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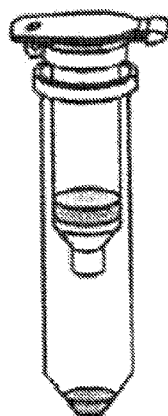


FIGURE 1

(57) Abstract: The invention relates to a genomic DNA isolation method, kit and production method thereof, that is economic, high quality and efficient, free of carcinogenic and mutagenic agents, with no negative impact on the environment and the researcher (applicator) in terms of health, and that can be used effectively on generative and vegetative plant parts and many plant varieties and species.



GENOMIC DNA ISOLATION METHOD AND KIT**Technical Field**

5 The invention relates to a genomic DNA isolation method, kit and production method thereof, that is economic, high quality and efficient, free of carcinogenic and mutagenic agents, with no negative impact on the environment and the researcher (applicator) in terms of health, and that can be used effectively
10 on generative and vegetative plant parts and many plant varieties and species.

State of The Art

DNA is a molecule that generally present a right handed helix
15 structure that occur as two parallel chains formed by phosphoric acid and deoxyribose units encoded in the sequence of the nucleotides where it carries the genetic information nucleotide are cross-linked by the purine and pyrimidine bases. The prerequisite at the molecular biology and epigenetic techniques
20 practices is to realize high-quality DNA isolation of adequate quantity from different gender, species, tissues or cells without damaging nucleotides and inducing any modifications. DNA in high-quality and quantity must be achieved before conducting the operations such as polymerase chain reaction (PCR) studies,
25 DNA sequencing studies (DNA sequence analyses), DNA amplification, hybridization and next generation DNA sequencing that involve molecular biology practices.

DNA isolation basically comprises of 2 phases as cell wall fractionation and dissolution of DNA-protein complex and
30 separation from other molecules, including the RNA. The cell wall must be fractionized in order to reach the genomic DNA at

the nucleus of the cell. Fractionation might be achieved through physical and chemical means. Physically heated cells are also subjected to chemical mixtures in order to ensure wall fractionation. The salt available within said chemical mixture permeate through the cell wall and separate the proteins from the DNA. The detergent that improve permeability of the cell wall enables release of the cell contents. Ethylene diamine tetra acetic acid (EDTA) attaches to the cofactors such as magnesium etc. and inhibit the DNase activity, thus allowing the DNA to remain stable. proteolytic enzymes, on the other hand, lyse the proteins in the cell content, and enable formation of homogenous mixture in terms of subsequent steps. As the RNAs within the cell can be stable in the form of single chain, those RNAs should also be removed, and RNase enzyme is used for this purpose. The chemicals used during fractionation phase and the reaction time might vary depending on the material you wish to extract the DNA. For instance, when extracting the DNA of some bacteria, a different enzyme called lysozyme can be used for cell wall having a different structure and the time can be prolonged. Likewise, the fractionation time spent for DNA isolation from the blood is less than the time spent for DNA isolation from the tissue.

The method of dissolving the DNA-protein complex and separation from other molecules generally predicates on dissolution and denaturation. However, the technological advancements today enabled use of spin columns that retain DNA. Phenol extraction process is frequently used in denaturation based chemical dissolution and the proteins and the DNA are separated and diverge from each other through use of phenol and chloroform and centrifugal power. In physical attachment where spin columns are used, on the other hand, the DNA adheres to matrix layer specifically placed inside the tubes by use of special solutions. As the molecules other than DNA fail to adhere to the matrix,

they are removed from the medium at the same phase though centrifugal or gravitational force. The spin columns are again subjected to the process so-called "washing" in order to completely remove the non-DNA substances though use of certain solutions. The last phase is the "elution" phase where the solutions that remove the DNA from the matrix are used, and with this last phase, the DNA gets its final form.

There are numerous other methods and kits available for DNA isolation. In the prior art, the applications no. EP2258845, US6027945, and US8729252 discloses methods for eDNA isolation, while the applications no. CN105002159, WO0040697 and WO2015132216 mentions about the kits used for DNA isolation.

In addition, the cDNA synthesized using the mRNA is another DNA source. In some cases, DNA should be isolated from PZR or hybridization studies. Regardless of the source, the DNA molecules are polymers resistant to degradation, and can be preserved for long time under suitable storage conditions and can be used at analyses recurrently. However, as the large fragments presents higher tendencies towards breaking and suffering damages compared to the small fragments, the DNA molecules intended to be isolated as large fragments should be processed very carefully. Isolation of large DNA fragments necessitate use of different methods.

The DNA to be purified can be genomic or non-genomic PZR or hybridization products as well as mitochondria (mtDNA) and chloroplast (chlDNA) DNA, or plasmid DNA from the bacteria. The increasing number and diversity of tests has brought up the necessity of automatic DNA isolation at the molecular diagnosis laboratories and automated instruments has been developed for this purpose. DNA isolation kits are standardized packages that

allow us to isolate the genomic and non-genomic DNA molecules. There are DNA isolation kits available today that are designed for various purposes. Some types of isolation kits allow us to separate the desired DNA from others from agarose gels after
5 PZR. This type of isolation kits comprises of platforms such as membrane, column, capillary, etc. that enable bonding of the DNA. The DNA isolation kits not only shorten the phases of the tests, but also are tools that allow more effective and easy DNA isolation.

10

The tissue fractionation process at the automatic DNA isolation systems generally uses magnetic ball technology. If, however, such magnetic particles are not removed from the medium at the elution step, they might affect the PZR amplification reaction
15 and might lead to unrealistic negative or positive PZR outcomes.

20

In the prior art, recently the DNA bonding columns so-called as DNA cartridges formed by processing the silica particles at the nano levels through special filters, are being developed wherein
the solid phase for DNA isolation is silica.

25

The kits developed for DNA isolation are generally capable of isolating adequate quantity and quality of genomic DNA from limited number of plant species. These kits which generally have
higher costs also require technical information and infrastructure. In addition, chemicals such as the beta mercapto ethanol, phenol, chloroform used at some genomic DNA isolation kits present the potential to have negative impact on human and environmental health.

30

Taking into consideration the disclosures provided hereinabove and the disadvantages experienced as a result of the studies, the genomic DNA isolation kit of the invention that allows DNA isolation in high quality and quantity with no negative impact in economic and health terms has been achieved.

The Problems that the Invention Aims to Solve

The objective of the invention is to develop genomic DNA isolation method, kit and production method thereof that is economic, high quality and efficient, free of carcinogenic and mutagenic agents, with no negative impact on the environment and the researcher (applicator) in terms of health, and that can be used effectively on generative and vegetative plant parts and many plant varieties and species.

Another objective of the invention is to ensure that DNA isolation can be achieved at live plants as well as from lifeless plant parts including the dried plant accessories and wheat and rice (paddy without embryo).

Another objective of the invention is to ensure that DNA isolation can be carried out in shorter time compared to the kits used for DNA isolation, at lower costs and without requiring high cost infrastructure.

Another objective of the invention is to form the DNA bonding column and to ensure reformation from used DNA bonding column parts.

Another objective of the invention is to reduce the number of disposable substances with the DNA isolation kit and to offer the opportunity to reuse the plastic materials used for DNA bonding column and kit.

5

Description of the Figures

Figure 1: DNA Bonding column

Figure 2: Agarose Gel Electrophoresis Analysis Image

Figure 3: Polymerase Chain Reaction Analysis Image

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Description of References in the Figures

The parts on the figures are enumerated, and their descriptions are provided hereunder:

15 1 : DNA Bonding column

Description of the Invention

The invention relates to a genomic DNA isolation method, kit and production method thereof, that is economic, high quality and
20 efficient, free of carcinogenic and mutagenic agents, with no negative impact on the environment and the researcher (applicator) in terms of health, and that can be used effectively on generative and vegetative plant parts and many plant varieties and species.

25

For DNA isolation, first of all, the DNA bonding column (1) should be formed and the tissue lesion solution, DNA bonding

solution and DNA washing and separation from silica solutions should be prepared.

FORMING THE DNA BONDING COLUMN

Three different types of DNA bonding columns (1) are formed at the DNA isolation kit of the invention developed from a multitude of live and lifeless plant species. The first column is the novel 1st DNA bonding column (1), and is illustrated in Figure 1.

1st DNA BONDING COLUMN

The chemicals required for column equilibrium solution (CES) used for the purpose of promoting higher quantity and quality of DNA molecules to bond to silica and ensure longer term availability of the columns for the 1st DNA bonding column (1) are 0,2-1,2 M 3-morpholino propane-1-sulfonic acid (MOPS, pH 6,5-7,5), 2,4-5,6 M sodium chloride (NaCl), stock Triton-X-100 and Stock isopropanol.

For example, in order to prepare 30 mL of CES, 17,55-21,55 mL distilled water, 3 mL MOPS, 4,5 mL NaCl, 0,45-0,65 mL Triton-X-100 and 2,5-6,5 mL isopropanol is added in order. 1st DNA bonding column (1) comprises the process steps of;

- o Placing the Filter papers within the tube using a sterile forceps and loading bar and adding 15-67 mg silica thereon,
- o After the silica surface equalizes, pressing the O-ring on at least one filter paper using the loading bar,
- o Adding 0,20-0,75 mL column equilibrium solution (CES) to the column,
- o Keeping the mixture at 22-26°C for 1-3 hours, and
- o Centrifuging the mixture for 1-2 minutes at the rate of 6000-12000xg.

2nd DNA BONDING COLUMN

2nd DNA bonding column (1) prepared for the DNA isolation kit is
5 obtained from full silica based DNA bonding column (1) used
previously. Obtaining 2nd DNA bonding column (1) from used DNA
bonding column (1) comprises the process steps of;

- Sorting the used DNA bonding column (1) according to the
type of components such as exterior tube, inner tube,
10 silica, supporting layers and O-ring,
- Keeping the components within 0,01-0,7 N hydrochloric acid
(HCl) at stoichiometric proportion of at least 3 times by
volume (3/1) for 25-36 hours,
- Rinsing the components with distilled water for at least
15 3 times and autoclaving,
- Adding, consecutively for 2-5 times, 0,5-0,75 mL 0,01-0,7
N HCl to the columns,
- Centrifuging the mixture for 1-2 minutes at the rate of
6000-12000xg,
- 20 • Centrifuging the empty DNA bonding column (1) for 1-2
minutes at the rate of 6000-12000xg,
- Adding 0,5-0,75 mL CES to the columns and keeping the same
at 22-26°C for 1-3 hours, and
- Centrifuging the mixture for 1-2 minutes at the rate of
25 6000-12000xg.

3rd DNA BONDING COLUMN

The 3rd DNA bonding column (1) prepared for the DNA isolation
kit, on the other hand, is for checking whether the DNA solutions
30 and methodology employed at the developed DNA isolation kit of
the invention are compatible at the commercially available DNA

bonding columns (1). Obtained results indicated that the solutions and methodology are compatible with all kinds of silica based DNA bonding column (1).

5 PREPARING THE TISSUE LESION SOLUTIONS

Tris(hydroxyl methyl)amino methane (Tris), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), sodium laurel sulfate (SLS), Sodium deoxycholate (SDC), polyvinyl polypyrrolidone (PVPP) and sodium azide (NaN_3) are used for preparing the tissue lesion solutions. The solution containing the specified chemicals is called M_1 . As the chemicals SDS, SLS and SDC present anionic characteristics, solutions M_{2A} and M_{2B} were formed in order to inhibit the bonding indicators to the silica particles that compete with the DNA molecules in DNA bonding column (1). These solutions should be used according to the condition of the samples, such as plant species and freshness, dryness, polysaccharide and oil contents. As the proteins fractionize by being processed by the proteinase K, the cellular RNA molecules (rRNA, mRNA, tRNA and other types of RNA) are washed with RNase enzyme in company with the lesion solution.

Preparation of Solution M_1 comprises the process steps of;

- Mixing 1-3 M Tris (pH 7,5-8,5) and 0,5-1,5 M (pH 7,5-8,5) EDTA,
- Adding Tris-EDTA mixture to distilled water,
- Autoclaving and sterilizing the mixture,
- Adding 12,5-25,5 mL 10-15% SDS, 12,5-25,5 mL 10-15% SLS, 12,5-15,5 mL 5-10% SDC, 25-50 mL 5-10% PVPP, 2,5-5,5 mL Triton-X-100, 2,5-5,5 mL Tween-20 and 1,2-2,75 mL 4-8% NaN_3 to the mixture,
- Filtering and sterilizing the solution.

The RNase and Proteinase that are added to solution M₁ as exogen have been tested by employing spectrophotometric and electrophoretic (agarose gel) methods where K enzymes show activity. it is possible to see from the agarose gel electrophoreses analysis image shown in Figure 2 that there are no RNA residue and protein/polysaccharide residues. Moreover, it is further possible to see from the polymerase chain reaction analysis image shown in Figure 3 that the isolated samples lack proteinase K activity and that there is no residue that would prohibit DNA polymerase activity. In addition, Table 1 gives the spectrophotometer reading values for DNA samples obtained from various plant parts.

Table 1. spectrophotometer reading values for DNA samples obtained from various plant parts

Plant parts	A230	A260	A280	A260/A280	A260/A230
Root	0,126	0,225	0,123	1,79	1,83
Seed	0,213	0,417	0,225	1,96	1,85
Leave	0,120	0,228	0,123	1,90	1,85
Placenta	0,072	0,126	0,063	1,75	2,00
Petal	0,120	0,234	0,123	1,95	1,90
Gemma	0,225	0,384	0,210	1,71	1,83
Radicle	0,261	0,519	0,270	1,99	1,92
Pedicle	0,111	0,192	0,102	1,73	1,88
Embryo	0,108	0,219	0,117	2,03	1,87
Pollen	0,225	0,429	0,207	1,91	2,07

An A260/A280 ratio above 1,6 indicates that it is a usable DNA, and a ratio in the range of 1,7-2,1 indicates that it is a very

high quality DNA. Moreover, if A260/A280 ratio is above 2,2, then some RNA molecules are present together with the DNA. An A260/A230 value in the range of 1,6-3,0 indicates that the DNA is of high quality, and if such ratio is above 3, then it indicates that some residues (some substances either isolated together with the DNA or added afterwards) affect absorbance. The spectrophotometric and agarose gel electrophoresis analyses clearly suggest that the RNase and Proteinase K enzymes added into the solution M₁ as exogen are active. When solution M₁ is used in conjunction with the exogen proteinase K and RNase enzymes, the proteins bonded to the cell wall and the cellular membranes and the DNA molecules are removed. RNase enzyme is used for the purpose of washing the RNA type nucleic acids thus preventing their isolation together with the DNA during DNA isolation. Moreover, the spectrophotometric analyses have confirmed that it doesn't involve microorganism activities at the room temperature for one year as it contains NaN₃.

Preparation of Solution M_{2A} comprises the process steps of;

- Dissolving 67,8-78,8 g potassium acetate (KCH₃COOH) in 60-80 mL distilled water,
- Adding 23-30 mL 12-14 M glacial acetic acid to the solution,
- Agitating the mixture,
- Adding 65-85 mL distilled water to the mixture.

Preparation of Solution M_{2B} comprises the process steps of;

- Mixing 42,8-56,2 g guanidine HCl and 30-42 mL 3,4-4,8 M NaCl,
- Adding distilled water until the volume of the solution reaches 100 mL.

PREPARING DNA BONDING SOLUTIONS

The solutions that enable the DNA to bond to the silica column are called as M_3 and M_4 , and some detergents, chaotropic agents and salts were used when preparing these solutions.

5

Preparation of Solution M_3 comprises the process steps of,

- Adding 50-80 mL M_2 solution to 105-115 g guanidine HCl,
- Adding 12,5-25,5 mL 80-100% Triton-X-100 and 67,5-87,5 mL distilled water to the mixture.

10

Preparation of Solution M_4 comprises the process steps of,

- Adding 165-195 mL 96-100% ethyl alcohol and 12,5-25,5 mL 80-100% Triton-X-100 to 37,8-54,3 g guanidine HCl,
- Adding ethyl alcohol to the mixture in order to increase the volume of the mixture to 200-250 mL.

15

PREPARING DNA WASHING AND SEPARATION FROM SILICA SOLUTIONS

The solutions that enable the DNA to remain bonded to the silica particles and other molecules to detach from the silica particles are called as M_5 and M_6 , and these solutions comprise of some detergents, chaotropic agent and alcohol. In addition, the solution denominated as M_7 is also used as DNA washing and separation from silica solution.

20

Preparation of Solution M_5 comprises the process steps of,

- Mixing 38,75-49,75 mL water with 25 mL 0,5-1,5 M MOPS (pH 7,2-7,9),

25

- Adding 50-75 mL 3,6-4,4 M NaCl and 1,25-2,50 mL 2-4% (w/v) NaN_3 to the mixture,
- Adding 25-50 mL M_{2A} solution and 50-100 mL 96-100% ethyl alcohol to the mixture.

5

Preparation of Solution M_6 comprises the process steps of,

- Mixing 8-12 mM Tris and 0,5-1,2 mM EDTA (pH 7,4-8,4),
- Adding 0,08-1,25 mL 5 M NaCl and 0,25-0,50 mL NaN_3 to the mixture,
- 10 • Adding 5,67-14,6 mL distilled water and 175-195 mL 96-100% ethyl alcohol to the mixture.

Preparation of Solution M_7 comprises the process step of,

- Mixing 25-35 mL 0,01-0,06 M Tris (pH 7,5-8,5) with 175-225
15 mL distilled water.

The DNA isolation process can be initiated upon preparation of the DNA solutions used at the DNA isolation kit. The DNA isolation process comprises of four phases, which are;

- 20 **A.** Tissue fractionation, expositing the cells and cell lesion,
- B.** Expositing the DNA molecules,
- C.** Bonding of the DNA molecules to the silica particles, and
- D.** Washing and separation of the DNA molecule from silica.

- 25 **A. Tissue fractionation, Expositing the cells and cell lesion;**

The tissue fractionation process that varies based on the plant species and the type of the tissue differ for dry and fresh plant

parts and plant seeds. Tissue fractionation, expositing the cells and cell lesion process comprises the process steps of;

- Fractionating the naturally dried or artificially dried plant parts, plant seeds and lyophilized samples at micro mills, and the fresh plant parts in liquid nitrogen,
- Collecting 0,03-0,08 g samples from dried plant parts, and 0,1-0,3 g samples from fresh tissue parts, and 0,1-0,15 g from plant seeds and placing the samples into 1,5-2,0 mL tubes,
- Adding 300-600 μ L M_1 solution and 10-20 μ L 20 mg/mL Proteinase K enzyme to the plant samples,
- Mixing the obtained solutions for 30-90 seconds,
- Keeping the mixtures at 50-70°C for 10-20 minutes and agitating the mixtures for 30-45 seconds once per every 5 minutes.

B. Expositing the DNA molecules

The expositing the DNA molecules step realized after incubation at the final step of tissue fractionation and cell lesion process comprises the process steps of;

- Adding solution M_{2A} or M_{2B} to the mixtures at half the stoichiometric ratio (1/2) of solution M_1 by volume,
- Mixing the obtained solutions for 30-60 seconds,
- Keeping the mixtures at 50-70°C for 10-20 minutes and agitating the mixtures for 15-45 seconds once per every 5 minutes,
- Following incubation, centrifuging the solution at 24-26°C for 10-20 minutes at the rate of 6000-12000xg.

C. Bonding of the DNA molecules to the silica particles

Bonding of the DNA molecules to the silica particles initiated by collecting 0.5 mL from centrifuged supernatant comprises the process steps of;

- 5 • Adding 0,25-0,5 mL solution M₃ and 0,25-0,5 mL ethanol or isopropanol to supernatant,
- Agitating the mixtures for 30-60 seconds,
- Transferring 0,5-0,7 mL to the DNA bonding column (1),
- Centrifuging the DNA bonding column (1) at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and decanting the solution collected at the exterior tube,
- 10 • Repeating transfer of 0,5-0,7 mL solution to the DNA bonding column (1) and bonding of the DNA molecules to the silica particles,
- Adding 0,25-0,5 mL solution M₄ solution to the inner chamber of the DNA bonding column (1) for the purpose of selective bonding of the DNA molecules to the silica particles and removal of the pigments, and
- 15 • Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and decanting the solution collected at the exterior tube.
- 20

D. Washing the DNA molecule and separation of it from silica

The step of washing and separation of the DNA molecule from silica required as proteins, lipids, polysaccharide and pigment residues are linked to the DNA molecules and the silica surface comprises the process steps of;

- Adding 500-750 µL solution M₅ to the DNA bonding column (1),
- Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and discharging the bottom chamber,
- 30 • Adding 500-750 µL solution M₆ to the mixture,

- Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and discharging the bottom chamber,
- Centrifuging the DNA bonding column (1) at 24-26°C for 1-2 minutes at the rate of 6000-11000xg,
- 5 • Separating the DNA bonding column (1) from the exterior chamber and transferring into the tube,
- Adding 30-200 µL solution M₇ kept at 50-70°C to the center of the silica column,
- Incubating the mixture at 50-70°C for 5-10 minutes, and
- 10 • Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg.

At the developed DNA isolation kit of the invention, the incubation periods of solutions M₁ and M₂ can be prolonged for
15 DNA isolation depending on the species and tissues. The incubation period of solution M₁ at the tissue fractionation and cell lesion phase can be in the range of 10-30 minutes, and the incubation period of solutions M_{2A} and M_{2B} at the exposing the DNA molecules phase can be in the range of 20-40 minutes.

20

The volume of solution M₇ can be reduced down to 0,03 mL at the phase of washing and separation of the DNA molecule from silica at the DNA isolation kit in cases where it is required to extract 0,5-1,0 micrograms of DNA per microliter.

25

The temperature can be increased up to 70-85°C at the DNA isolation kit in order to achieve effective decomposition for plant species such as Origanum, Thymus, hemp, cumin, etc. at the phase of washing and separation of the DNA molecule from silica.

30

It is possible to add chloroform at identical stoichiometric ratios (1/1) by volume after addition of solution M₂ at the DNA isolation kit at the phase of expositing the DNA molecules for the tissues such as pericarp, petal, placenta, sepal, etc. that are rich in pigment and polysaccharides. Furthermore, for these tissues, the phase expositing the DNA molecules comprises the following process steps after the centrifuging process;

- Adding 2,4-5,6 M NaCl at stoichiometric ratio of 0,1 (1/0,1) by volume and isopropanol at stoichiometric ratio of 0,9 (1/0,9) by volume on 0,5 mL supernatant,
- Centrifuging the solution at 24-26°C for 10 minutes at the rate of 6000-11000xg, and
- Dissolving the pellets obtained in 0,25-0,39 mL solution M₇. After these process steps, the phase of bonding of the DNA molecules to the silica particles can be maintained without any changes.

DNA isolation has been performed at the DNA bonding columns (1) using the DNA solutions especially from seed, radicle and pedicle parts of the members of *Poaceae* family such as wheat, barley, oat, paddy, triticales, corn, stolon *agrostis tenuis*; from ovule, seed, micro tuber, pedicle, root, leaf, pericarp, placenta, tuber, and stolon parts of the member of *Solanaceae* family, such as aubergine, potato, tomato, pepper; from leave, ovule, seed, root, sepal, petal, pollen parts of the cotton species (*Gossypium hirsutum* L. and *G. barbadense* L.); from the seeds of the member of *Leguminosae* family, such as lentil, beans, pea, trefoil and the leaves of medical aromatic plants, such as laurel, raziyane, sage, thyme etc. The quality and quantity of the isolated DNA samples have been determined through spectrophotometric measurements. In addition, the samples have been subjected to agarose gel electrophoresis technique and determined using ethidium bromide dyeing technique.

Form of Implementing the Invention in the Industry:

The genomic DNA isolation method, kit and production method of the invention, that is economic, high quality and efficient,
5 free of carcinogenic and mutagenic agents, with no negative impact on the environment and the researcher (applicator) in terms of health, and that can be used effectively on generative and vegetative plant parts and many plant varieties and species, can be qualified as having superior characteristics than other
10 DNA isolation kits and methods.

CLAIMS

1. The invention relates to a genomic DNA isolation method and kit, characterized in that the DNA isolation method comprises
5 the process steps of;

A. Tissue fractionation, Expositing the cells and cell lesion,

- Fractionating the naturally dried or artificially dried plant parts, plant seeds and lyophilized samples
10 at micro mills, and the fresh plant parts in liquid nitrogen,
- Collecting 0,03-0,08 g samples from dried plant parts, and 0,1-0,3 g samples from fresh tissue parts, and 0,1-0,15 g from plant seeds and placing the samples
15 into 1,5-2,0 mL tubes,
- Adding 300-600 μ L M_1 solution and 10-20 μ L 20 mg/mL Proteinase K enzyme to the plant samples,
- Mixing the obtained solutions for 30-90 seconds,
- Keeping the mixtures at 50-70°C for 10-20 minutes and
20 agitating the mixtures for 30-45 seconds once per every 5 minutes,

B. Expositing the DNA molecules,

- Adding solution M_{2A} or M_{2B} to the mixtures at half the stoichiometric ratio (1/2) of solution M_1 by volume,
25
- Mixing the obtained solutions for 30-60 seconds,
- Keeping the mixtures at 50-70°C for 10-20 minutes and agitating the mixtures for 15-45 seconds once per every 5 minutes,
- Following incubation, centrifuging the solution at 24-
30 26°C for 10-20 minutes at the rate of 6000-12000xg,

C. Bonding of the DNA molecules to the silica particles,

- Adding 0,25-0,5 mL solution M₃ and 0,25-0,5 mL ethanol or isopropanol to supernatant,
- Agitating the mixtures for 30-60 seconds,
- Transferring 0,5-0,7 mL to the DNA bonding column (1),
- 5 • Centrifuging the DNA bonding column (1) at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and decanting the solution collected at the exterior tube,
- Repeating transfer of 0,5-0,7 mL solution to the DNA bonding column (1) and bonding of the DNA molecules to the silica particles,
- 10 • Adding 0,25-0,5 mL solution M₄ solution to the inner chamber of the DNA bonding column (1) for the purpose of selective bonding of the DNA molecules to the silica particles and removal of the pigments, and
- 15 • Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and decanting the solution collected at the exterior tube,

D. Washing and separation of the DNA molecule from silica,

- Adding 500-750 µL solution M₅ to the DNA bonding column (1),
- 20 • Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and discharging the bottom chamber,
- Adding 500-750 µL solution M₆ to the mixture,
- 25 • Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg and discharging the bottom chamber,
- Centrifuging the DNA bonding column (1) at 24-26°C for 1-2 minutes at the rate of 6000-11000xg,
- 30 • Separating the DNA bonding column (1) from the exterior chamber and transferring into the tube,

- Adding 30-200 μ L solution M₇ kept at 50-70°C to the center of the silica column,
- Incubating the mixture at 50-70°C for 5-10 minutes, and
- Centrifuging the mixture at 24-26°C for 1-2 minutes at the rate of 6000-11000xg,

Through obtaining DNA bonding column (1) that is economic, high quality and efficient, free of carcinogenic and mutagenic agents, with no negative impact on the environment and the researcher (applicator) in terms of health, and that can be used effectively on generative and vegetative plant parts and many plant varieties and species.

2. A DNA isolation method and kit as mentioned in Claim 1, characterized in that the method for obtaining the 1st DNA bonding column (1) comprises the process steps of;

- Placing the Filter papers within the tube using a sterile forceps and loading bar and adding 15-67 mg silica thereon,
- After the silica surface equalizes, pressing the O-ring on at least one filter paper using the loading bar,
- Obtaining column equilibrium solution (CES) by mixing 3 mL 0,2-1,2 M 3-morpholino propane-1-sulfonic acid (MOPS, pH 6,5-7,5), 4,5 mL 2,4-5,6 M sodium chloride (NaCl), 17,55-21,55 mL distilled water, 0,45-0,65 mL Triton-X-100 and 2,5-6,5 mL isopropanol in a separate tube,
- Adding 0,20-0,75 mL column equilibrium solution (CES) to the column,
- Keeping the mixture at 22-26°C for 1-3 hours, and,
- Centrifuging the mixture for 1-2 minutes at the rate of 6000-12000xg.

3. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, the method for obtaining 2nd DNA bonding column (1) from used DNA bonding column (1) comprises the process steps of;

- 5 • Sorting the used DNA bonding column (1) according to the type of components such as exterior tube, inner tube, silica, supporting layers and O-ring,
- Keeping the components within 0,01-0,7 N hydrochloric acid (HCl) at stoichiometric proportion of at least 3 times by
10 volume (3/1) for 25-36 hours,
- Rinsing the components with distilled water for at least 3 times and autoclaving,
- Adding, consecutively for 2-5 times, 0,5-0,75 mL 0,01-0,7 N HCl to the columns,
- 15 • Centrifuging the mixture for 1-2 minutes at the rate of 6000-12000xg,
- Centrifuging the empty DNA bonding column (1) for 1-2 minutes at the rate of 6000-12000xg,
- Adding 0,5-0,75 mL CES to the columns and keeping the same
20 at 22-26°C for 1-3 hours, and
- Centrifuging the mixture for 1-2 minutes at the rate of 6000-12000xg.

4. A DNA isolation method and kit as mentioned in Claim 1,
25 characterized in that, preparation of Solution M₁ used at the phase of tissue fractionation and cell lesion comprises the process steps of;

- Mixing 1-3 M Tris (pH 7,5-8,5) and 0,5-1,5 M (pH 7,5-8,5) EDTA,
- 30 • Adding Tris-EDTA mixture to distilled water,
- Autoclaving and sterilizing the mixture,

- Adding 12,5-25,5 mL 10-15% SDS, 12,5-25,5 mL 10-15% SLS, 12,5-15,5 mL 5-10% SDC, 25-50 mL 5-10% PVPP, 2,5-5,5 mL Triton-X-100, 2,5-5,5 mL Tween-20 and 1,2-2,75 mL 4-8% NaN_3 to the mixture,
- 5 • Filtering and sterilizing the solution.

5. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of Solution M_{2A} used at the phase of exposing the DNA molecules comprises the process steps
10 of;

- Dissolving 67,8-78,8 g potassium acetate (KCH_3COOH) in 60-80 mL distilled water,
- Adding 23-30 mL 12-14 M glacial acetic acid to the solution,
- Agitating the mixture,
- 15 • Adding 65-85 mL distilled water to the mixture.

6. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of solution M_{2B} used at the phase of exposing the DNA molecules comprises the process steps
20 of;

- Mixing 42,8-56,2 g guanidine HCl and 30-42 mL 3,4-4,8 M NaCl,
- Adding distilled water until the volume of the solution reaches 100 mL.

7. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of solution M_3 used at the phase of bonding the DNA molecules to the silica particles comprises the process steps of;

- 30 • Adding 50-80 mL M_2 solution to 105-115 g guanidine HCl,

- Adding 12,5-25,5 mL 80-100% Triton-X-100 and 67,5-87,5 mL distilled water to the mixture.

8. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of solution M₄ used at the phase of bonding the DNA molecules to the silica particles comprises the process steps of;

- Adding 165-195 mL 96-100% ethyl alcohol and 12,5-25,5 mL 80-100% Triton-X-100 to 37,8-54,3 g guanidine HCl,
- Adding ethyl alcohol to the mixture in order to increase the volume of the mixture to 200-250 mL.

9. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of solution M₅ used at the phase of washing and separation of DNA molecule from silica comprises the process steps of;

- Mixing 38,75-49,75 mL water with 25 mL 0,5-1,5 M MOPS (pH 7,2-7,9),
- Adding 50-75 mL 3,6-4,4 M NaCl and 1,25-2,50 mL 2-4% (w/v) NaN₃ to the mixture,
- Adding 25-50 mL M_{2A} solution and 50-100 mL 96-100% ethyl alcohol to the mixture.

10. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of solution M₆ used at the phase of washing and separation of DNA molecule from silica comprises the process steps of;

- Mixing 8-12 mM Tris and 0,5-1,2 mM EDTA (pH 7,4-8,4),
- Adding 0,08-1,25 mL 5 M NaCl and 0,25-0,50 mL NaN₃ to the mixture,

- Adding 5,67-14,6 mL distilled water and 175-195 mL 96-100% ethyl alcohol to the mixture.

11. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, preparation of solution M₇ used at the phase of washing and separation of DNA molecule from silica comprises the process steps of;

- Mixing 25-35 mL 0,01-0,06 M Tris (pH 7,5-8,5) with 175-225 mL distilled water.

10

12. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, the incubation period of solution M₁ at the tissue fractionation and cell lesion phase can be in the range of 10-30 minutes, and the incubation period of solutions M_{2A} and M_{2B} at the expositing the DNA molecules phase can be in the range of 20-40 minutes for DNA isolation depending on the species and tissues used.

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13. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, the volume of solution M₇ can be reduced down to 0,03 mL at the phase of washing and separation of the DNA molecule from silica in cases where it is required to extract 0,5-1,0 micrograms of DNA per microliter.

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14. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, the temperature can be increased up to 70-85°C in order to achieve effective decomposition for plant species such as Origanum, Thymus, hemp, cumin, etc. at the phase of washing and separation of the DNA molecule from silica.

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15. A DNA isolation method and kit as mentioned in Claim 1, characterized in that, adding chloroform is possible at identical stoichiometric ratios (1/1) by volume after addition of solution M₂ at the DNA isolation kit at the phase of expositing the DNA molecules for the tissues such as pericarp, petal, placenta, sepal, etc. that are rich in pigment and polysaccharides.

16. A DNA isolation method and kit as mentioned in Claim 15, characterized in that, at the phase of expositing the DNA molecules, the expositing the DNA molecules comprises the following process steps after the centrifuging process;

- Adding 2,4-5,6 M NaCl at stoichiometric ratio of 0,1 (1/0,1) by volume and isopropanol at stoichiometric ratio of 0,9 (1/0,9) by volume on 0,5 mL supernatant,
- Centrifuging the solution at 24-26°C for 10 minutes at the rate of 6000-11000xg, and
- Dissolving the pellets obtained in 0,25-0,39 mL solution M₇.

1/2

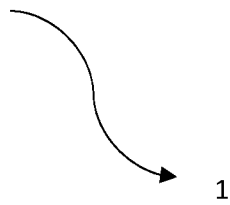
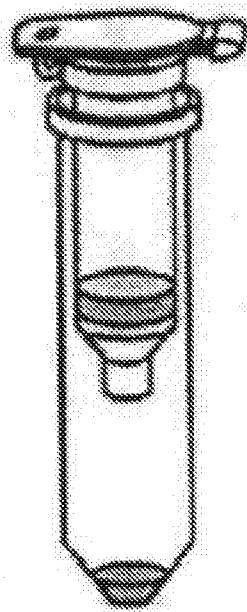


FIGURE 1

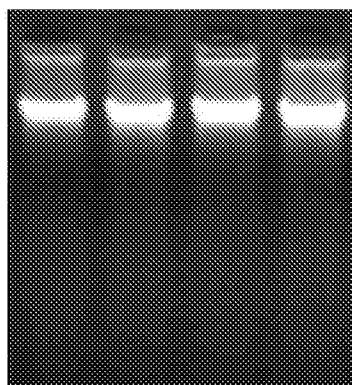


FIGURE 2

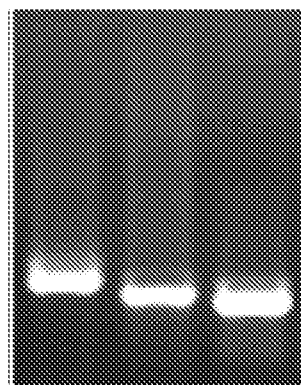


FIGURE 3