Seed segregation of five South Indian *Rauvolfia* L. species by electrophoretic protein profiling

Seed identification based on electrophoretic profiling[1] is possible as storage proteins remain stable irrespective of environmental or age factors. This technique which has been employed in systematic studies of *Lotus*[2] and *Vigna*[3] is tested with the *Rauvolfia* genus among which *R. serpentina* belongs. The Government of India has banned the export of this species which warrants legitimate species segregation even at the seed level. Seeds of some *Rauvolfia* species look almost alike and hence the five species selected for demarcation based on seed storage protein profile are *R. hookeri* Srinivas. *et.* Chithra, *R. micrantha* Hook.f., *R. serpentine* (L.) Benth. Ex Kurtz, *R. tetraphylla* L. and *R. verticillata* (Lour.) Baill.

Seeds collected from the third generation at the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) campus at Thiruvananthapuram were used for the study. Decoated seeds each of 100 mg were ground and extracted with chilled 0.3 ml 0.1 M Tris-HCl buffer, pH 8.4. The homogenate was centrifuged at 12,000 rpm for 20 min at 4°C and the supernatant[4] was either used immediately for electrophoresis or stored at 0°C.

Protein content in the sample was defatted with acetone before estimation by Lowry's method[5] using BSA as standard. Samples corresponding to 100 μg each of protein were loaded into the wells. Samples were run on a constant voltage of 150 V at 10°C for 4 h and soluble proteins were separated using native[6] and SDS–PAGE[7] on vertical 8% polyacrylamide slab gels. Gels with similar samples were replicated thrice to ensure repeatability in results.

For SDS–PAGE, sample and marker dye (20% w/v sucrose, 1% v/v bromo phenol blue (BPB), 1% w/v SDS in 75 mM Tris-HCl, pH 7.8) were kept in a water bath for 10 min at 100°C and loaded into the gel[8]. The tank buffer was supplemented with 0.1% SDS. The samples were run on a slab gel electrophoresis unit with 150 mm × 100 mm × 1 mm gel plate, along with the marker.

After separation, the sample gels were stained overnight in 0.1% Coomassie blue in 40 ml alcohol, 10 ml acetic acid and 50 ml distilled water. Destaining was done with 7% acetic acid and 40% alcohol.

Electrophorograms were prepared by plotting the gel on a transparency sheet. Relative mobility (*Rf*) and molecular weight of each protein band were calculated[9]. Band intensity was assessed visually and classified into light, medium and heavy[10], whereas band width was grouped into narrow and broad[11].

Seeds of *R. verticillata* recorded the highest soluble storage seed protein content (69.69 mg/g plant tissue), whereas the lowest (45.07 mg/g plant tissue) was recorded with *R. serpentina* (Table 1).

In native PAGE with 8% acrylamide separating gel, protein polymorphism and banding pattern varied from two (*R. verticillata* and *R. micrantha*) to five (*R. serpentina*). Molecular weight of the protein band ranged from 22.6 to 48.6 kDa. The highest range of protein, 22.6–48.4 kDa was present in *R. tetraphylla* (Table 1).

Band numbers varied in SDS–PAGE from 12 (*R. hookeri* and *R. verticillata*) to a minimum of six in *R. tetraphylla*. *R. hookeri* had the highest range of protein bands from 46.65 to 3.3 kDa, whereas lowest range of bands was exhibited by *R. tetraphylla* from 20.1 to 3.15 kDa (Table 1).

Protein bands of all species fall under 32 different molecular weights (from 46.65 to 3 kDa). *R. hookeri* and *R. micrantha* have maximum number of similar bands (five bands of MW 45.5, 43, 15.9, 6.6 and 3 kDa respectively). Bands of the same molecular weight were absent between *R. serpentina* and *R. micrantha*. All species had 1–5 unique bands, which could be observed as species identity key (Table 2).

Total bands were classified into three groups; Group A (46.65–20.1 kDa), group B (19.5–10 kDa) and group C (9.3–3 kDa). Group A displayed less protein polymorphism consisting eight bands, among which *R. micrantha* and *R. hookeri* had four bands each, *R. verticillata* had two bands, whereas *R. serpentina* and *R. tetraphylla* consist of one band each. Group B consists of totally ten bands, among which two bands each in the case of *R. verticillata* and *R. serpentina* differed in band width and intensity of staining. *R. micrantha* and *R. hookeri* each had three bands and *R. tetraphylla* had two bands.

In group C, protein polymorphism was maximum consisting of totally 14 bands. In this region, *R. verticillata* had six bands; *R. hookeri* and *R. serpentina* had five bands each, whereas *R. micrantha* and *R. tetraphylla* had three bands each. This group of bands is ideal for systematic studies among the species especially between *R. micrantha* and *R. hookeri* (Table 2).

Native PAGE revealed only few bands for higher molecular weight, which were similar among the species (Figure 1 *a*). But in SDS–PAGE, more number and range of protein bands were present along with the highest inter-specific protein polymorphism, which is quantitatively expressed in terms of staining

Table 1. Soluble protein content and polymorphism of storage protein bands

Plant material	Amount of soluble protein (mg/g)	Total number of bands in SDS–PAGE	Range of proteins in SDS–PAGE (kDa)	Total number of bands in native PAGE	Range of proteins in native PAGE (kDa)
R. hookeri	56.9	10	46.65–3.3	4	47.8–37.2
R. micrantha	65.79	12	46.35–3.3	2	48.6–45.6
R. serpentina	45.07	10	40.5–3.15	5	48.2–28.2
R. tetraphylla	61.312	6	20.1–3.15	3	48.4–22.6
R. verticillata	69.69	12	45.45–3	2	47.8–47

Table 2. SDS–PAGE seed storage protein profile of five *Rauvolfia* species

Molecular weight (kDa)	Relative mobility (*Rf*)	*R. verticillata*	*R. serpentina*	*R. tetraphylla*	*R. micrantha*	*R. hookeri*
Group A						
46.65	0.06					+*
46.35	0.07				+*	
45.45	0.09	+			+	+
43	0.13				+	+
40.5	0.15		+*			
34.2	0.2					+*
29.7	0.24				+*	
20.1	0.32	+		+		
Group B						
19.5	0.34	+*				
18.3	0.39		+	+		+
18	0.41				+*	
15.9	0.51			+	+	+
15.6	0.53	+	+			
14.3	0.57	+*				
12.9	0.6		+*			
11.6	0.63		+			+
10.8	0.64				+*	
10.35	0.65	+*				
Group C						
9.3	0.67			+*		
8.7	0.68		+*			
7.65	0.7	+	+	+		
6.6	0.73	+			+	+
6.3	0.74				+*	
6.15	0.75	+				+
5.4	0.8	+*				
5.1	0.82	+				+
4.95	0.83		+*			
4.35	0.87					+*
4.05	0.89		+*			
3.3	0.95				+	+
3.15	0.96		+	+		
3	0.97	+*				

*Denotes species-specific proteins.

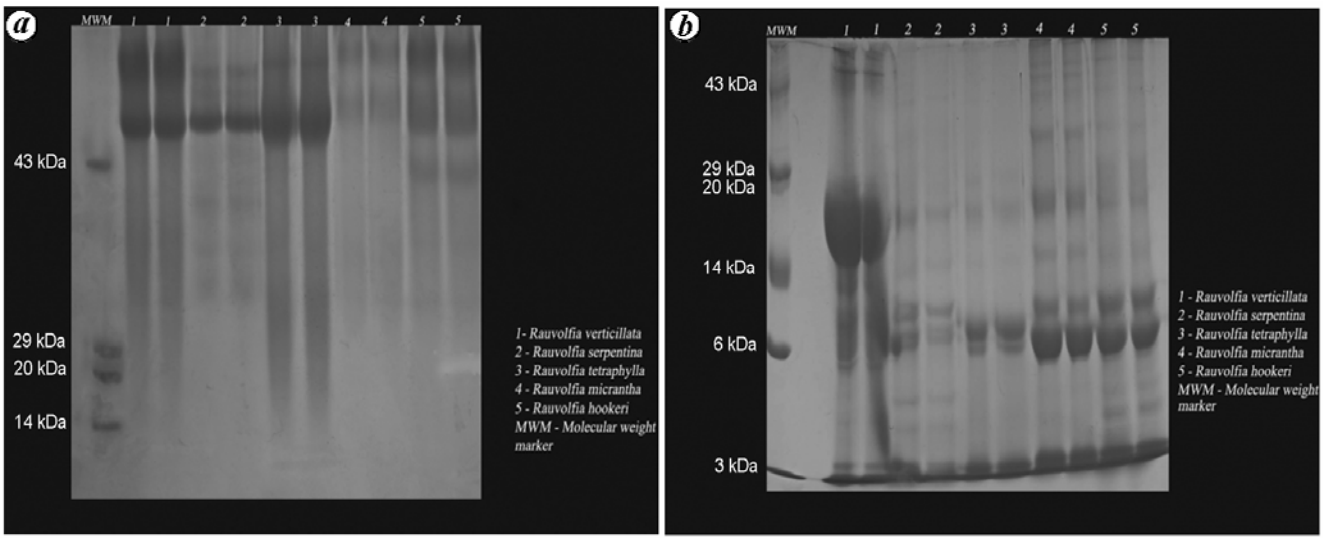

Figure 1. Native PAGE (***a***) and SDS–PAGE (***b***) of seed storage proteins of *Rauvolfia* L. species.

intensity facilitating species identification (Figure 1 *b*). It is evident from the present study that the segregation of *Rauvolfia* seeds through storage protein profiling by SDS–PAGE is reliable and reasonable.

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C. ANILKUMAR*
CHRIS IPE
S. BINDU
C. R. CHITRA
P. J. MATHEW
P. N. KRISHNAN

Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, India
**For correspondence.*
e-mail: tsbpalode@gmail.com

Evaluation of millicompost versus vermicompost

Increase in the human population, indiscriminate growth of cities, industrialization and agricultural practices have led to an increased accumulation of waste materials[1]. As nature is unable to degrade the huge quantity of wastes in a short period of time, these materials can act as a secondary host of diseases and pests[2]. All these problems forced us to revive the old traditional techniques of compost production and organic farming, which are nature's ways for the renewal of life. This results in loss of potentially valuable materials that can be processed as fertilizer, fuel and fodder. The biological treatment of these wastes appears to be most cost-effective and carries a less negative environmental impact[3].

To restore soil health, the practice of organic farming and encouraging the activities of soil invertebrates in agriculture is an essential step. The principal economic values of soil macroinvertebrates include soil turn-over, incorporation of organic matter into the mineral horizons, improvement of soil aeration by creating cavities, conversion of organic nitrogen and phosphorus into plant-assimilable forms, stimulation of soil respiratory enzymes, dispersion of microbial propagules and preservation of soil structure through humification. Biocomposting is an environmentally sound technology (EST) according to the criteria defined by the United Nations Environmental Programme (UNEP). Composting improves soil structure, texture and aeration and increases the water-holding capacity of the soil. The organic matter in the compost provides food for microorganisms, which keep the soil in a healthy, balanced condition; nitrogen, potassium and phosphorus will be produced naturally[4].

Earthworms are major component of the soil system and represent a key component in nutrient cycling of soils. The role of earthworms in organic matter decomposition, nutrient recycling of soil structure and plant productivity has been studied in detail[5]. The microorganism present in the gut of earthworm helps in degradation of organic materials[6]. Hence, the use of earthworms for waste conditioning is widely practiced all over the world for vermicomposting technology[7]. Vermicomposting is an eco-friendly technique involving no pollution and hence is the most suitable method for solid waste disposal when compared to conventional methods like land-filling, incineration, biogas production, etc.

Similarly, millipedes are known to be macrodetrivores terrestrial arthropods feeding on decaying vegetables matter and mineral soil and are represented by more than 80,000 species. They are essentially soil-dwelling and in some ecosystems, they are more important than worms as agents of soil and nutrient turnover. Although millipedes are often called 'thousand leggers', they actually have far fewer legs, and each body segment has two pairs of short legs. Millipedes do not bite or pose any danger to humans. Martens *et al*.[8] reported that earthworms and millipedes are important members of the detritus food web in the agricultural ecosystem and both use manure as the food source. Microorganism plays a crucial role in the digestion of millipedes by breaking down the cellulose into simple sugar. Further, it is reported that the degradation of organic matter and recycling of detritus energy are favoured by the gut microbes of millipedes[9]. Like earthworms, millipedes improve the soil structure and enrich the soil with nutrients. Even though millipedes are the major saprophagous fauna, so far no sufficient information is available on using millipedes for compost production. Hence, the present study is made to explore the efficiency of millipedes in converting the organic waste into useful fertilizers and comparing them with earthworms.

The raw materials for the present study, flowers of the discarded garlands, were collected from the historic Meenakshi Sundareswarar Temple in Madurai city. The predominant flower wastes of the garland include: *Jasminum sambac* (jasmine), *Calendula officinalis* (marigold), *Gomphrena globosa* (Globe Amaranth or Bachelor Button), *Celosia spicata* (cockscomb) and petals of *Nelumbo nucifera* (lotus). They were shredded into