



### Somoclonal variation studies on *Cassia occidentalis* Linn using SDS-PAGE

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#### Abstract:

The present study was aimed to reveal the somoclonal variation in *Cassia occidentalis* Linn using SDS- PAGE as a tool. For the electrophoresis studies, mother plant and leaves segments derived calli and nodal segment derived plants young leaves were harvested and ground on ice cold mortar and pestle with 0.1 M phosphate buffer (pH 7.0). The SDS- PAGE gel electrophoresis was performed by Anbalagan method. The protein banding profile showed the different banding profile in leaves segments derived calli. The mother plants and nodal segments derived plantlets showed the similar banding pattern in the gel system. The SDS-PAGE protein banding patterns of the somoclonal variant is used as a biochemical marker for the future plant breeding or genetic improvement programme.

**Keywords:** SDS-PAGE; Somoclonal variation; Biochemical marker.

#### Introduction

Medicinal plants are of great interest to the researchers in the field of biotechnology, as many of the drug industries depend on plants for the production of valuable compounds used in health applications<sup>1</sup>. Biotechnology

involves modern tissue culture, cell biology and molecular biology offers the opportunity to develop new germplasms that are well adapted to changing demands. Biotechnological tools are also equally important for multiplication and genetic enhancement of the medicinal plants by adopting various techniques such as *in vitro* regeneration and genetic transformation. It can also be harnessed for the production of secondary metabolites using plant as bioreactors<sup>2</sup>. Somaclonal variation, a frequent occurrence in plant cell cultures, illustrated by phenotypic variation of either

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genetic or epigenetic origin, may be a rationalization for the wide range of inhibitor activity found. The types of variation (genetic or epigenetic) that are normally observed may be varied from species to species, and it is more complicated to find out the genetic nature of the observed variation<sup>3</sup>. To reveal the variation occurrence in the cell line / *in vitro* derived plants, numerous critical analyses have been performed on the phenotypic, genetic (e.g., aneuploidy, polyploidy, or mixoploidy), cytogenetic and molecular (DNA methylation) alterations found in plant cells and/or tissues in crop breeding programs<sup>4</sup>. Several studies have shown that somaclonal variation can be assessed by analysis of phenotype, chromosome number and structure, proteins or direct DNA evaluation of plants<sup>5</sup>. Analyses of the occurrence of variants in plant cell cultures concerning biochemical phenotype have been undertaken to a lesser extent.

*Cassia occidentalis* Linn, a native plant of southern India, belongs to family Caesalpiniaceae. *C.occidentalis* is used for fever, menstrual problems, tuberculosis, diuretic, anemic, liver complaints<sup>6</sup>. The parts of the plant used are roots, leaves and seeds. *C. occidentalis* is used for fever, menstrual problems, tuberculosis, diuretic anemic, liver complaints and as a tonic for general weakness and illness<sup>6</sup>. It is also used to cure sore eyes, hematuria, rheumatism, typhoid, asthma, disorder of haemoglobin, leprosy and diabetes<sup>7</sup>. *C. occidentalis* leaf extracts possess antibacterial<sup>8,9</sup>, antimalarial<sup>10</sup>, antimutagenic<sup>11</sup>, antiplasmodial<sup>12</sup>, anticarcinogenic<sup>13</sup> and hepatoprotective properties<sup>14</sup>. A wide range of chemical compounds including achrosin, aloemodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol etc. have been isolated from this plant. The tools of modern biotechnology are being increasingly applied for plant diversity characterization<sup>15</sup>. The modern molecular approaches have been adopted to classify the medicinal plants in order to distinguish the taxon. Proteomic analyses are used not only as markers of *inter-specific* and *intra-specific* taxa, but also they may be used as markers of different

developmental stages of the same plant. It is necessary to understand the molecular differences among the selected medicinal plants for successful utilization and identification. Polyacrylamide Gel Electrophoresis (PAGE) is a versatile biochemical technique to detect genetic variation. In recent years, there has been explosion in the availability of different types of genetic markers<sup>16</sup>. With this knowledge, the present study was aimed to assess the somaclonal variation among the mother plant, nodal region and leaves derived calli of *C. occidentalis* using SDS-PAGE as a tool.

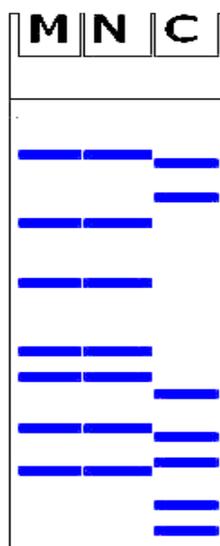
### Materials and Methods

For the electrophoresis studies, mother plant young leaves, leaves segments derived calli and nodal segment derived plants young leaves of *Cassia occidentalis* Linn were harvested and ground on ice cold mortar and pestle with 0.1 M phosphate buffer (pH 7.0). The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4°C in cooling centrifuge and the supernatant was stored at -70°C before use. SDS-PAGE was carried out by standard protocol described by Anbalagan<sup>17</sup>. After electrophoresis the gel was observed using a Vilber Loubermat gel documentation system and banding profiles of protein was compared by zymogram. Protein profiling was observed among mother plant, *in vitro* propagated nodal region and leaves derived calli of *C. occidentalis* documented using cladogram.

### Results and Discussion

Multiple regions of activity were obtained from protein electrophoretic system of mother plant, nodal region and leaves derived calli of *C. occidentalis*. The selected plants expressed bands at different regions and positions with various MW-RF values (Table 1). *Cassia occidentalis* expressed twenty one bands (Mother plant-7, Nodal region-7 & leaves derived calli - 7) in SDS-PAGE system. Based on the occurrence of protein bands in the gel system, the protein profiles of *C. occidentalis* were classified into seven regions. Region 1 showed four bands, PP1<sup>1</sup> (0.11) was shared by mother plant and nodal region of *C. occidentalis*. PP1<sup>2</sup> (0.12) and PP1<sup>3</sup> (0.16) expressed its unique presence in leaves derived calli of *C. occidentalis*. Region 2 depicted four

bands, PP2<sup>1</sup> (0.2) and PP2<sup>2</sup> (0.28) was contributed to the mother plant and nodal region of *C. occidentalis*. Region 3 expressed two bands, PP3<sup>1</sup> (0.37) was shared by both mother plant and nodal region of *C. occidentalis*. Similar to that, region 4 also revealed two bands, PP4<sup>1</sup> (0.42) was contributed to the mother plant and nodal region of *C. occidentalis*. PP4<sup>2</sup> (0.44) was specific to leaves derived calli of *C. occidentalis*. Region 5 expressed six bands, PP5<sup>1</sup> (0.5) and PP5<sup>4</sup> (0.58) were shared by mother plant and nodal region of *C. occidentalis*. PP5<sup>2</sup> (0.51) and PP5<sup>3</sup> (0.57) showed its unique expression in leaves derived calli of *C. occidentalis*. Region 6 and 7 revealed two bands, PP6<sup>1</sup> (0.67) and PP7<sup>1</sup> (0.75) were distinct to the leaves derived calli of *C. occidentalis*. The proteins with MW-Rf values of 0.12, 0.16, 0.44, 0.51, 0.57, 0.67 and 0.75 were expressed their presence in the *in vitro* culture. The presence of these proteins confirmed the somaclonal variation occurrence in the SDS-PAGE system of *C. occidentalis*. Based on the banding pattern of *C. occidentalis* protein profile, the zymogram was constructed using MS-Excel (Fig. 1).



**Fig. 1: Zymogram of *Cassia occidentalis* Linn**

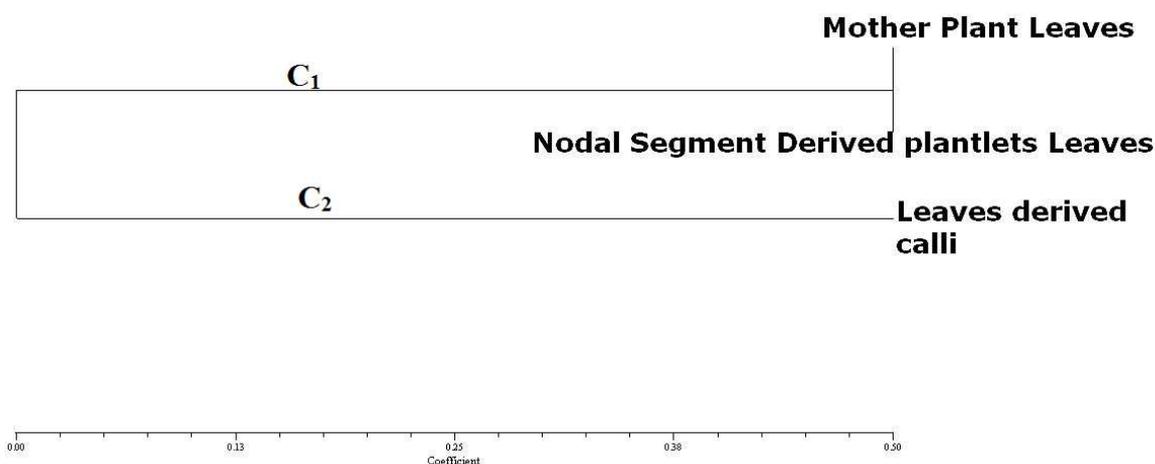
Note: M - Mother Plants Leaves; N - Nodal segment derived plantlets Leaves; C- Leaves derived Calli

**Table 1: SDS-PAGE Protein profile of *C. occidentalis***

MW-RF	<i>C. occidentalis</i>		
	M	N	C
0.11	+	+	
0.12			+
0.16			+
0.2	+	+	
0.28	+	+	
0.37	+	+	
0.42	+	+	
0.44			+
0.5	+	+	
0.51			+
0.57			+
0.58	+	+	
0.67			+
0.75			+

Note: M - Mother Plants Leaves; N - Nodal segment derived plantlets Leaves; C- Leaves derived Calli

Based on the protein expression of mother plant, nodal region and leaves derived calli of *C. occidentalis*, the cladogram was constructed by UPGMA method using NTSys. The cladogram of *C. occidentalis* showed two major clusters (Cluster 1 and Cluster 2). The cluster 1 showed the similarity and variation between the mother plant and nodal region of *C. occidentalis* and cluster 2 showed the uniqueness of leaves derived calli of *C. occidentalis*, it is varied from mother plant and nodal region of *C. occidentalis* and thus confirms the somaclonal variation of *in vitro* propagated leaves derived calli from the mother plant and nodal region of *C. occidentalis* (Fig. 2).



**Fig. 2: Cladogram of *C. occidentalis* based on the Protein Profiles**

Environment is an important criterion for the morphological variation, a product of genotype, but much diversity which remains unexpressed morphologically can be revealed by biochemical methods<sup>18</sup>. Studies of protein variation are important tool (PAGE) that has often been employed for morphological and somaclonal variation. The general pattern of appearance and disappearance of bands can be explained on the basis of gradual shifts of protein patterns in samples taken in the course of development due to differential activation of genes involved in synthesis at different stages of development<sup>18</sup>. The patterns of genetic variation within and among populations are of great interest to diverse fields in plant biology including *in vitro* propagation, population genetics and plant systematics. In the present study, the SDS-PAGE analysis was used to reveal the morphological and biochemical variation. Similar to the present observations, Agarwal<sup>19</sup>, Johnson<sup>20</sup> and Johnson<sup>21</sup> also observed the somoclonal variations in *Centella asiatica*, *Rhinacanthus nasutus* and *Phyllanthus amarus* and confirmed the variations using SDS –PAGE as a tool. The present study also revealed the somaclonal

variation very clearly by the banding positions occupied by the leaves derived calli in the SDS-PAGE system which will be very much useful in plant breeding and pharmaceutical industries.

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