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EVOLUTIONARY RELATIONSHIP OF *CODIAEUM VARIEGATUM* SPECIES BASED ON SDS-PAGE

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Abstract:

SDS-PAGE is a technique used to separate macromolecules based on their migration through an electric field. Protein may vary in their charge, molecular weight and shape, all of these characteristics will influence their movement in an electric field. The evolutionary relationship based on SDS-PAGE of croton species were observed using SDA-PAGE method. The biochemical and molecular marker enable detailed analysis of genetic relationship, physiological adaptation and variation within population and historical continuity in phylogenetic relationship between various species. The results were recorded based on Nie and lie formula. According to this formula all the varieties were closely related. SDS-PAGE provided valueable information for the identification and evolutionary relationship of croton species.

Key word: *SDS-PAGE, Codiaem variegatum, Evolutionary relationship.*

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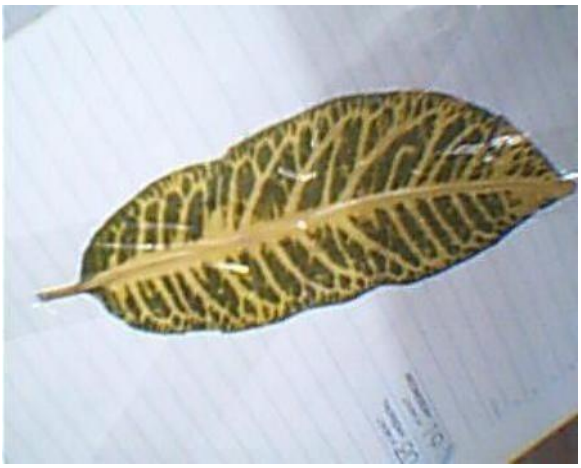
INTRODUCTION:

Croton Codiaeum variegatum, commonly known as the Croton and sometimes called Joseph's Coat,belongs to the family Euphorbiaceae and one of the most popularDecorative foliage plants.
Croton is a very popular houseplant and its attraction is because of vivid foliage colors and varied leaf shapes. (Plant-ideas.net). Croton plant type is shrub and its flower is yellow, without information on the number of petals. Its height is about 1.2 m and grows at altitude of about
270-660m.



Croton Specie 1

Croton Specie 2

Taxonomy

Kingdom Plantae	Plants
Subkingdom Tracheobionta	Vascular plants
Super division Spermatophyta	Seed plants
Division Magnoliophyta	Flowering plants
Class Magnoliopsida	Dicotyledons
Subclass	Rosidae
Order	Euphorbiales
Family Euphorbiaceae	Spurge family
Genus CodiaeumJuss.	Codiaeum
Species	Codiaeum variegatum

SDS-PAGE is an abbreviation for sodium dodecyl sulfate (SDS) polyacrylamide gelelectrophoresis.SDS-PAGE is a molecular biology technique used to separate proteins accordingly by size. SDS-PAGE also can separate DNA and RNA molecules.Various types of biochemical and molecular markers have been used to investigate the evolution and systematic relationship between various Codiaeum species. The biochemical and molecular markers enable detailed analysis of genetic relationship, physiological adaptations and variation within population, and historical continuity in phylogenetic relationship between various species (Werhahn et al., 2002).

Electrophoresis may be the main technique for molecular separation in today's cell and molecular biology laboratory. It is a very powerful technique and reasonably easy and inexpensive,and has become very common. In spite of the many physical arrangements for the apparatus, and regardless of the medium through whichm olecules are allowed to migrate, all electrophoretic separations depend upon thecharge distribution of the molecules being separated. (Deyl et al., 1979)

SDS-PAGE USES

SDS-PAGE has a number of uses, which include:
- Establishing protein size.
- Protein identification.
- Determining sample purity.
- Identifying disulfide bonds.
- Quantifying proteins.
- Blotting applications.

SDS-PAGE stands for sodium dodecyl (lauryl) sulfate-polyacrylamide gelelectrophoresis. The SDS portion is a detergent. You may recognize it if you read the ingredients lists on your shampoo, soap, or toothpaste. The purpose of the SDSdetergent is to take the protein from its native shape, which is basically a big globular, and open it up into a linear piece. It's kind of like taking a wadded up ball of string and untangling it into one straight, long piece. This will allow it to run more efficiently downthe gel and will get you better results, since it's easier to compare

two linear pieces of something rather than towards of the same thing. In more scientific terms, it is an anionic detergent that binds quantitatively to proteins, giving them linearity and uniformcharge, so that they can be separated solely on the basis if their size. The SDS has a high negative charge that overwhelms any charge theprotein may have, imparting all proteins with a relatively equal negative charge. TheSDS has a hydrophobic tail that interacts strongly with protein (polypeptide) chains. The number of SDS molecules that bind to a protein is proportional to the number of aminoacids that make up the protein. Each SDS molecule contributes two negative charges,over whelming any charge the protein may have. SDS also disrupts the forces that contribute to protein folding (tertiary structure), ensuring that the protein is not only uniformly negatively charged, but linear as well.The polyacrylamide gel electrophoresis works in a similar fashion to an agarose gel,separating protein molecules according to their size. In electrophoresis, an electriccurrent is used to move the protein molecules across a polyacrylamide gel. The polyacrylamide gel is a cross-linked matrix that functions as a sort of sieve to help"catch" the molecules as they are transported by the electric current. The polyacrylamide gel acts somewhat like a three-dimensional mesh or screen. The negatively charged protein molecules are pulled to the positive end by the current, butthey encounter resistance from this polyacrylamide mesh. The smaller molecules areable to navigate the mesh faster than the larger one, so they make it further down the gel than the larger molecules. This is how SDS-PAGEseparates different protein molecules according to their size.

MATERIAL AND METHODS:

SDS-PAGE Methodology

Preparation of Samples

● Leaves of three different varieties of croton were collected as samples ,coded as1,2,3.

● Washed with water and cleaned to remove any dust or impurity on the surface ofthe leaf.

● Weighed up to 0.2 gm.

● Grinding was then done in the autoclaved mortar and pestle.

● Sample buffer was used 800ul and grinded till the paste formed.

● Clean eppendorf tube used for sample storage and placed on ice.

● Same procedure repeated for the remaining samples.

● Samples were centrifuged at 10,000 rpm for 10 min. at 4^{o}C.

● The supernatant was stored.

● Eppendorfs stand kept in the refrigerator.

● Mix protein 1:1 with the sample buffer. Heat sample by Boiling at 65 degrees C for10 minutes

Preparation of Gel Assembly

● The apparatus required for gel assembly was washed with distilled water anddried using cotton.

● The seal made by using rubber tubing to make a watertight chamber.

● Spacers were then placed in between two plates to make space for gels. Twoclean plates with two teflon spacers make a single assembly.

● Both plates were clipped and distilled water passed in between plates to check
leakage and then proceed further.

Gel Preparation

● Preparations involve casting of two different layers of acryl amide between glassplates. The lower layer (separating, or resolving, gel) is responsible for actually separating polypeptides by size.

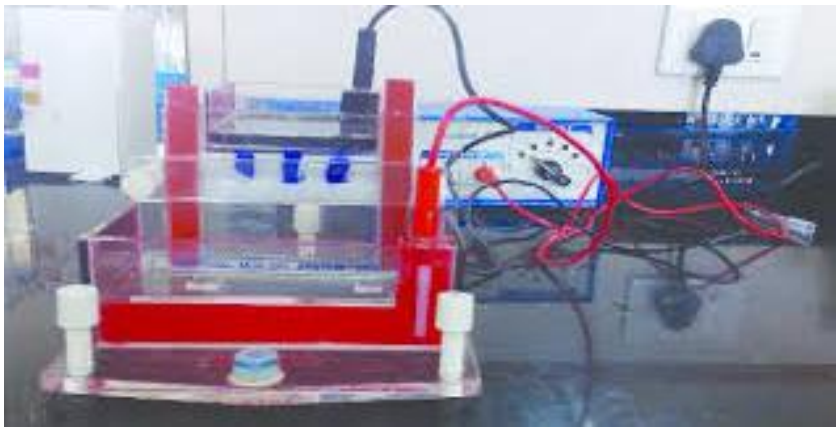
● The upper layer (stacking gel) includes the sample wells, and of a compositionthat causes the samples to become compressed (stacked).

Separating Gel Preparation

● The gel was poured immediately between the plates.

● Immediately after pouring the gel mix, the bubbles removed with water-saturatedbutanol. The purpose of butanol is to produce a smooth, completely level surfaceon top of the separating gel, so that bands are straight and uniform.

● Polymerization confirmed by pulling some of the remaining gel mix and allowing itto stand, and checked after 15 min.



Gel preparation used in SDS-PAGE.

Stacking Gel Preparation

● The butanol was removed completely.
● Stacking gels poured quickly up to the top edge of the plate and combs wereinserted as stacking gel polymerizes more quickly.
● The comb was inserted carefully trying not to get bubbles stuck underneath.
● The polymerization occurred within 20 min.

Assembly, Loading and Running of Gels

● The comb was taken out slowly, to minimize the chance of air bubble in the wells.
Good wells can result in good electrophoresis.
● Then the plates were clamped to the system.
● Both buffer chambers filled with gel running buffer
● The samples were then loaded and 20ul was loaded in each well by using pipette.
● Power leads then attached after filling both buffer chambers.
● The electrophoresis done at 100 milliamps, 50 watts, 300 volts for five hours.
● When the blue dye front reached the bottom, the power turned off and cables
removed.
● The plates were separated and the gel was dropped into a staining dish containingwater.
● After a quick rinse, the water poured off and stain added.

Gel Staining

● For staining the gel we use two different techniques i.e. Coomassie Blue Stainingand Silver Staining.

Coomassie Blue Staining

● 500 ml of freshly prepared Coomassie Blue Stain was poured in the gel-stainingtray.
● The gel was separated from the glass plates and dipped in the stain and coveredwith foil.
● Left the gel for at least 15 min or preferably overnight.

Destaining

● 50 ml of destaining solution poured after removing staining solution.
● Left the gel for 15 minutes.
● The gel destained for at least six times.

Gel Fixation

● The gel placed in fixative solution for about 20 minutes.

Gel Observation

● The fixative solution poured out and gel placed on a clean transparency.
● The gel observed and scored on a florescent light illuminator.

Gel Preparation

Then prepared 12.5% polyacrylamide gel (Resolving) with the following ingredients:

Ingredients Amounts

● Acrylamide-bis 12,500 µl
● DH2O 10,500 µl
● Separating buffer 4,000 µl
● 10% SDS 3,000 µl
● TEMED 20 µl
● Ammonium phosphate 300 µl

Some amount of butanol had been added quickly to remove the bubbles.After polymerization, prepared the 12.5% polyacrylamide gel (Stacking) with thefollowing ingredients:

Ingredients Amounts

● Acrylamide-bis 2,500 µl
● DH2O 11,000 µl
● Stacking Gel 5,000 µl
● 10% SDS 2,000 µl
● TEMED 20 µl
● Ammonium phosphate 300 µl

After Preparation of Resolving gel I had few drops of butanol, also add few staking geland pour in plates. Insert the comb when solidifies. The comb had been removed before polymerization of the gel.After

polymerization, the comb had been removed from the gel.

Electrophoresis

The apparatus tanks had been filled with the running buffer.The voltage set at 300 volt. 20-30 µl of the samples were loaded in the wells.Constant current was applied and allowed to run for at least 4hours or till the samplereached to the other end.

Gel Staining

For staining the gel we use two different techniques i.e. Coomassie Blue staining andSilver Staining.

Coomassie Blue Staining

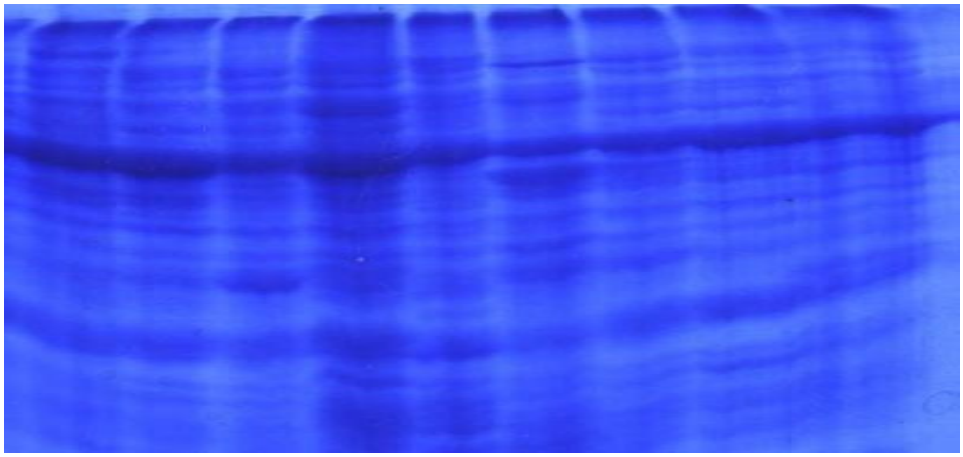
500 ml of freshly prepared Coomassie Blue Stain was poured in the gel staining tray.The gel had been separated from the glass plates and dipped in the stain.Covered the tray with the foil.Left the gel for at least 15 min or preferably overnight.The stain had been poured out and around 50 ml of destain solution has been added.Left the gel for 15 minutes. Destained the gel for at least three times.Place in fixative. Bands formed in the gel.

RESULTS AND OBSERVATION:

The gel was scored the basis of Nei and Li's co-efficient formula.

A	B	C
_	+	_
_	+	+
+	+	_
_	_	_
_	+	+
_	+	_
+	_	+
+	+	+
+	+	+
_	+	+
+	+	_
+	+	+
+	+	+
+	+	+
+	+	+
+	+	+
+	_	+
_	_	_
_	+	+
11	15	13

Coomassie Blue Staining. The gel picture shows the bands appear after SDS-PAGE. The sample s were extracted from the three varities of croton**.**



DENDOGRAM

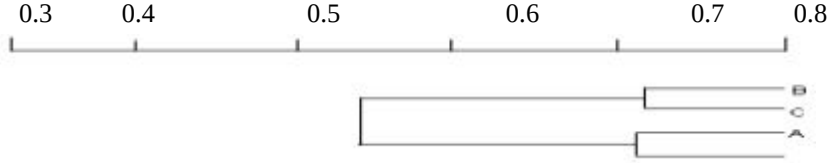


Fig Showing the resultant Dendrogram and evolutionary relationship among thedifferent varities.

DISCUSSION:

It is clear from the dendrogram three varities are identified on the basis of proteinby SDS-PAGE.

The dendogram values suggested that the varities B and C are Closely related, B,C andA are Some what related.In genral all three varities are evolutionary related to each other.

SDS is novel technique of protein finger printing.It is a common tool used by the biochemist to discover the molecular weight of protein in protein sample. The technique separates the different protein in sample by their size.The purpose of the SDS detergent is to take the protein from its native shape, which is basically a big glob, and open it up into a linear piece.In procedure the sample to be analyzed first mixed with detergent SDS, SDSbinds all protein and break up all weak bonds and smooth them out so that theyexist in long rope like chains. SDS binds (14g SDS/1g) protein and each boundSDS has one negative charge this give all the polypeptide sample rougly the same charge to mass ratio.The sample (SDS-protein solution) is placed on top of a polyacrylamide gel. Thegel contains Tris HCl buffer and is covered at the top and bottom with liquid Trisglycinerunning buffer. An electric field (– to +) is applied across the gel from thetop phase (stacking gel) to the bottom phase (resolving gel), causing thenegatively charged "current-carrying" anions to migrate down through the geltoward the positive electrode. From our resulted dendrogram by the gel we can compare this dendogram with other varities of crotons.

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