The present invention relates to the field of nucleic acid extraction from the plant material. Particularly, the invention provides a high yielding DNA extraction process from oil crops. The said process is a quick, simple, cheaper and an efficient process which is capable of generating large amount of DNA from very small starting plant material with highly purified *DNA content.*

Field of the Invention:

The present invention relates to the field of nucleic acid extraction from the plant material. Particularly, the invention provides a high yielding DNA extraction process from oil crops. The said process is a quick, simple, cheaper and an efficient process which is capable of generating large amount of DNA from very small starting plant material with highly purified DNA content.

Background of the invention:

India has a wide range of oilseed crops grown in different agro climatic zones. Sesame, Sarson, Taramira, Jatropa and Groundnut are traditionally cultivated oilseeds crops. Modern tools like molecular techniques could be employed for enhancing the efficiency of resistance breeding to make oil seed production competitive and sustainable. However, molecular studies on these plants have some limitations due to the presence of phenolic acids, polysaccharides and flavonoid contents that make DNA extraction difficult. The currently existing DNA extraction protocols are not suitable for oilseed crops because of the presence of high level of polyphenols & essential oils therein. As various protocols are known such as CTAB and salt extraction methods (Doyle and Doyle, 1987) have been described earlier, but those tend to use liquid nitrogen for crushing the plant material, which is quite expensive and require extensive precautions and such methods does not yield high amount of DNA with good quality. Existing protocols require large quantities of tissue (in grams) to be crush, which requires long time for plant growth.

Thus, there exists need for a quick, simple, cheaper and efficient protocol for the isolation of DNA which can provide good yield in terms of purity and quantity of DNA content from oil crops.

Object of the Invention:

A primary object of this invention is to overcome the disadvantages/drawbacks of the known art of process for the extraction of DNA.

Another object of this invention is to provide a DNA extraction process which is capable of producing large quantity of DNA from small quantity of plant material.

Another object of this invention is to provide a DNA extraction process which yields highly purified DNA.

Another object of this invention is to provide for a quick, simple, cheaper and efficient DNA extraction process.

These and other advantages of the present invention will become readily apparent from the following detailed description read in conjunction with the accompanying drawings.

Summary of the invention:

The present invention relates to the field of nucleic acid extraction from the plant material. Particularly, the invention provides a high yielding DNA extraction process from oil crops. The said process is a quick, simple, cheaper and an efficient process which is capable of generating high yield of DNA from very small starting plant material and simultaneously the process enables the production of high quality and purified DNA.

Detailed description of the invention:

The following description is of exemplary embodiments only and is not intended to limit the scope, applicability or configuration of the invention in any way. Rather, the following description provides a convenient illustration for implementing exemplary embodiments of the invention. Various changes to the described embodiments may be made in the function and arrangement of the elements described without departing from the scope of the invention.

The present invention provides an efficient process of extraction of DNA from the oil crops, which is capable of producing large quantity of DNA with high purity.
The process yields DNA with A_{260}/ A_{280} (purity content) ranging from 1.37-2.0. The process takes less time as it requires very small amount (0.5 g) of plant material and produces high yield of DNA on an average of 3222µg/gm of DNA with maximum of 6935 µg/gm and minimum of 655 µg/gm tissue.

The process also eliminates the necessity of phenol, which makes the method less hazardous. Thus, the method is quite safe and cost effective.

The invention employs 1% SDS buffer, high concentration of Polyvinyl pyrrolidone (PVP), NaCl, β-mercaptoethanol (instead of liquid N_2), fixing solutions like Alcohol, Chloroform, EDTA and mixtures thereof are used in the instant process of extraction process. Further, the addition of high concentration of PVP and β-mercaptoethanol are helpful in removing the polyphenols. The problem arising from the presence of high levels of polysaccharides has also been overcome by using NaCl at a higher concentration.

The major steps of protocol are:

1. Preheating 5 ml SDS buffer (1% SDS, 100mM NaCl, 100 mM Tris-base, 100 mM EDTA, pH-8.5) in 50 ml flask at 60 ° C in a water bath.
2. Taking 0.5 g plant tissue and dipping in different fixing solutions for 30 min. Then grinding the fixed tissue in chilled mortar pestle to fine paste. The cell walls must be broken or digested away in order to release the cellular constituents. This is usually done by grinding the tissue in dry ice or liquid nitrogen with a mortar and pestle. The use of different fixing solutions (instead of liquid nitrogen) like alcohol, alcohol: chloroform and alcohol:EDTA in varying ratio plays a significant role in the extraction process leading into the production of high amount of DNA with high A_{260}/ A_{280} indicating high purity content of DNA.
3. Transferring the grind tissue into a pre-chilled (with dry ice) centrifuge tube followed by the addition of preheated (60° C) 5 ml SDS buffer (1% SDS, 100mM NaCl, 100 mM Tris-base, 100 mM EDTA, 4% PVP and 4% β- mercaptoethanol). PVP and β-mercaptoethanol must be added before use. The cell membranes must be disrupted so that the DNA is released into the extraction buffer. This is accomplished by using a detergent like sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS).
4. Incubating the tubes at 60° C water bath for 1 hr. Mixing gently in between the incubation period for uniform mixing.
5. Now taking out the centrifuge tubes out of water bath and leaving for 10 min for cooling at room temperature.

6. Adding equal volume (equal to the current total volume of the contents of the tube) of chloroform/isoamyl alcohol (a 24: 1 solution, the chloroform extracts proteins, the isoamyl alcohol decreases foaming of the emulsion). Mixing thoroughly to form a complete emulsion until the two separate phases will not be seen (i.e., no chloroform phase should form at the bottom of the tube until centrifugation).
7. Centrifuging tubes at 12000 rpm for 10 min at 4° C.
8. Transferring the supernatant solution from the top (aqueous) phase into a new autoclaved centrifuge tube.
9. Discarding the lower (chloroform) phase which contains cell debris.
10. The upper aqueous phase will be clear, though often colored;
11. Centrifuging the upper aqueous phase.
12. Repeating 6, 7, 8 and 9 steps thrice.
13. Precipitating the DNA by adding 5 M NaCl and 2/3 volume of chilled isopropanol. Leave for 2 hrs at -20° C or overnight for complete precipitation.
14. P 1000 (1 ml pipette) is used because DNA in solution is a long, gentle molecule that is easily sheared when it passes through a narrow opening. It will improve the quality of DNA.
15. If possible, spool out nucleic acids with a glass rod.
16. Now centrifuging precipitated DNA at 10000 rpm for 10 min at 4° C. DNA pellet is obtained.
17. Usually in case of oil seed crops obtained pellet is yellowish in color instead of white.
18. The pellet is further washed with 70% ethanol. This step is repeated twice.
19. In some cases centrifugation can be done for washing pellet with 70% ethanol.
20. Air drying the pellet at room temperature (leave overnight for better yield).
21. Resuspending the air dried pellet in 200 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
22. Checking the DNA quality by running on 0.8% agarose gel and quantity by taking absorbance at 260 nm and 280 nm.

The below stated table the differentiating features of DNA isolation protocol of present invention with the other existing protocol:

S.No.	Components used in Extraction buffer	Doyle & Doyle protocol (1987)	Present Invention
1.	**Liquid nitrogen**	Used	Not used
2.	**NaCl**	1.4M	100mM
3.	**Tris-Hcl or Tris-base**	100mM Tris-Hcl	100mM Tris-base
4.	**EDTA**	20mM	100mM
5.	**Detergent**	CTAB-2%	SDS-1%
6.	**β-ME (β-Mercaptoethanol)**	.2%	4%
7.	**PVP (Polyvinylpyrrollidone)**	-	4%

Table-1- showing modifications with earlier DNA isolation protocol

Below mentioned is the table showing the DNA yield and A_{260}/ A_{280} ratio in different plants.

Plant	Modifications	A_{260}	A_{280}	A_{260}/ A_{280}	Conc. (µg/µl)	Yield (µg/g tissue)
Sarson	A	0.967	0.532	1.82	2.42	4835
	B	0.575	0.338	1.7	1.44	2875
	C	0.996	0.595	1.67	2.49	4980
	D	0.127	0.12	1.06	0.32	635
Sesame	A	0.721	0.527	1.37	1.8	3605
	B	0.799	0.514	1.55	2.0	3995
	C	0.609	0.38	1.6	1.52	3045
	D	0.735	0.469	1.57	1.84	3675
Jatropa	A	0.623	0.358	1.74	1.56	3115
	B	1.222	0.6	2.0	3.06	6110
	C	1.387	0.695	2.0	3.47	6935
	D	0.932	0.504	1.85	2.33	4660

Taramira	A	0.077	0.054	1.43	0.19	385
	B	0.234	0.15	1.56	0.59	1170
	C	0.173	0.115	1.5	0.43	865
	D	0.131	0.075	1.74	0.33	655
Groundnut	A	0.281	0.164	1.7	0.70	1404
	B	0.409	0.251	1.62	1.02	2045
	C	0.228	0.112	2.03	0.57	1140
	D	0.697	0.441	1.58	1.72	3485

Table- 2- Average values of DNA yield in µg/g tissue

In the above table:

A denotes -Liquid nitrogen;

B denotes -Alcohol;

C denotes -Alcohol: Chloroform;

D denotes -Alcohol: EDTA

Below mentioned is the comparative analysis of DNA yield of present invention with the existing methods in Table 3. The data shows that the present invention provides higher purity and quantity of DNA as compared to the other existing methods used for extraction of DNA in oil crops.

Table 3- Comparative analysis showing higher purity and quantity of DNA yield:

Modifications	Doyle and Doyle method, 1987		Dellaporta *et al.*, 1983		Sharma *et al.*, 2003		Dhakshanamoorthy and Selvaraj, 2009		Sharma *et al.*, 2010		Present Invention	
With different fixing solutions:	**A_{260}/A_{280}**	**DNA yield (µg/g tissue)**	**A_{260}/A_{280}**	**DNA yield (µg/g tissue)**	**A_{260}/A_{280}**	**DNA yield (µg/g tissue)**	**A_{260}/A_{280}**	**DNA yield (µg/g tissue)**	**A_{260}/A_{280}**	**DNA yield (µg/g tissue)**	**A_{260}/A_{280}**	**DNA yield (µg/g tissue)**
Absolute Alcohol (100%)	-	-	-	-	1.98-2.02	330-340	1.83-1.99	2330-2710	1.10-1.49	1066-2083	**1.55-2.0**	**1170-6110**
Alcohol:chloroform (70: 30)	-	-	-	-	2.04-2.05	345-360	-	-	1.10-1.54	700-2066	**1.5-2.03**	**865-6935**
Alcohol:EDTA(70:30)	-	-	-	-	1.96-1.98	295-372	-	-	1.10-1.54	983-2608	**1.06-1.86**	**635-4660**
With liquid nitrogen	+	+	+	+	1.84-2.00	327-401	1.88-2.02	2260-2500	1.3-1.6	1060-1550	**1.37-1.82**	**385-4835**
Extraction buffer	2 % CTAB, 100 mM Tris, 1.4 M NaCl, and 20 mM EDTA, 0.2% β-mercaptoethanol		1% SDS, 50 mM Tris/HCl, 100 mM NaCl, 10 mM EDTA		2% CTAB 100 mM Tris, 20 mM EDTA , 1.4 M NaCl, , 0.2 % β-mercaptoethanol		2 % CTAB, 100 mM Tris, 1.4 M NaCl, and 20 mM EDTA, 2% PVP, β-mercaptoethanol		2 % CTAB, 100 mM Tris, 1.4 M NaCl, and 20 mM EDTA, 0.2% β-mercaptoethanol, 1.5-2.5% PVP		1 % SDS, 100 mM Tris, 100mM NaCl, and 100 mM EDTA, 4% PVP, 4% β-mercaptoethanol	

(-) denote absence of that modification, (+) denotes presence of that modification.

Dated this 16th day of April 2012

Suvarna Pandey
Of S. Majumdar & Co.
(Applicant's Agent)