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Original Research Article

Proteomic Analysis of *Mukia maderaspatana* (L.) M.Roem.

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Abstract:
The present study was aimed to identify the somoclonal variants of *Mukia maderaspatana* (L.) M.Roem. using protein profiles. For the electrophoresis studies, young leaves of mother plant, nodal segment derived plants and calli mediated plants were harvested and ground on ice cold mortar and pestle with 0.1 M Tris buffer (pH 7.0). The SDS- PAGE gel electrophoresis was performed by Anbalagan method. The protein banding profile confirmed the somoclonal variants presence in the calli mediated plantlets. The SDS- PAGE gel system of *M. maderaspatana* displayed twenty one bands of which six bands were present in mother plant, eight bands displayed their presence in plantlets raised from nodal expalnts and seven bands demonstrated their occurrence in the calli mediated plantlets. These protein profiles will be used as a biochemical marker for the future plant breeding or genetic improvement programme.

Keywords: SDS-PAGE; Somoclonal variation; Biochemical marker

Introduction

Mukia maderaspatana (L.) M.Roem. (Cucurbitaceae) is a slender, scabrous climber useful in vitiated conditions of pitta, burning, sensation, dyspepsia, flatulence, colic, constipation, ulcers, cough, asthma, neuralgia, nostalgia, odontalgia and vertigo[1]. *M. maderaspatana* is also reported to have the activities like hepatoprotective, antiheumtic, antiflatulent, anti-inflammatory, anticancer, antidiabetic, diuretic and stomachic and used for toothache and recommended in vertigo and biliousness also[2]. Decoctions of leaves of *M. maderaspatana* are being used by siddha practitioners in Tamil Nadu for the treatment of hypertension[3].

Somaclonal variation is a well-known incidence in plant cell and tissue cultures,

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embraces all kinds of variations that can be present among plants or cells derived from "*in vitro*" cultures[4]. Somaclonal variation is not limited to, but is predominantly occured in plant regenerated from callus. The variations can be genotypic or phenotypic, which in the later case can be either genetic or epigenetic in origin. *In vitro* propagation through indirect organogenesis studies on medicinal plants showed the somoclonal variations occurrence[5]. Number of studies proved that somaclonal variation can be assessed by analysis of phenotype, chromosome number and structure, proteins or direct DNA evaluation of plants[6, 7]. The types of variation that are commonly observed may vary from species to species, and it is often difficult to find out the genetic nature of the observed variation. Analyses of the occurrence of variants in plant cell cultures concerning biochemical phenotype have been undertaken to a lesser extent[8]. Since 1930's, electrophoresis coupled with the zymogram technique have been the tool of choice for studies of heritable variation by geneticists, systematists and population biologist. Proteomic analysis constructed a pathway to study the genetic differentiation in plant populations. In recent years proteomic analysis using SDS-PAGE is used as a powerful tool to understand the genetic variability within and between the population of plants and animals[9-12]. The presence and absence of protein bands has been found useful in the placement of the plant in taxonomic categories[13, 14]. With this knowledge, the present investigation was aimed to identify the somoclonal variants of *Mukia maderaspatana* (L.) M.Roem using SDS-PAGE.

Materials and Methods

For the electrophoresis studies, young leaves from mother plant, *in vitro* plantlet raised thorough the nodal segments and plantlets obtained from the callus of *M. maderaspatana* were harvested and grounded using ice cold mortar and pestle with 0.1 M Tris buffer (pH 7.0). The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4ºC in cooling centrifuge and the supernatant was stored at -70ºC before use. SDS-PAGE was carried out by standard protocol described by Anbalagan[15]. After electrophoresis the gel was observed using a Vilber Loubermat gel documentation system and the zymogram was constructed based on banding profiles in the gel system. For the identification of somoclonal variants, the protein profiles were converted into a "1" and "0" matrix, to indicate the presence or absence of the Rf values, respectively. Genetic similarities (GS) were estimated according to Nei and Li[16]. To identify the somoclonal variants, a cladogram was constructed by UPGMA using NTSYSpc-2.0

Result and Discussion

The relative positions of the protein bands were revealed by SDS-PAGE using three samples viz., young leaves from mother plant, *in vitro* plantlets from the nodal explants and the plantlets obtained from the callus of *M. maderaspatana* (Table 1; Fig. 1). The SDS-PAGE gels system of *M. maderaspatana* displayed twenty one bands of which six bands were present in mother plant, eight bands displayed their presence in plantlets raised from nodal expalnts and seven bands demonstrated their occurrence in the calli mediated plantlets. Based on the occurrence of protein bands in the gel system, the protein profiles of *M. maderaspatana* were classified into six active regions. Region 1, 2 and 8 were failed to express the protein bands in SDS-PAGE gel system of *M. maderaspatana*. Region 3 revealed three bands, $PP3^1$ (0.27) was shared by mother plant, *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus. Region 4 expressed with five bands, $PP4^1$(0.33) were showed its presence jointly in mother plant, *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus. $PP4^2$ (0.36) was shared by *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus. Similar to region 3, Region 5 depicted with three bands, $PP5^1$ (0.43) was shared by mother plant, *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus. Region 6 displayed with five bands, $PP6^1$ (0.52) were showed its presence commonly in mother plant and *in vitro* plantlet from the

nodal explants. $PP6^2$ (0.59) was shared by mother plant, *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus. Region 7 showed two bands, $PP7^1$ (0.66) were showed its presence jointly in *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus. Region 9 expressed three bands, $PP9^1$ (0.85) were showed its presence commonly in mother plant, *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus.

Based on the protein expression of mother plant, *in vitro* plantlet from the nodal explants and the regenerated plantlets obtained from the callus of *M. maderaspatana*, the cladogram was constructed by UPGMA method using NTSys. The cladogram of *M. maderaspatana* showed two major clusters (C_1 and C_2). The C_1 showed the closeness of the mother plant and *in vitro* plantlet derived from the nodal explants of *M. maderaspatana* and C_2 showed the uniqueness of leaves derived calli of *M. maderaspatana*, it is varied from mother plant and nodal region of *M. maderaspatana* and thus confirms the somaclonal variation of *in vitro* propagated leaves derived calli from the mother plant and *in vitro* plantlet derived from the explants of *M. maderaspatana* (Fig. 2).

Table 1: SDS-PAGE Protein profile of *M. maderaspatana*

MW-RF	Regions	Positions	*M. maderaspatana*		
			M	N	C
0.27	3	$PP3^1$	+	+	+
0.33	4	$PP4^1$	+	+	+
0.36		$PP4^2$		+	+
0.43	5	$PP5^1$	+	+	+
0.52	6	$PP6^1$	+	+	
0.59		$PP6^2$	+	+	+
0.66	7	$PP7^1$		+	+
0.85	9	$PP9^1$	+	+	+

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

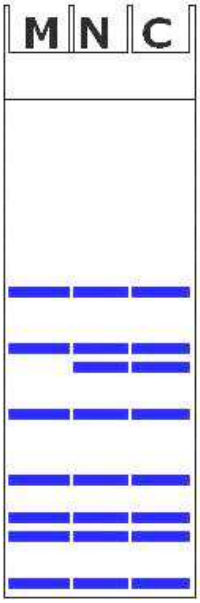

Fig. 1: Zymogram of *Mukia maderaspatana* (L.) M.Roem

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

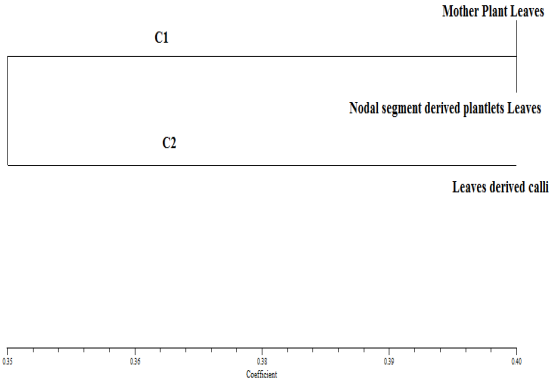

Fig. 2: Cladogram of *C. occidentalis* based on the Protein Profiles

Genetic markers are observable traits that are classified into five broad groups morphological, cytological, chemicals, protein and DNA. The use of protein as genetic marker has increased dramatically over the last decade as it has number of important advantages over more conventional morphological markers. Several workers have observed qualitative changes in insoluble proteins during development and differentiation. Changes in the protein bands during development of tissue can be detected

most conveniently by pattern shift on zymogram subsequently to electrophoresis[9-12&17]. Similar to the previous observations, in the present also we distinguised the mother plant, nodal segment derived plantlets and calli mediated plantlets using proteomic profiles. The results of present study showed the presence of variation in calli mediated plantlets. The genetic variability in the culture regenerated plants has great potential to be used for improvement of *Mukia maderaspatana* germplasm.

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