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DNA isolation protocol for Vigna radiata with free of phenolics

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Method Article

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Abstract

Introduction

Pulses are major constituents of human diet and are mainly grown in marginal lands under rainfed conditions. Among the pulses, blackgram and mungbean are the major pulses contributing much for the total production. Mungbean \[_Vigna radiata_ \(L.) Wilczek] \(2n=22), primarily an inbreeding species is cultivated throughout tropical Asia including India which accounts for 45% of the world production. _Vigna_ species are an important source of protein for people, particularly in tropical Africa and Asia and several _Vigna_ species have been domesticated in Asia. Species in Leguminosae \(Fabaceae) genus _Vigna_ subgenus _Ceratotropis_ are called the Asian _Vigna_ because of their natural distribution. Recently the taxonomy of the subgenus has been revised \(Tomooka et al., 2002c). On the basis of these taxonomic revisions, a new taxonomic system was proposed for subgenus _Ceratotropis_ that consists of 21 species, of which eight are used as food, fodder or cover crops \(Tomooka et al., 2001, 2002a). In the new system, the subgenus was divided into three sections, Angulares, Ceratotropis and Aconitifoliae consisting of 12, 4 and 5 species, respectively.

Reagents

The materials used for DNA isolation are agroinoculaed leaves, seeds and embryos. a. Cetyl Trimethyl Ammonium Bromide \(CTAB) Extraction Buffer CTAB 2% W/V Tris HCl \(pH 8.0) 200 mM Sodium Chloride 1.4 M EDTA 20 mM 2-mercaptoethanol 0.1% v/v \(Mercaptoethanol was added immediately prior to use) b. Tris EDTA \(TE) Buffer Tris HCl \(pH 8.0) 10 mM EDTA 1 mM c. Ice-cold Isopropanol d. Chloroform: Isoamylalcohol \(24:1 V/V) e. Phenol: chloroform \(1:1) f. Sodium acetate \(3.0 M) pH 5.2 \(pH adjusted using glacial acetic acid) g. Ethanol \(100% and 70%) h. PVP \(1%)

Equipment

1.Waterbath 2. Centrifuge

Procedure

1) Grind the leaves with 600 µl of CTAB buffer, transfer into an eppendorf tube and incubate for 30 min. at 65 °C with occasional mixing after adding PVP and mercaptoethanol. 2) Remove the tubes from the water bath and allow to cool at room temperature. 3) Add equal volume of chloroform: isoamyl alcohol mixture \(24:1) and mix by inversion for 15 min. 4) Centrifuge at 4000 rpm for 20 min and transfer the clear aqueous phase to a new sterile tube. 5) Add equal volume of phenol: chloroform \(1:1. Centrifuged at 4000 rpm for 20 min and transfer the clear aqueous phase to a new sterile tube. 6) Add 1/3 volume of ice-cold isopropanol and 3M Sodium acetate and mix gently by inversion. 7) Then, centrifuge at 4000 rpm for 20 min to pellet the DNA and discard the supernatant. 8) Wash the DNA pellet with 70 per cent alcohol. After washing with 70% alcoholair dry the DNA pellet. 9) Discard the alcohol and air dry the DNA

pellet completely. Depending upon the size of the pellet, dissolve the DNA in 100-200 µl of 1X TE \(pH 8.0) and store at 4 ºC.

Timing

2 hours

Critical Steps

The supernatant in each step should be carefully transferred without disturbing the other layers.

Troubleshooting

Repeat the purification steps if there is a presence of phenolics in DNA.

Anticipated Results

PCR compatible DNA \(highly purified DNA) can be isolated by this method.