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OFFICE OF THE DIRECTOR OF AGRICULTURE AND FOOD PRODUCTION, ODISHA, BHUBANESWAR

No: 2M (05)60/2014/ 41086 Dt. 01.11.2014

To

The Dean Research
Odisha University of Agriculture and Technology
Bhubaneswar

Sub: DNA finger printing protocol and the list of SSR primers.

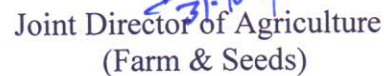
Ref: This office L. No. 2M (05)60/2014/40550 dated 29.10.2014

Sir,

In continuation to this office letter no. and subject cited above, please find enclosed herewith the list of SSR primers and the protocol for DNA finger printing in Rice.

This is for your kind information and necessary action.

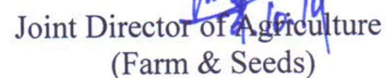
Yours faithfully,



Joint Director of Agriculture
(Farm & Seeds)

Memo No: 41087 **Dt.** 01.11.2014

Copy forwarded to the SCO, State Seed Testing Laboratory, Bhubaneswar for information and necessary action.



Joint Director of Agriculture
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DNA FINGERPRINTING PROTOCOL

Genomic DNA Extraction

Solution preparation

1. Preparation of CTAB (cetyltrimethyl ammonium bromide) buffer:

		Final Concentration
CTAB	= 2 gm	2%
NACl	= 8.18 gm	1.4 M
Tris (1M, pH , 8.0)	= 10 ml (or Tris = 1.21 gm)	100 mM
EDTA (0.25 M, pH 8.0)	= 8 ml (or EDTA = (0.744 gm)	20 mM

Dissolve in 70 ml sterile water, adjust pH to 8.0 with dilute HCl/ NAOH and adjust volume to 100 ml. Autoclave at 15psi for 20mins. Store at room temperature for short term use and store in freezer for long term use.

2. TE buffer:

For preparing 100 ml of TE, 0.2 ml of EDTA (0.5M) and 1ml of Tris (1M) & 98.8ml of water was added and autoclaved.

3. Chloroform: Isoamyl alcohol (24:1)

Chloroform (HPLC grade)	= 96 ml
Isoamyl alcohol (HPLC grade)	= 4 ml
Total volume	= 100 ml

Mix well & stored bottle at room temp/ in freezer

Procedure (Mini preparation)

- Collect healthy rice leaf sample (500 mg to 1gms) from young seedlings in 2ml micro-centrifuge tube and keep in ice.
- Cut leaf into small pieces and place in a well of plate/ mortar
- Add 300 µl CTAB buffer to sample and ground with a thick glass rod /pestle. Further add 300µl CTAB buffer and transfer mixture into 2ml eppendorf tube.
- Add 600µl of chloroform: Isoamyl alcohol (24:1), mix properly by inverting and centrifuge at 10- 17ºC at 8000 rpm for 10mins.

- Transfer top aqueous phase to another 2 ml micro-centrifuge tube.
- Then add 2µl of RNAase A (DNAse free) to the sample, mix and keep at 37ºC in water bath for 1-1.5 hour.
- Add equal volume of chloroform: Isoamyl alcohol (24:1) to the sample, mix by inverting and centrifuge at 8000 rpm for 15mins. Then transfer the supernatant into another 1.5 ml micro centrifuge.
- Add 1ml(2.5 volume) of pre-chilled Ethanol and kept it in -20ºC for 1hour to overnight. Centrifuge at room temperature at 8000 rpm for 15mins. Then supernatant decant supernatant with taking care that clot does not dislodge.
- Wash pellet with 70% of Ethanol & air dry.
- Suspended DNA in 100-200µl of 1X TE buffer & stored in -20ºC till further use.

PCR (Polymerase Chain Reaction)

DNA amplification is performed in a Gradient Thermal Cycler (Verity, Applied BioSciences) with a reaction volume of 20µl containing 1.5mM Tris HCL (pH 8.75), 50mM KCL, 2mM $MgCl_2$, 0.1% TrotonX-100, 200µM each of dATP, dCTP, dTTP, dGTP, 4pMole of each forward and reverse primers, 1 unit of Taq Polymerase and 30ng of genomic DNA. The reaction mixture was first denatured for 4 mins at 94^0C and then subjected to 35 cycles of 1 min denaturation at 94^0C, 1 min annealing at 55^0C, and 1.5 min extension at 72^0C; and then a final extension for 5 mins at 72^0C.

Visualization of PCR product/ genomic DNA

Solution preparation

1. 10X TBE buffer

For preparation of 500 ml of 10x TBE buffer,

Tris	= 54 gm
Boric Acid	= 27.5 gm
EDTA	= 4.6 gm

Dissolve chemical in distilled water, adjust pH 8.0 with concentrate HCL/ NAOH and make the final volume upto 500ml. Then solution autoclaved and stored at room temperature.

2. 1X TBE buffer (gel running buffer)

10X TBE buffer = 100 ml

Distilled Water = 900 ml

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3. **6X Loading dye**

Bromophenol blue = 100 mg

Tris (1M , pH 8.0) = 5 ml

Glycerol = 60 ml

0.25M EDTA = 8 ml

Sterile distilled water = 27 ml

Final volume 100 ml and store in micro centrifuge in freezer

Electrophoresis

Procedure

1.For quantification of genomic DNA, run 2µl DNA sample along with 2µl of loading dye in in 0.8% of agarose gel,

2. Add 7 µl loading dye to PCR amplified products, mix well and load 10µl sample to well in 2.5% agarose gel containing 0.10µg/ml Ethidium Bromide. Run in 1X TBE (pH 8.0) for 3-5 hr. Use 50bp DNA ladder for comparing molecular weight of PCR products. Photograph using a Gel Documentation System (SynGene).