Jc-16***	17.00	41.00	91.00	983.00	1319.00	5325.00	15.00	47.00	103.00	65.53:1	28.00:1	51.70:1
17.	***Jc-17***	18.00	25.00	86.00	576.00	840.00	3260.00	18.00	25.00	177.00	32.00:1	33.60:1	18.42:1
18.	***Jc-18***	10.00	34.00	90.00	581.00	1987.00	3710.00	09.00	40.00	97.00	64.56:1	49.49:1	38.24:1
19.	***Jc-19***	13.00	20.00	56.00	787.00	1136.00	7482.00	09.00	18.00	157.00	87.44:1	63.10:1	47.65:1
20.	***Jc-20***	19.00	32.00	50.00	943.00	2227.00	3875.00	16.00	35.00	113.00	58.94:1	63.84:1	34.29:1
21.	***Jc-21***	12.00	36.00	47.00	807.00	2097.00	2797.00	08.00	82.00	111.00	100.87:1	25.66:1	25.20:1
22.	***Jc-22***	10.00	32.00	97.00	530.00	907.00	1939.00	10.00	72.00	141.00	53.00:1	12.60:1	13.75:1
23.	***Jc-23***	09.00	23.00	95.00	500.00	1047.00	1278.00	09.00	26.00	174.00	55.56:1	40.28:1	07.34:1
24.	***Jc-24***	09.00	22.00	64.00	487.00	1021.00	4270.00	10.00	25.00	168.00	48.70:1	40.84:1	25.42:1
Mean		14.21	33.92	84.37	619.83	1768.29	3540.75	13.13	48.63	140.13	49.84:1	39.83:1	26.83
SD		03.38	07.19	21.66	182.07	734.70	1263.55	03.03	19.93	32.75	18.53	20.17	10.77
CV%		23.79	21.19	25.67	29.41	41.55	35.68	23.09	40.98	23.37	37.18	50.64	40.14
± SEM		00.69	01.47	04.42	32.16	149.93	257.86	00.62	04.07	06.68	03.78	04.12	02.20

SD= Standard deviation; CV= Coefficient of variance; SEM= Standard error of mean.

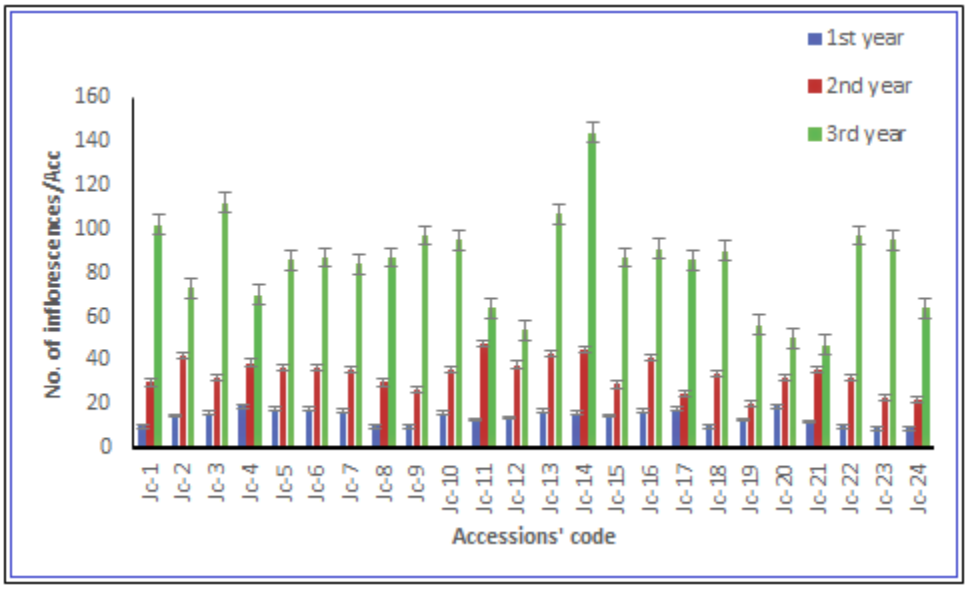

Fig.4.3: Graph presenting year wise total no. of inflorescences per accession for 24 *J. curcas* accessions studied.

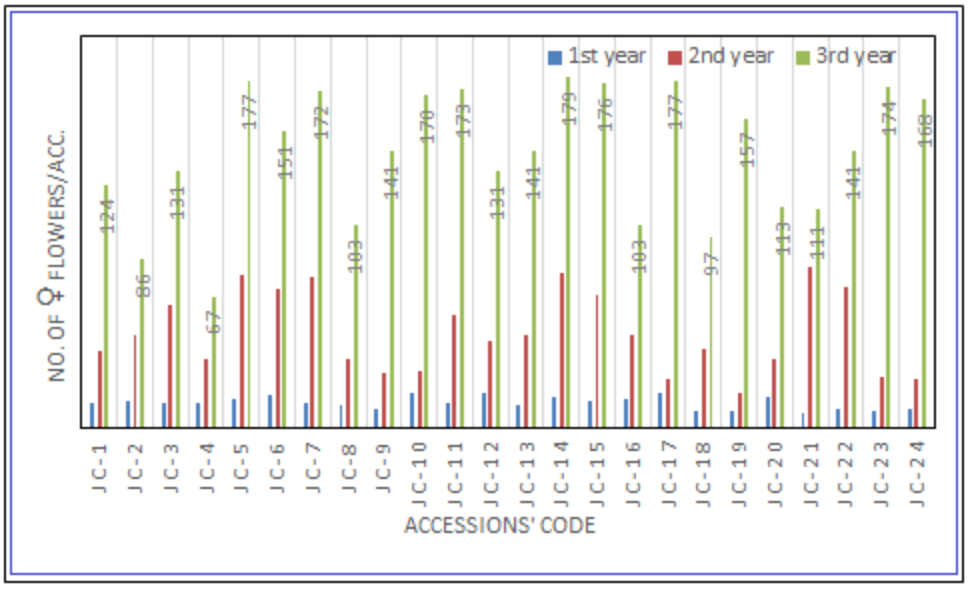

Fig.4.4: Year wise presentation for total number of female flowers per accession of 24 *J. curcas* accessions studied.

Under the physiological parameters the data were recorded for total leaf area per plant, photosynthetic rate per accession, stomatal conductance and transpiration rate per accession. Variation in physiological parameters reflects the extent or seasonal integral of light interception or mineral and water conductance among the accessions which may be directly correlated with seed yield. There was a vast difference between the total leaf area size of first year and third year data. It was ranged from 3687.42 (*Jc-18*) to 5683.49 cm^2/plant (*Jc-14*). Highest photosynthetic rate recorded in the first year was 11.48 µmol CO_2 $m^{-2}s^{-1}$ for *Jc-10*. While in the second year highest rate was recorded in the accession *Jc-08* (13.67 µmol CO_2 $m^{-2}s^{-1}$) and then second highest is of 13.33 for *Jc-04*. But in the third year the highest photosynthetic rate was counted in the accession *Jc-14* (15.50) followed by *Jc-08* & *Jc-09* with the rate of 15.30 & 15.48 µmol CO_2 $m^{-2}s^{-1}$ respectively. The average rate recorded in the third year was of 6.96 µmol CO_2 $m^{-2}s^{-1}$ (±1.09) with CV% 76.58. Table 4.3 showing the corresponding mean values for all the physiological parameters studied. Fig. 4.5 (a, b & c) showing the graphical representation of photosynthetic rate, stomatal conductance and transpiration rate respectively. In case of stomatal conductance there was no significant differences between the measurements of all the three years recorded. From the first year to the third year the average rate increases from 0.37 cm/s to 0.56 cm/s. In the third year *Jc-14* and *Jc-15* both showed highest stomatal conductance of 0.95 cm/s, whereas lowest recorded in *Jc-21* with 0.11 cm/s. On the other hand the maximum transpiration rate in the first year was recorded in the accessions *Jc-09* and *Jc-14* which was of 1.65 mmol/m^2/s with average of 1.33±0.03 and CV% of 11.28. In the second year this measurement was significantly increases to 2.73 mmol/m^2/s in the accession *Jc-14*, where the average was recorded as 1.80 mmol/m^2/s with CV% 23.33. But in the third year this rate was slightly increased upto 2.95 as the highest measurement in the same accession (*Jc-14*). The average transpiration rate measured in the third year was 2.12±0.06 mmol/m^2/s with CV% of 15.09.

4.3 Determination of accession wise seeds yield and oil content

The evaluation and comparison of seed yield and oil content of the accession sources is the main objective of this study. The *J. curcas* plants yield seeds in two season times *i.e*. in spring-summer and in autumn season. Thus the seed yield data had been prepared by combining both the seasons for all the three years. In the first year the highest seed yield of 42.12 gm was shown by accession no. *Jc-10* & *Jc-17*, whereas in the third year *Jc-10* & *Jc-14* showed highest seed yield with more than 389 gm of seeds per accession followed by *Jc-17* with about 387 gm of seeds. Besides these, other two accessions viz. *Jc-11* & *Jc-15* also yielded more than 380 gm of seeds in the third year. The lowest seed yield in the third year was recorded in *Jc-23* which was of only 113.19 gm. Thus the average seed yield was 301.72 gm in the third year.

Oil extraction had been done by using soxhlet method where hexane was used as solvent. For each accession three replicates of oil samples had been extracted and the average of the triplicates was taken as the final. The average 100 seeds weight and oil density was also measured for the oil samples of each and every accession. The average 100 seed weight was recorded as 78.46 gm in the third year, whereas the average oil density was found as 0.92 only. All the corresponding data were presented in the Table 4.4. the estimation of oil%, its density and 100 seed weight were done only in the final year (third year) of this study. The highest oil % was recorded at *Jc-14* with 35.17 which was followed by *Jc-04* and *Jc-10* with more than 33% oil yield and thus the average seed oil was 28.18%.

Pictures in the Plate 4.1 showing the *J. curcas* plant bearing the fruits, some harvested fruits and dry seeds and lastly the transverse section of a fruit where the detail parts of the fruit *viz.* fruit cover, seed coat and the kernel were labelled. The kernel is the major part of the seed where highest percent of crude oil is stored. Then

Table 4.3. Year wise Physiological data (Mean data):

Sl. No.	Accession Code	Total Leaf area (cm^2 $plant^{-1}$)			Photosynthetic Rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)			Stomatal Conductance ($cm\ s^{-1}$)			Transpiration Rate (mmol m-2 s-1)		
		1st year	2nd year	3rd year	1st year	2nd year	3rd year	1st year	2nd year	3rd year	1st year	2nd year	3rd year
1.	***Jc-01***	1912.90	2634.15	4134.11	02.10	03.37	03.73	0.33	0.35	0.39	1.20	2.35	2.50
2.	***Jc-02***	1812.79	3140.83	4340.84	01.02	01.25	01.61	0.31	0.39	0.46	1.35	2.33	2.55
3.	***Jc-03***	1347.89	3127.50	3946.57	03.48	04.67	05.60	0.76	0.78	0.86	1.30	2.15	2.35
4.	***Jc-04***	1490.77	3160.39	5610.90	11.23	13.33	14.96	0.66	0.83	0.86	1.33	1.87	2.00
5.	***Jc-05***	1885.36	3245.67	4901.32	03.73	04.75	06.96	0.39	0.50	0.59	1.35	1.45	2.00
6.	***Jc-06***	1024.76	3144.45	4441.45	01.61	04.50	05.40	0.52	0.65	0.67	1.25	1.37	2.00
7.	***Jc-07***	1466.74	2896.35	4270.17	01.60	03.25	03.89	0.25	0.33	0.41	1.15	1.28	2.00
8.	***Jc-08***	1197.33	3458.75	5438.78	05.40	13.67	15.30	0.66	0.75	0.90	1.50	1.63	2.33
9.	***Jc-09***	1058.52	2549.65	5249.50	10.71	12.25	15.48	0.35	0.45	0.50	1.65	2.00	2.25
10.	***Jc-10***	1346.51	3655.27	5365.21	11.48	13.18	14.71	0.69	0.87	0.93	1.55	2.49	2.87
11.	***Jc-11***	1230.12	3764.74	4064.43	02.39	02.65	03.71	0.11	0.15	0.14	1.30	2.53	2.67
12.	***Jc-12***	1060.73	3543.85	4757.85	01.99	03.27	05.01	0.19	0.25	0.33	1.25	1.94	2.00
13.	***Jc-13***	1740.94	2965.93	4565.97	10.89	12.15	12.87	0.28	0.33	0.44	1.45	1.65	2.00
14.	***Jc-14***	1978.55	3837.45	5683.49	11.27	12.34	15.50	0.47	0.67	0.95	1.65	2.73	2.95
15.	***Jc-15***	1240.56	2953.80	4993.88	05.61	08.56	12.49	0.43	0.65	0.95	1.33	1.93	2.00
16.	***Jc-16***	1691.87	3264.67	4204.66	00.86	01.33	01.61	0.37	0.50	0.68	1.27	1.78	2.00
17.	***Jc-17***	1028.88	2864.33	4602.34	00.75	01.98	02.18	0.16	0.25	0.29	1.37	1.50	1.78
18.	***Jc-18***	1717.59	2767.25	3687.42	02.18	08.67	11.14	0.31	0.33	0.37	1.00	1.35	1.87
19.	***Jc-19***	1962.50	2984.73	4884.80	02.70	04.87	06.70	0.54	0.67	0.77	1.25	1.65	1.93
20.	***Jc-20***	1767.65	2676.45	4116.48	00.49	01.95	02.10	0.17	0.20	0.23	1.20	1.55	1.87
21.	***Jc-21***	1857.54	2194.71	4291.19	00.85	01.00	01.02	0.07	0.10	0.11	1.15	1.30	1.65
22.	***Jc-22***	1987.87	2394.50	4293.06	00.69	01.05	01.48	0.42	0.50	0.58	1.33	1.33	1.67
23.	***Jc-23***	2331.44	3782.57	5287.83	00.83	01.78	02.39	0.29	0.67	0.75	1.25	1.25	1.83
24.	***Jc-24***	1761.98	2574.63	4257.85	00.95	01.00	01.27	0.23	0.25	0.33	1.50	1.75	1.95
Mean		1579.24	3065.94	4641.25	03.95	05.70	06.96	00.37	00.47	00.56	01.33	01.80	02.12
SD		363.09	440.98	553.54	03.91	04.57	05.33	00.21	00.17	00.17	00.15	00.42	00.32
CV%		22.99	14.38	11.93	99.00	80.17	76.58	56.75	36.17	30.36	11.28	23.33	15.09
± SEM		74.10	89.00	112.97	00.80	00.93	01.09	00.04	00.03	00.03	00.03	00.08	00.06

SD= Standard deviation; CV= Coefficient of variance; SEM= Standard error of mean.

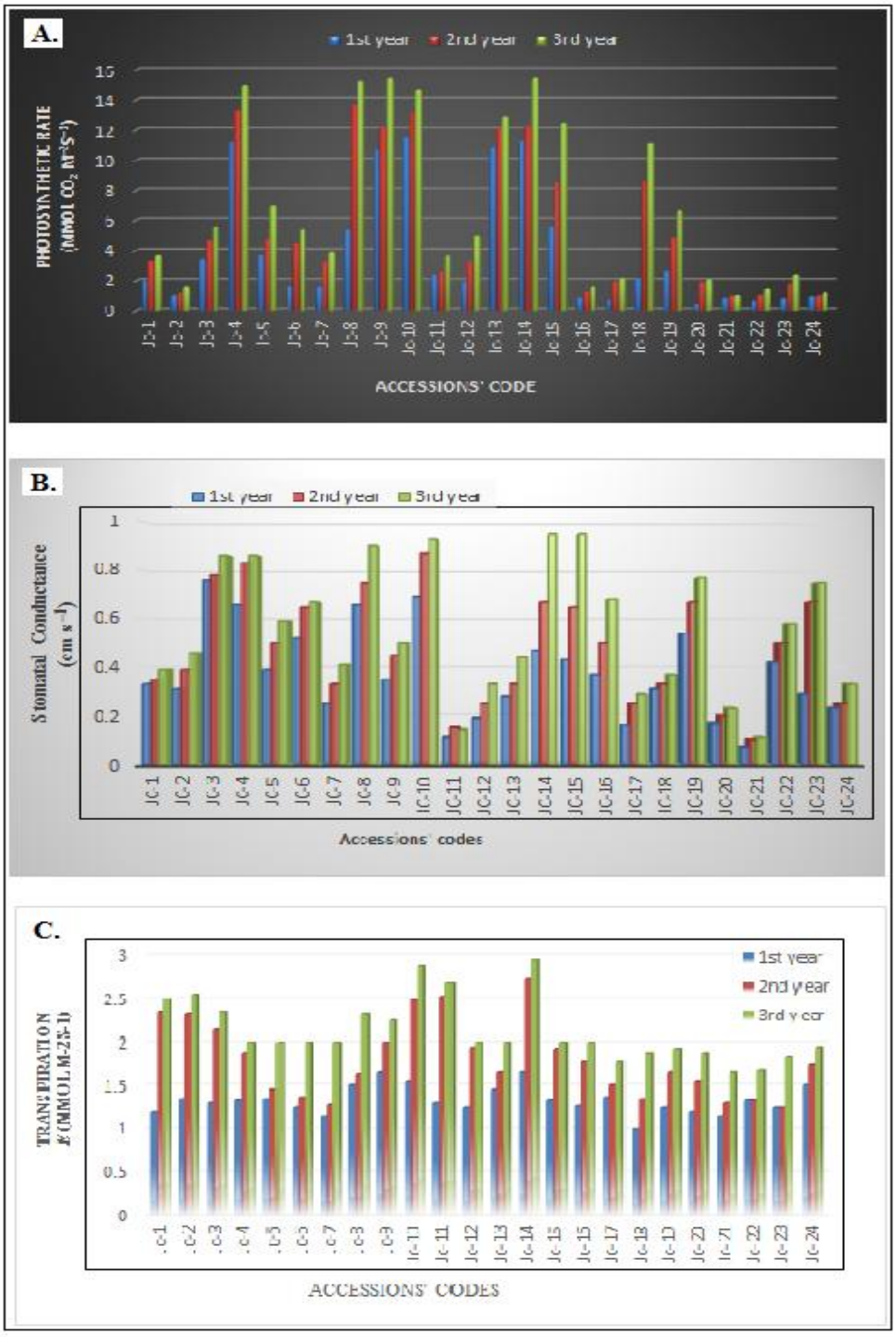

Fig.4.5. Graphs showing the variation in physiological parameters in three years for 24 *J. curcas* accessions studied: **A.** Average photosynthetic rate; **B.** Average stomatal conduction rate & **C.** Average transpiration rate per accession.

Table 4.4. Accession wise average quantification of Seed yield, crude oil %, Oil density and Tocopherol (3rd year data):

Sl. No.	Accession Code	Seed yield (gm)/ Accession			100 Seed Weight (gm)	Oil (% w/w)	Oil density	Tocopherol (ng/g)
		1st year	2nd year	3rd year				
1.	***Jc-01***	30.32	120.83	298.88	77.55	27.21	0.92	377.41
2.	***Jc-02***	31.76	119.31	207.24	79.15	25.32	0.93	156.53
3.	***Jc-03***	30.35	136.79	311.47	75.28	25.43	0.92	369.38
4.	***Jc-04***	30.37	98.35	160.78	73.15	33.53	0.92	573.01
5.	***Jc-05***	35.18	181.34	305.56	88.86	25.64	0.92	194.08
6.	***Jc-06***	39.58	163.43	353.37	80.54	24.57	0.93	73.50
7.	***Jc-07***	30.45	175.41	365.64	76.75	25.74	0.92	275.63
8.	***Jc-08***	28.03	81.25	253.57	76.35	28.86	0.91	253.11
9.	***Jc-09***	23.41	115.24	331.14	85.15	30.92	0.92	294.31
10.	***Jc-10***	42.12	138.57	389.57	83.65	33.62	0.92	403.50
11.	***Jc-11***	28.33	131.11	381.83	71.95	29.48	0.91	353.09
12.	***Jc-12***	37.32	102.22	309.54	76.15	26.38	0.91	368.13
13.	***Jc-13***	28.08	104.51	331.73	83.25	28.26	0.92	247.41
14.	***Jc-14***	37.44	180.07	389.83	84.75	35.17	0.92	246.45
15.	***Jc-15***	32.76	152.44	383.83	71.15	26.39	0.92	328.49
16.	***Jc-16***	35.19	103.51	251.35	72.50	25.03	0.92	428.43
17.	***Jc-17***	42.12	134.25	387.28	77.85	25.40	0.92	219.05
18.	***Jc-18***	21.11	93.29	222.93	80.15	27.83	0.92	78.09
19.	***Jc-19***	19.06	141.94	363.35	77.25	29.37	0.91	112.48
20.	***Jc-20***	37.44	81.55	261.47	76.95	28.00	0.91	116.23
21.	***Jc-21***	18.72	180.06	253.08	82.65	31.02	0.91	241.27
22.	***Jc-22***	23.14	163.76	323.43	78.90	31.28	0.92	326.15
23.	***Jc-23***	21.06	55.58	113.19	76.65	29.85	0.89	218.42
24.	***Jc-24***	20.24	93.25	291.17	76.55	22.15	0.92	907.58
Mean ± SEM		30.15±1.45	127.00±7.10	301.72±14.93	78.46±00.89	28.18±00.57	00.92	298.41±29.13
SD		7.12	34.78	73.15	04.38	02.81	--	142.75
CV%		23.62	27.38	24.24	05.58	09.97	--	47.83

SD= Standard deviation; CV= Coefficient of variance; SEM= Standard error of mean

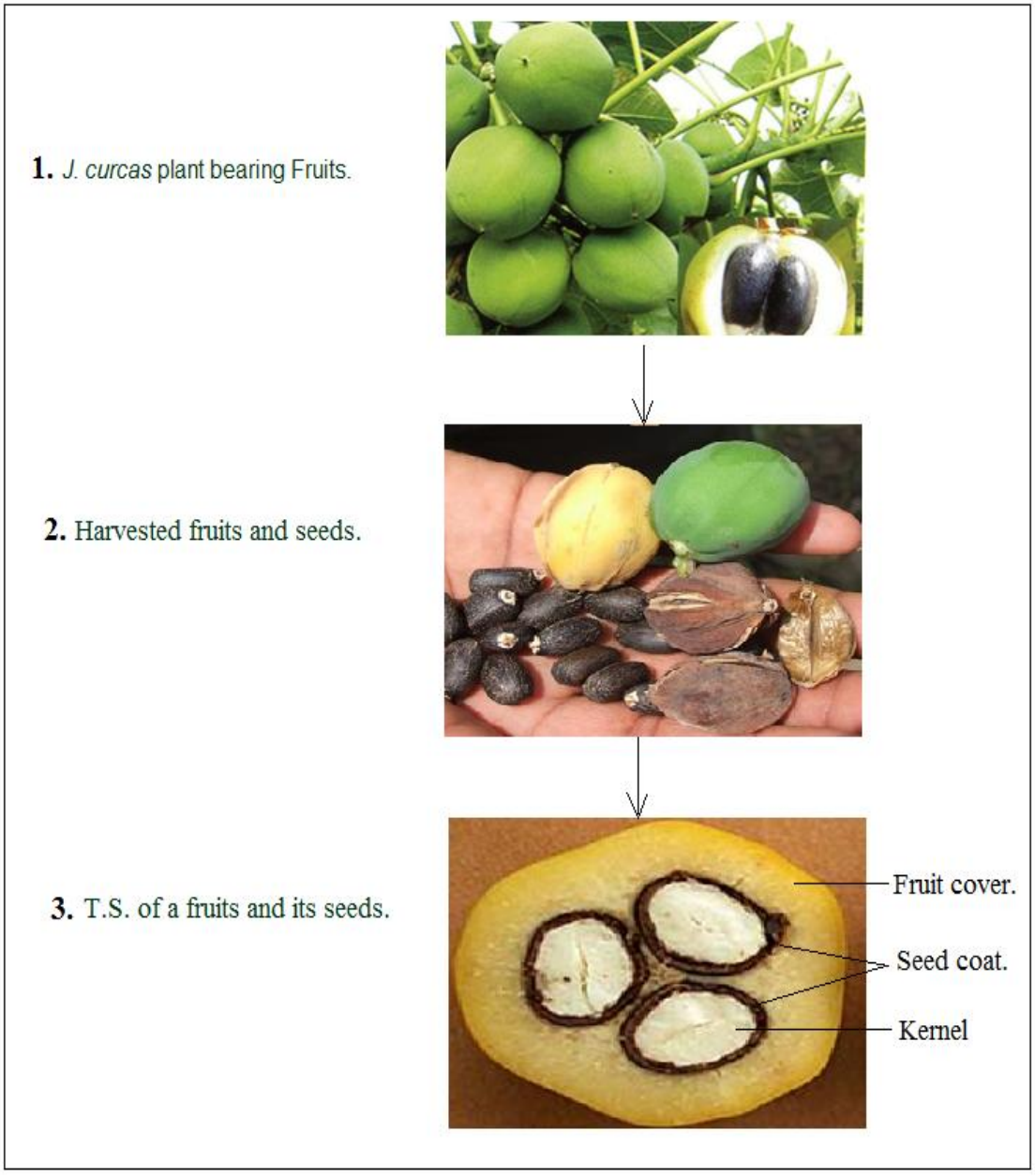

Plate 4.1. Figures showing harvesting of fruits and seeds and their transverse section with different parts.

the Plate 4.2 showing some pictures of harvested dry seed samples and some crude oil samples extracted from such dry seed samples.

4.4 Biochemical analysis of crude seed oil

The biochemical analysis i.e. the quantification of free fatty acid amount, tocopherol and the phorbolester in oil samples of each and every *J. curcas* accession of this study were done in the third year only by biochemical processes and HPLC method as described in the Chapter 3. To estimate the amount of these chemicals the experiment for extraction was carried out for three times and hence, the HPLC method was also done for three times for each and every oil samples of each and every accession and then the average was taken.

The tocopherols or Vitamin E are lipo-soluble and are totally hexane extracted together with oil. Table 4.4 showing the accession wise amount of tocopherol estimated. The HPLC graph for tocopherol estimation is presented in the Fig. 4.8. on the other hand the Fig. 4.7 and Fig. 4.9 showing the general chemical structure of tocopherol and phorbolester respectively. Fig. 4.10 showing the HPLC separation graph for phorbolester obtained by Makkar *et. al.* in 2009 in the left panel, while the right panel showing the graph obtained in this study for the same. The estimated data for phorbolester was presented in the Table 4.5. The highest amount of tocopherol was recorded in the accession no. Jc-24 which was of 907.58 ng/gm of oil, while the lowest was of 73.50 in Jc-06 and thus the average tocopherol amount in this study was measured as 298.41 ng/gm of oil. On the other hand from the quantification of the toxic material *i.e.* phorbolester we found that, the highest is in Jc-16 with 3.25 mg/gm of oil and the lowest amount in Jc-21 with 1.09 mg/gm of oil. The average amount for phorbolester was recorded as 2.07 mg/gm of oil.

The analysing of oil fractions for fatty acid composition of triglycerides revealed the presence of four main components viz. linoleic, oleic, stearic and palmitic acids. The overall corresponding data for FFA and all the triglycerides were presented

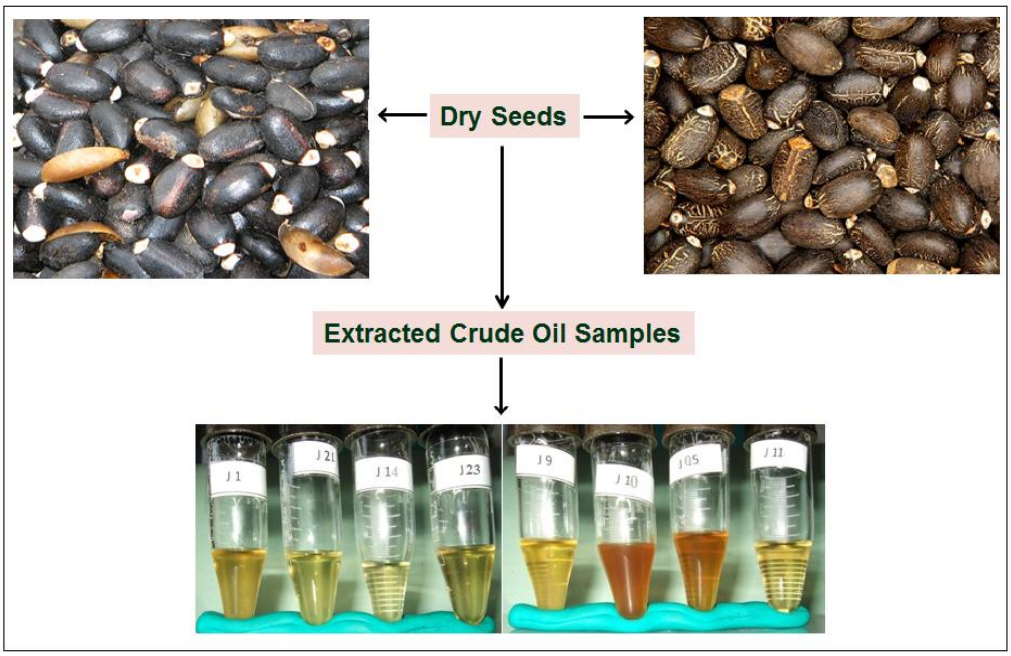

Plate 4.2. Figures showing *Jatropha curcas* oil samples extracted from the different accessions analyzed.

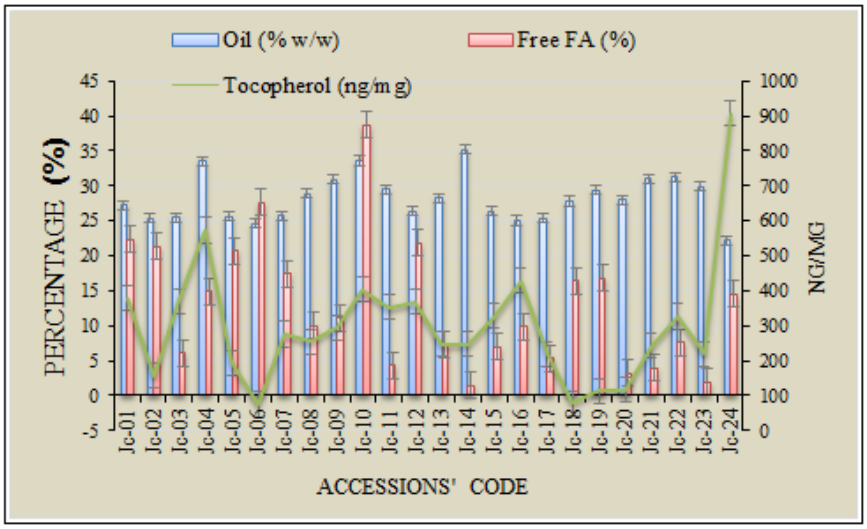

Fig.4.6. Graph showing the variation in oil % and its constituents (*viz.* Free FA and Tocopherol) according to the data collected in third year for all the 24 *J. curcas* accessions studied.

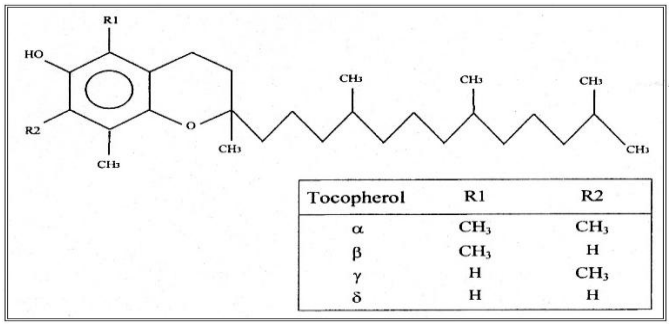

Fig.4.7: General formula and structure of tocopherols.

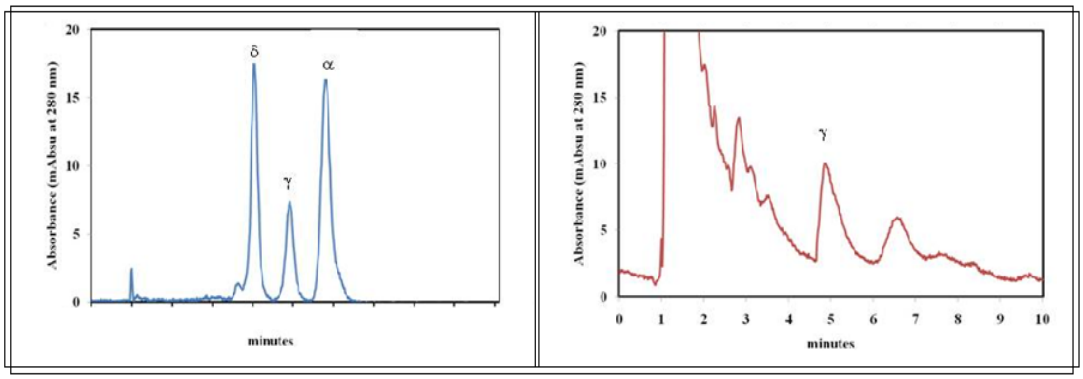

Fig.4.8: Tocopherol isomers separation analysis on HPLC:

Left panel: standard δ, γ and α isomers;

Right panel: tocopherol analysis in jatropha oil in this study.

(*Tocopherol was identified by retention time and by comparing with online UV spectra*.**)**

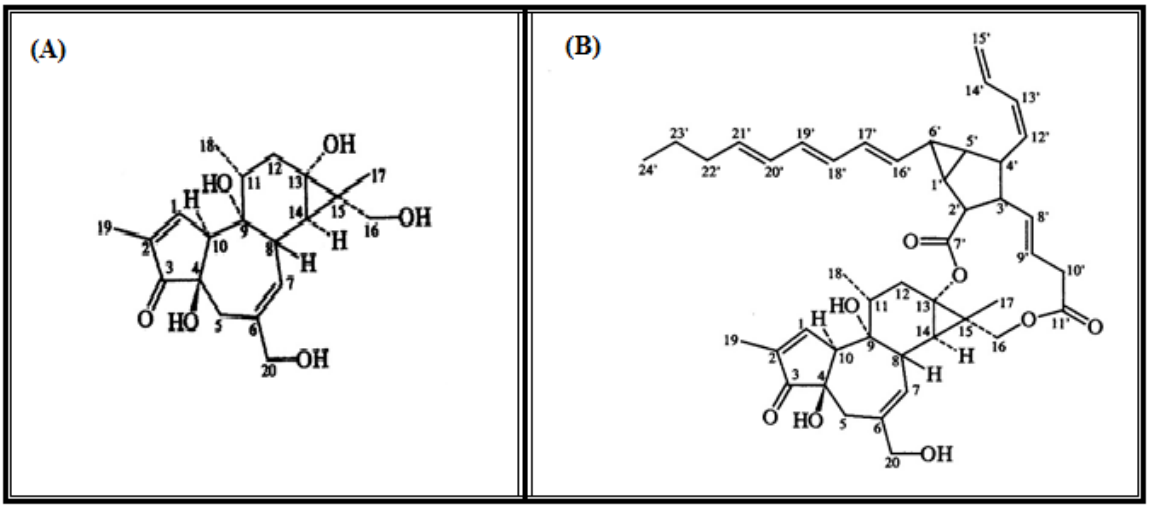

Fig.4.9: General chemical structure of phorbolester**.** (**Source:** Haas and Mittelbach, 2000)

(A) 12- deoxy-16-hydroxyphorbol.
(B) 12- Deoxy-16- hydroxyphorbol- 4%- [12%,14%- butadienyl]- 6%-[16%,18%, 20%- nonatrienyl]- bicycle [3.1.0] hexane- (13-*O*)- 2% - [Carboxylate] - (16-*O*)-3%- [8%- butenoic- 10%] ate (DHPB).

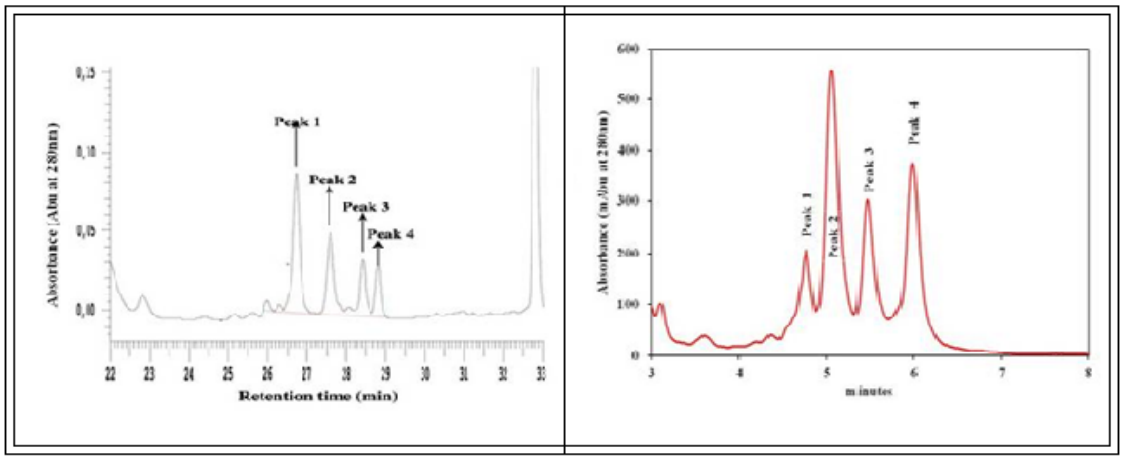

Fig.4.10: Comparison of HPLC phorbol esters separation obtained by Makkar *et al.*, 2009 (left panel) and with Kinetex column used in this work (right panel). The reduction of elution time was evident without change in compound resolution.

Table 4.5. Accession wise HPLC quantification of Phorbolester and its peak wise distribution (Mean±SEM):

Sl. No.	Accession Code	Phorbol (mg/g)	Peak I	Peak II	Peak III	Peak IV
			(Percentage distribution of total phorbols)			
1.	***Jc-01***	02.89±0.03	07.36±0.27	39.35±0.10	23.15±0.08	30.14±0.26
2.	***Jc-02***	01.80±0.03	05.88±0.41	41.23±0.14	23.84±0.14	29.04±0.14
3.	***Jc-03***	01.86±0.09	04.85±0.35	44.79±0.40	24.44±0.23	25.92±0.42
4.	***Jc-04***	01.64±0.21	05.60±0.84	41.24±3.12	29.28±2.47	23.89±1.43
5.	***Jc-05***	02.49±0.34	03.48±0.19	23.28±0.45	37.01±0.26	36.22±0.49
6.	***Jc-06***	01.85±0.02	08.98±0.37	50.58±0.29	21.38±0.20	19.07±0.05
7.	***Jc-07***	01.63±0.24	02.44±0.10	47.08±0.43	25.24±0.24	25.23±0.18
8.	***Jc-08***	02.74±0.02	10.59±0.21	40.21±0.21	21.61±0.07	27.59±0.06
9.	***Jc-09***	01.59±0.02	05.35±0.47	46.23±0.32	23.67±0.24	24.76±0.31
10.	***Jc-10***	02.61±0.12	01.03±0.03	25.27±0.35	39.16±0.14	35.57±0.23
11.	***Jc-11***	02.52±0.01	06.66±0.11	41.58±0.09	25.14±0.11	26.62±0.06
12.	***Jc-12***	01.71±0.04	04.56±0.13	38.33±0.47	26.93±0.23	30.19±0.15
13.	***Jc-13***	01.75±0.04	11.61±0.19	42.40±0.54	20.24±0.31	24.65±0.27
14.	***Jc-14***	02.30±0.42	07.47±0.25	41.37±0.21	23.11±0.46	28.06±0.01
15.	***Jc-15***	02.12±0.06	06.47±0.15	43.72±0.17	21.73±0.14	28.08±0.09
16.	***Jc-16***	03.25±0.12	04.74±0.20	44.92±0.44	21.66±0.40	28.68±0.24
17.	***Jc-17***	01.51±0.05	02.20±0.30	48.15±0.43	25.49±0.24	24.53±0.18
18.	***Jc-18***	01.94±0.13	07.12±2.75	45.45±1.06	21.83±0.48	25.61±1.27
19.	***Jc-19***	01.21±0.04	08.33±0.31	42.73±0.44	25.72±0.51	23.22±0.16
20.	***Jc-20***	02.53±0.09	06.34±0.41	42.81±0.41	23.67±0.40	25.82±0.35
21.	***Jc-21***	01.09±0.01	06.39±0.08	46.89±0.43	25.06±0.19	21.67±0.33
22.	***Jc-22***	01.27±0.06	05.55±0.10	41.52±0.51	26.37±0.12	26.56±0.48
23.	***Jc-23***	02.82±0.03	06.52±0.32	44.93±0.18	20.71±0.19	27.84±0.23
24.	***Jc-24***	02.72±0.06	05.08±0.04	43.02±0.92	21.68±0.72	30.22±0.18
Mean± SEM		02.07±0.15	06.02±0.44	41.96±1.22	24.92±0.95	27.04±0.75
SD		00.73	02.16	05.97	04.66	03.68
CV%		35.26	35.88	14.23	18.70	13.61

SD= Standard deviation; CV= Coefficient of variation; SEM= Standard error of mean.

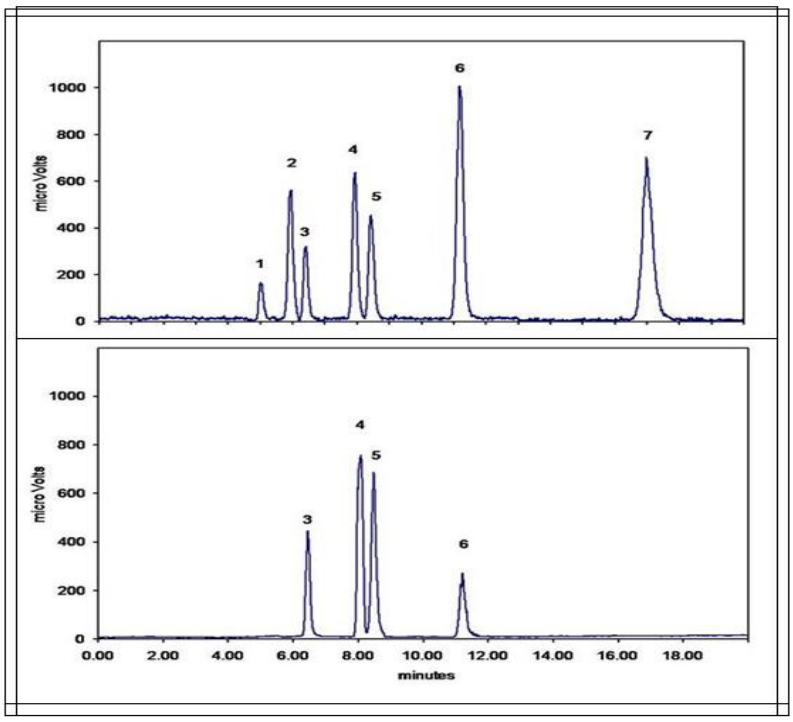

Figure 4.11. Fatty acid analysis on HPLC-ELS detector, standard chromatogram (top), Typical fatty acid chromatogram for jatropha oil (bottom)

Peak 1 = linolenic acid (C18:3); Peak 2 = palmitoleic acid (C16:1);
Peak 3 = linoleic acid (C18:2); Peak 4 = eicosatrienooic acid (C20:3);
Peak 5 = palmitic acid (C16:0); Peak 6 = oleic acid (C18:0);
Peak 7 = stearic acid (C18:0); Peak 8 = erucic acid (C22:1).

in the Table 4.6. The HPLC chromatograms for the FFA estimation were shown in the Fig. 4.11. Among the accessions, *Jc-14* which has the highest oil content possess low free fatty acid content (1.43%), slightly higher than the lowest reported value for Jatropha. The accession *Jc-10* showed maximum FFA of 38.73%, which was significantly higher of reported value for Jatropha. On the other hand the accessions *Jc-01, Jc-02, Jc-05, Jc-06* and *Jc-12* had FFA amount of more than 20%. In case of triglyceride composition the average data for linoleic acid, oleic acid, stearic acid and palmitic acid are 19.25%, 45.57%, 10.92% and 21.44% respectively.

4.5 Analysis of genetic variability among the accessions

It has been stated earlier that the assessment of genetic diversity is becoming most important criteria for the study of biodiversity, population dynamics and ecological relationship. For that purpose different types of molecular markers are now widely used tool to assess the genetic diversity. In this study also we have used different versions of TBP i.e. TBP and hTBP methods to assess the genetic diversity if present among the accessions of *J. curcas* used for this study. The results of these methods were presented in the Plate 4.4 and 4.5.

Besides the general TBP and hTBP method the amplification were also done by TBP method after digesting the genomic DNA by restriction enzyme *AatII* in the absence of DNA methylation. The result is shown in the Plate 4.6.

Moreover, the RAPD method was also used to assess the genetic diversity among the 24 *J. curcas* accessions studied by restricting the genomic DNA using restriction enzymes viz. *HpaII* and *MspI*. The corresponding result was shown in the Plate 4.7.

Table 4.6. Accession wise Free FA% and Triglyceride Oil Composition in 3rd year (Mean±SEM):

Sl. No.	Accession Code	Free FA (%)	Triglyceride Oil Composition %			
			Linoleic Acid	Oleic Acid	Stearic Acid	Palmitic Acid
1.	***Jc-01***	22.35	18.85±0.92	52.52±0.75	10.78±2.73	17.84±1.03
2.	***Jc-02***	21.37	16.21±0.18	37.05±0.35	10.30±1.05	21.25±0.15
3.	***Jc-03***	06.02	23.85±1.17	53.29±0.36	13.44±0.59	09.42±0.93
4.	***Jc-04***	14.93	19.14±0.45	27.09±2.79	13.59±0.97	40.18±4.22
5.	***Jc-05***	20.66	15.64±1.74	52.39±6.92	10.91±2.06	21.06±3.14
6.	***Jc-06***	27.61	27.61±0.07	63.61±0.11	01.40±0.15	07.38±0.11
7.	***Jc-07***	17.45	25.15±0.03	56.35±0.37	01.50±0.03	05.33±0.05
8.	***Jc-08***	09.98	13.96±0.24	30.49±0.53	10.15±0.14	45.40±0.14
9.	***Jc-09***	11.14	22.58±0.88	51.40±1.11	10.15±1.77	15.87±3.77
10.	***Jc-10***	38.73	16.19±0.24	48.59±0.34	17.38±0.14	17.84±0.53
11.	***Jc-11***	04.27	23.46±0.27	49.68±1.81	16.57±0.22	10.29±2.31
12.	***Jc-12***	21.82	14.21±0.16	35.03±0.35	09.30±0.01	41.45±0.19
13.	***Jc-13***	07.37	15.39±6.52	43.44±6.80	08.55±1.86	22.61±1.59
14.	***Jc-14***	01.43	14.16±0.97	32.52±0.74	11.76±0.86	41.56±1.09
15.	***Jc-15***	06.93	24.61±0.14	58.06±1.20	08.14±1.89	09.20±0.54
16.	***Jc-16***	09.87	22.36±0.08	58.37±0.87	10.40±1.21	08.87±0.41
17.	***Jc-17***	05.27	21.19±0.19	53.50±0.33	07.33±0.45	04.19±0.05
18.	***Jc-18***	16.44	16.59±0.21	27.30±0.34	13.68±0.11	42.43±0.02
19.	***Jc-19***	16.83	17.10±0.30	34.05±0.37	20.54±0.28	28.31±0.20
20.	***Jc-20***	03.16	11.73±1.65	25.13±0.33	08.05±0.05	38.14±1.92
21.	***Jc-21***	03.92	19.84±0.22	50.78±0.31	15.52±0.26	13.85±0.17
22.	***Jc-22***	07.60	22.04±0.36	52.41±0.29	11.94±0.18	13.61±0.25
23.	***Jc-23***	01.96	15.43±0.74	36.95±0.79	16.20±0.45	31.42±0.40
24.	***Jc-24***	14.55	24.85±0.01	63.66±1.66	04.51±2.39	06.98±0.70
Mean± SEM		12.98±1.72	19.25±0.74	45.57±0.73	10.92±0.93	21.44±2.74
SD		08.42	3.65	03.59	04.55	13.41
CV%		64.87	18.96	07.88	41.67	62.55

SD= Standard deviation; CV= Coefficient of variation; SEM= Standard error of mean.

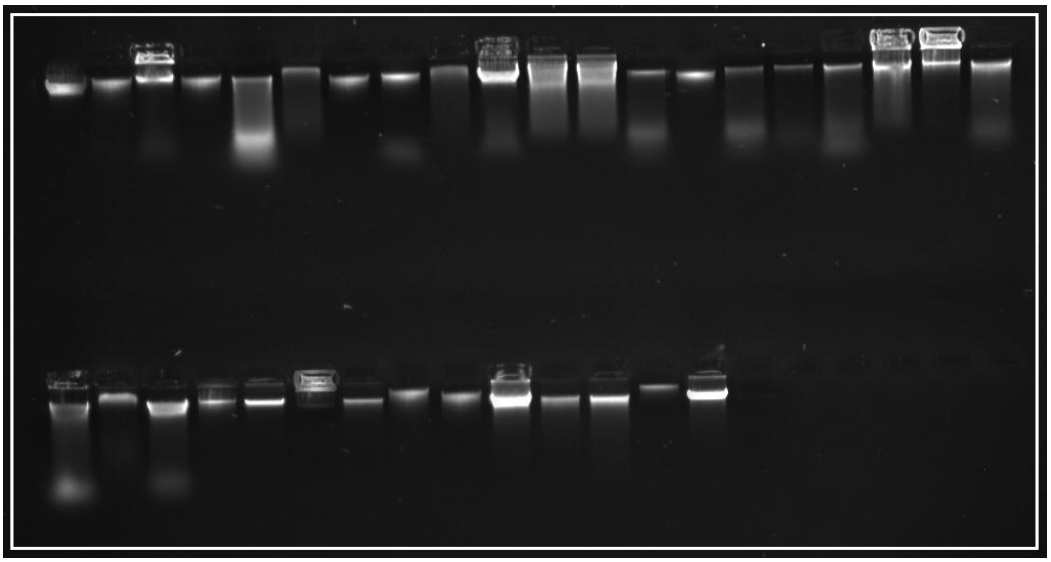

Plate 4.3. Extracted DNA samples of different *J. curcas* L. accessions analyzed.

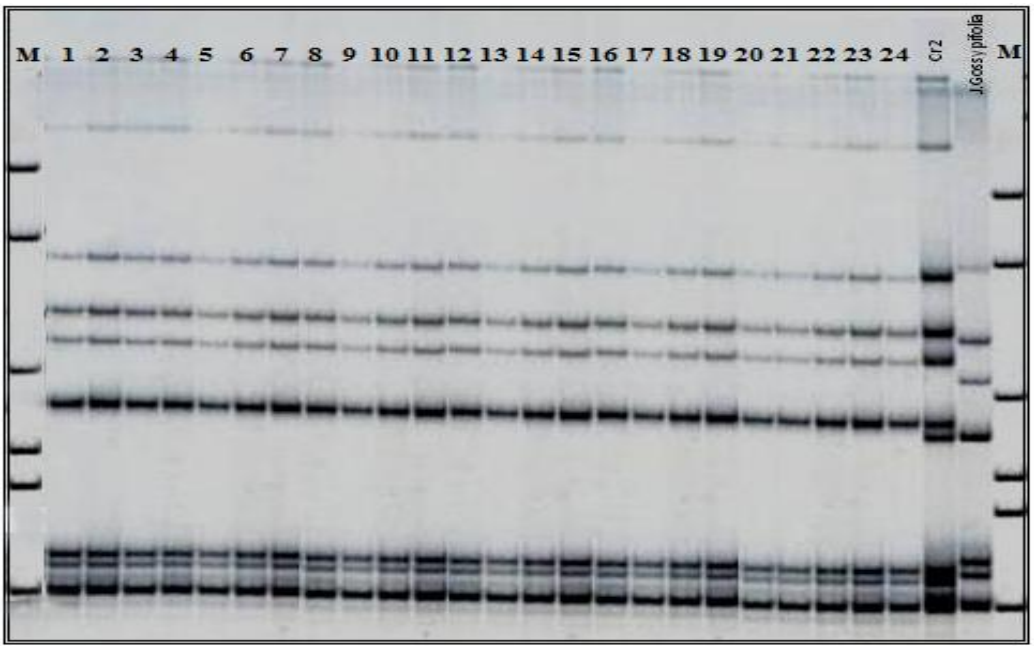

Plate 4.4. Polyacrylamide gel electrophoresis showing the TBP amplification profile of 24 accessions. Molecular marker sizes are on the extreme sides.

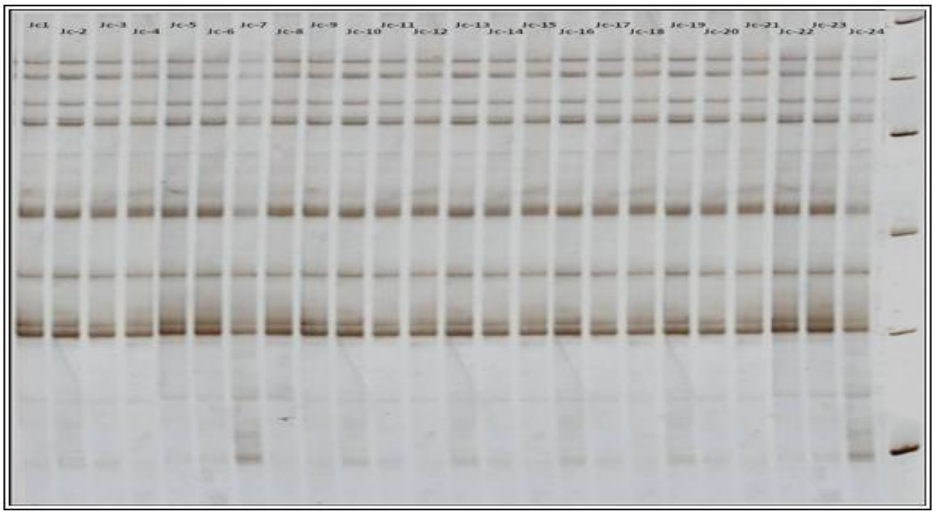

Plate 4.5. hTBP banding pattern for the 24 *J. curcas* accessions. Intron I and II were amplified together with the second exon of the coding region thus yielding amplicons ranging from 700 to 3000 base pairs in size. No polymorphism in length was detected. Molecular marker size is on the right.

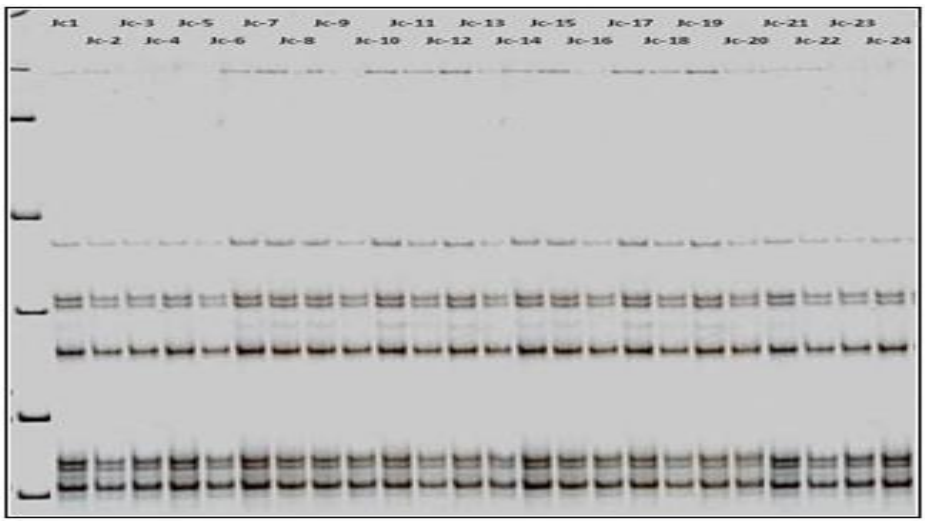

Plate 4.6. TBP amplification after genomic DNA digested with enzyme *Aat*-II. No polymorphism was observed among the 24 *J. curcas* accessions. Molecular marker sizes are on the extreme left.

But in all the molecular experiments done for the assessment of the genetic variability of these 24 *J. curcas* accessions in this study, there were no any variations found in the amplifications bands among the samples studied and hence among the accessions too.

4.6 Statistical analysis

The statistical analysis was done to find out the correlation among all the parameters studied in this study by using the "Pearson co-efficient correlation" method. The Corresponding statistical data were presented in the Table 4.7. Except three cases, remaining all showed positive correlation between each and every parameters. Among all, between the no. of female flower and seed yield showed highest correlation of 12.59 and on the other hand the correlation between the no. of female flower and primary branch no. showed the lowest value of -0.15.

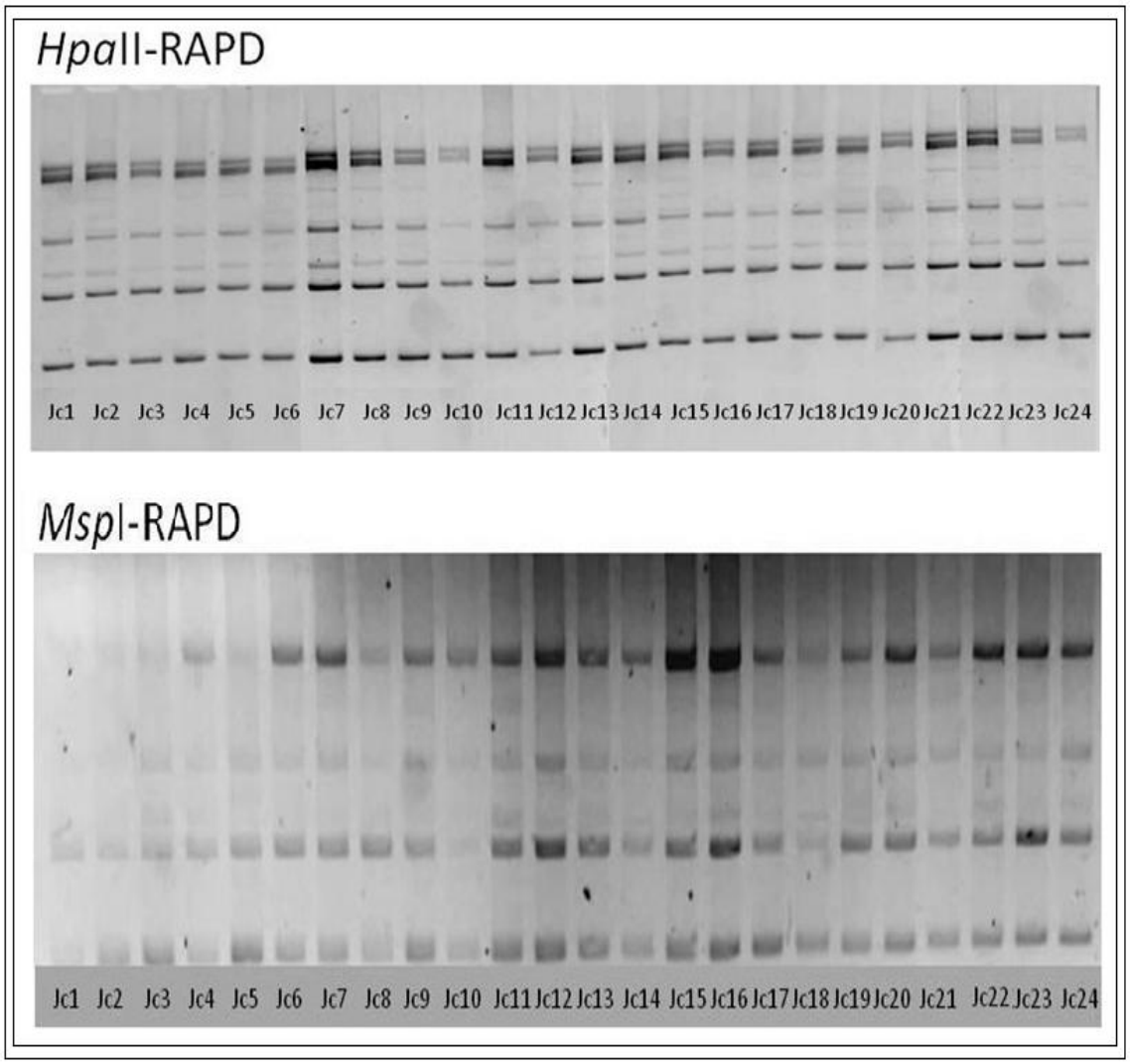

Plate 4.7. Pattern of RAPD amplification of 24 *J. curcas* accessions by LBJ6 primer after restricting the DNA samples with *Hpa*ll (upper) and *Msp*l (lower) enzymes.

Table.4.7. Study of correlation between the parameters by 'Pearson co-efficient correlation' method:

Parameters	Height	Branch	Leaf Area	Photosynthetic Rate	Inflorescence Nos.	Female Flower	Oil%	Seed yield
Height	**+1.00**							
Branch	0.27	**+1.00**						
Leaf Area	0.25	0.23	**+1.00**					
Photosynthetic Rate	0.34	0.23	0.66	**+1.00**				
Inflorescence Nos.	0.22	0.35	0.26	0.44	**+1.00**			
Female Flower	0.08	-0.15	0.17	0.03	0.26	**+1.00**		
Oil%	0.51	0.17	0.52	0.28	0.11	0.20	**+1.00**	
Seed yield	0.12	-0.13	1.92	-0.02	3.09	12.59	0.83	**+1.00**