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AN EFFICIENT METHOD FOR THE ISOLATION OF PUREST FORM OF DNA FROM THE OKRA PLANT

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Abstract:

Abelmoschusenculentus (L.) does not have only great nutritional value but also possess some pharmacological activities. This plant is useful for the treatment of genito-urinary disorders, chronic dysentery, curing ulcers and gave relief from hemorrhoids. There are no significant levels of an anti-nutritional factor or endogenous toxins reported in this plant to date. In this study,a new method developed to isolate the purest form DNA from the okra leaves. The young leaves of Okra were collected into the centrifuge tube and several steps have taken to isolate the DNA from the okra plant leaves. It is difficult to isolate genomic DNA from the green leaves of the okra because the tissue of okra contains high mucilaginous acidic polysaccharides content. The DNA isolated by this method was tested for yield, purity, and ability to be amplified by PCR. In this experiment sodium citrate was used for the removal of mucilaginous acidic polysaccharides contain in Okra leaves. these give best result in the highest yields of total genomic DNA.

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INTRODUCTION:

Abelmoschus esculentus (L.), also known as Bhendi or Lady's finger, belongs to the Malvaceae family. The most common name of this plant is okra. *Abelmoschus esculentus* (L.) Plant can be cultivated throughout the tropical, subtropical and high temperate zones of the world. Okra has comparatively high nutrition value and it is agood component in developing country [1].Okra plant is the most popularly grown in Aisa, among the different species of genus *Abelmoschus*. This plant has great commercial value due to its great nutritional value[2].The height of the Okra plant in nearly about two meters. The leaves of the Okra is around 10-20 cm long and broad, palmately lobed with 5-7 lobes. The flower of this plant is about 4-8 cm in diameter, contains five petals which are white to yellow in color[3]. The fruits of this plant should be harvested when immature and eaten as a vegetable. This is commercially grown in many countries like India, Iran, Turkey, etc[4].

For the isolation of the purest form of DNA requires molecular techniques. Due to this reason, the modified CTAB methods are used for the isolation of the DNA [5].Polysaccharides are the viscous glue in nature which makes the DNA unmanageable in pipetting[6].

MATERIAL AND METHODS:

The experiment was conducted in January- June 2017 in Adithya Biotech lab & Research PVT. Ltd. Raipur, Chhattisgarh, India. All the experiments were conducted in triplicates.

DNA extraction of okra plant:

DNA extraction was done by following the standard methods of Meena et.al 2014 [1] with slight modification.

Sodium citrate methods of DNA extraction:

The 100mg of young leaves of Okra were collected into the centrifuge tube and 10mg of PVP (Polyvinyl pyrrolidone) was added to the foliar sample. The leaves were crushed in the presence of 2ml liquid nitrogen (Liq.N2). After the crushing,1.8 ml of sodium citrate buffer was added to the solution, the solution was allowed to centrifuge at 10000 rpm (i.e. rotation per minute) for three minutes. After the centrifugation process, the supernatant was discarded. Again the sodium citrate buffer solution was added to the tube and allowed to centrifuge at 10000 rpm for three minutes. When stickiness(Latex) of the sample is removed, 600 µl C-TAB buffer solution was added to the solution, put in the water bath for 40 minutes at 65ºC then add CI (i.e. chloroform: isoamyl:: 24:1, 600µl) in the equal volume as a buffer and centrifuge the sample at 10000 rpm for ten minutes. After the centrifugation collects the supernatant in the separate tube and discards the pellet, add an equal volume of CI as supernatant, centrifuge the solution for 10 minutes at 10000 rpm. Add sodium citrate one by ten of the supernatant and absolute alcohol double to the volume of the supernatant, incubate the solution at -20ºC for 20 minutes and after incubation centrifuge the solution the solution for 10 minutes at 10000rpm. After centrifugation, discard the supernatant and add 200 µl of 70 % alcohol for washing. After the addition of 70% alcohol, the solution was centrifuged for 10 minutes at 10000 rpm. Discard the supernatant and dry the pellet and add 60 µl of TE (i.e. Tris EDTA) buffer and perform gel electrophoresis.

RESULTS AND DISCUSSION:

DNA Isolation:

It is difficult to isolate genomic DNA from the green leaves of the okra because the tissue of okra contains high mucilaginous acidic polysaccharides content. This study shows the new method to extract high quality and quantity genomic DNA from the dried leaves of lady's finger. The DNA isolated by this method was tested for yield, purity,and ability to be amplified by PCR.

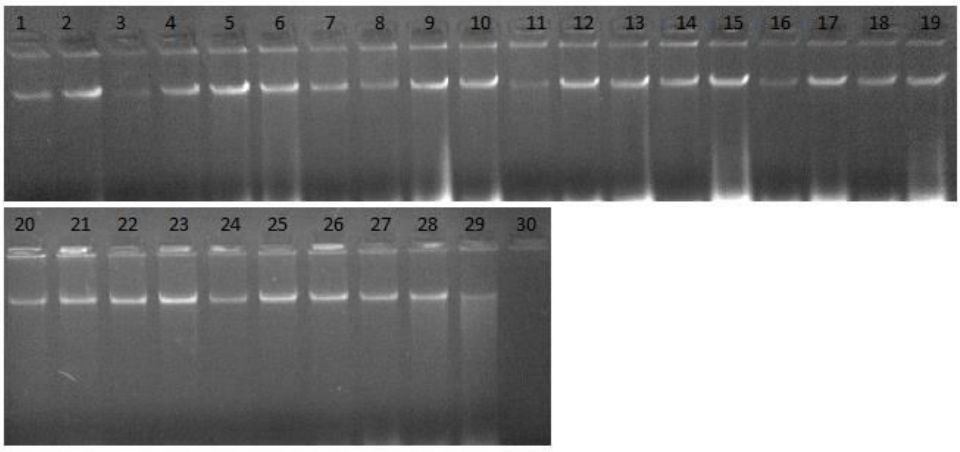

Fig 1- Isolated DNA of the okra plant

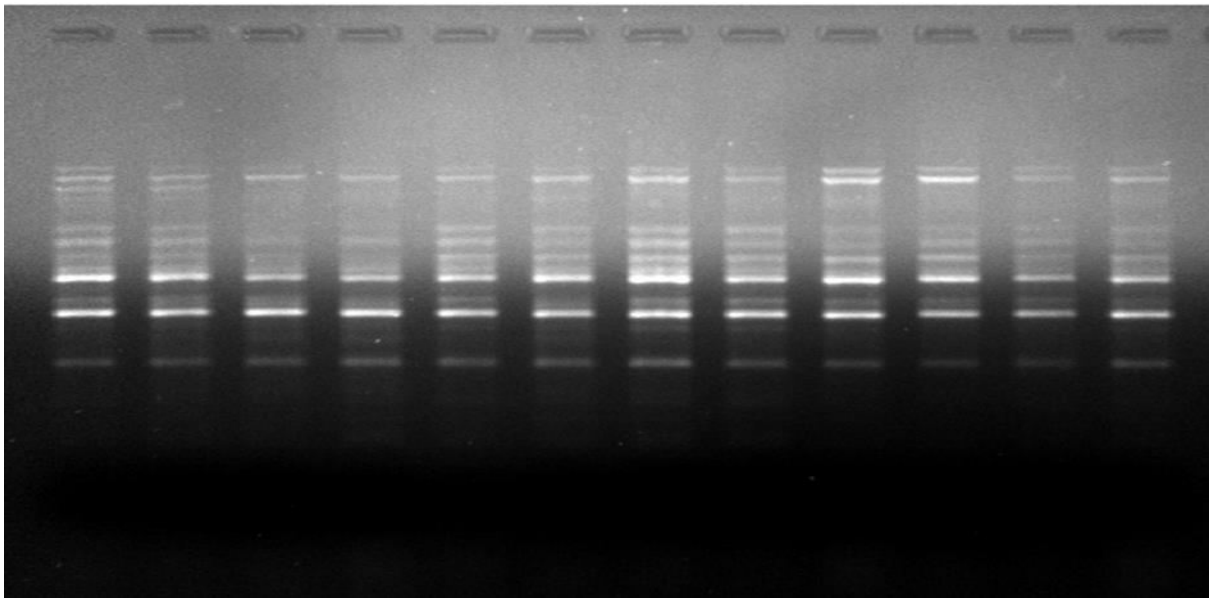

Fig2:Amplification result of Okra DNA sample by using ISSR primer(UBC 807)

CONCLUSION:

Okra plant is best known for its edible green seed pods. The green seed pods rapidly become fibrous and woody and, to be palatable as a vegetable, must be harvested when immature. The fresh Okra contains 90% water, 2% protein, 7% carbohydrates, and fat in a negligible amount. It is difficult to isolate genomic DNA from the green leaves of the okra because the tissue of okra contains high mucilaginous acidic polysaccharides content. This study exhibited the new methods for the extraction of genomic DNA. In this experiment sodium citrate was used for the removal of mucilaginous acidic polysaccharides contain in Okra leaves. These give best result in the highest yields of total genomic DNA. The Salt use during precipitation of DNA increases the solubility of polysaccharides in ethanol thus preventing co-precipitation with DNA.

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