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Analysis of protein diversity among five different varieties of Rosa species through SDS-PAGE

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Abstract : The work was done to analyze the relationship between five different varieties of *Rosa indica* belonging to the family Rosaceae collected from Tirunelveli District of Tamilnadu. The five varieties of rose were characterized by analyzing protein variability in leaf tissues through SDS PAGE. The electrophorogram showed a total of 54 bands of proteins with molecular weight ranging from 4.6 KDa to 152.5 KDa. The protein diversity analysis was conducted on the basis of presence or absence of bands and interpreted using NTSYS software; their molecular weights were also calculated. The protein variability analysis clearly showed that there was divergence among these varieties of rose.

Keywords : *Rosa indica*, SDS-PAGE, Protein variability.

1 Introduction

A rose is a woody perennial of the genus*Rosa*, within the family Rosaceae. There are over 100 species. They form a group of erect shrubs, and climbing or trailing plants, with stems that are often armed with sharp prickles. Flowers are large and showy, in colours ranging from white through yellows and reds. Most species are native to Asia, with smaller numbers native

to Europe, North America, and northwest Africa. Species, cultivars and hybrids are all widely grown for their beauty and fragrance. Rose plants range in size from compact, miniature roses, to climbers that can reach 7 meters in height. Different species hybridize easily, and this has been used in the development of the wide range of garden roses [1]. Rose tea (petals and leaves brewed as a tea) can bring down fever. It works as a diuretic to flush the toxins from the body. It can also relieve bronchial and chest congestion, provide relief from sore throat and stop runny nose. Rose water is known for its antiseptic properties and is used as an eye wash to treat eye irritation. Rose oil is used for skin treatment to smooth and moisturize the skin and to relieve skin irritation [2].

The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties [3]. The classification between various subgenera, species and subspecies are based primarily on morphological attributes. However, these morphological characters may be unstable and influenced by environmental condition [4]. Plant identification is often obscured by high species variation. Re-evaluation of the morphological variation within taxa and populations is therefore necessary [5].

Among several biochemical techniques, SDS-PAGE (Sodium Dodecyl Sulphate) has been used successfully to resolve taxonomic and evolutionary problems of several plants [6, 7]. In this technique protein is separated according to their molecular weights. Resolution of this technique is very high and therefore it could be used as a reliable tool for taxonomic purposes [8]. Its banding pattern is very stable which advocated for identification purpose in medicinal plants. It has been widely suggested that such banding patterns could be used as important supplemental method for medicinal plant identification [9, 10]. Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for its use in practical plant breeding technique [11]. Hence, in this work five different varieties of roses were chosen and their relationship was studied using SDS-PAGE.

2 Materials and Methods

Plant materials: The five different varieties of roses, a wild variety (Whitish rose) and four variedly coloured hybrid varieties (yellow, white, pink and red) were collected from Tirunelveli district of Tamilnadu. The leaf samples were maintained in deep freezer at -70° C.

Protein profiling: In each species fresh young leaves were taken for protein isolation. The separation of protein was carried out at 50°C at 100V thereafter for 3-5 hours. SDS-PAGE electrophoresis preparation was followed [12].

Data analysis: The molecular weight of the unknown proteins were calculated with reference to the molecular marker using TL 100 software and a dendrogram was constructed based on the similarity index using NTSYS software

3 Results and Discussion

Proteins were isolated from the leaves of five different varieties of rose. The total leaf protein extracts were subjected to SDS-PAGE analysis. A total of 54 bands were observed. The molecular weight of the proteins ranged from 4.6 KDa to 152.5 KDa (Table 1). The similarity coefficients were calculated on the basis of presence and absence of bands and it ranged from 0.38–0.81 (Table 2). Using Total Lab 100 protein analyzer software the molecular weight and the Rf value of each band was calculated. The least molecular weight protein (4.6 KDa) was present in red coloured variety with highest Rf value 0.89 and highest molecular weight protein (152.5 KDa) was found in yellow coloured variety with lowest Rf value 0.15 (Table 1).

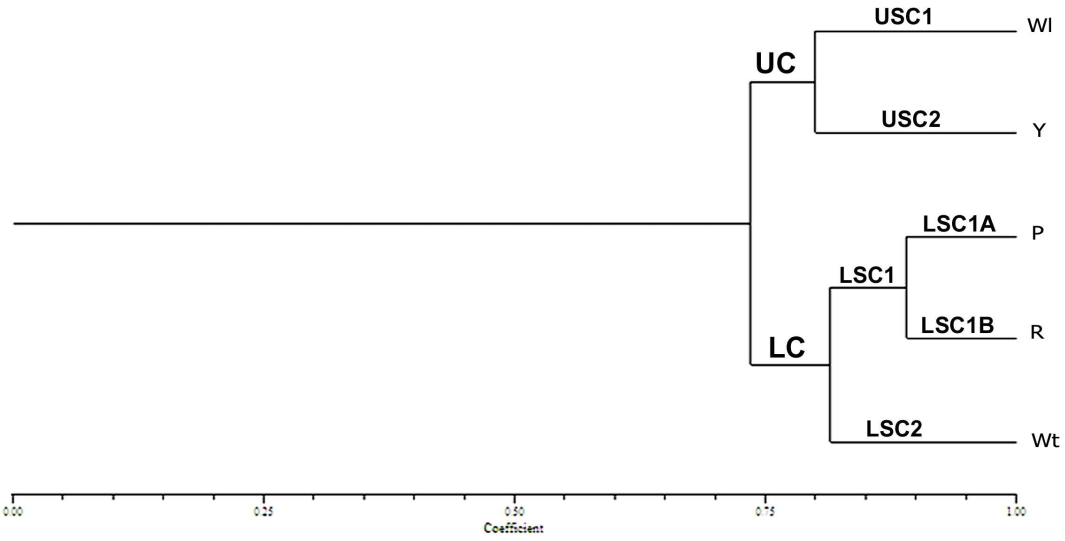
Table 1. Molecular weight and Rf value of proteins in five Rose varieties

Band No.	**Wild variety**		**Yellow coloured**		**White coloured**		**Pink coloured**		**Red coloured**	
	MW	Rf	MW	Rf	MW	Rf	MW	Rf	MW	Rf
1.	-	-	152.544	0.15	-	-	-	-	-	-
2.	-	-	118.689	0.204	-	-	119.174	0.203	114.834	0.211
3.	-	-	-	-	-	-	94.63	0.252	-	-
4.	82.402	0.282	-	-	-	-	78.17	0.293	83.639	0.279
5.	68.519	0.322	64.648	0.334	-	-	64.574	0.334	-	-
6.	57.352	0.36	-	-	-	-	53.695	0.374	52.68	0.378
7.	47.69	0.399	-	-	-	-	-	-	-	-
8.	38.879	0.443	-	-	-	-	-	-	-	-
9.	33.193	0.477	34.069	0.471	32.889	0.479	32.543	0.481	32.746	0.479
10.	26.706	0.523	-	-	25.903	0.53	26.012	0.529	26.16	0.528
11.	18.466	0.602	20.22	0.583	19.866	0.587	19.594	0.59	19.693	0.588
12.	16.08	0.632	16.807	0.622	16.281	0.629	15.974	0.633	16.154	0.631
13.	13.818	0.664	14.155	0.659	12.994	0.678	12.518	0.685	13.786	0.665
14.	11.266	0.708	-	-	11.082	0.712	-	-	-	-
15.	9.81	0.738	-	-	9.577	0.743	10.072	0.732	10.447	0.724
16.	8.542	0.767		-	-	-	-	-	-	-
17.	7.293	0.801	6.624	0.822	6.518	0.825	-	-	6.366	0.83
18.	5.315	0.869	4.888	0.887	-	-	-	-	4.668	0.897

Table 2. Similarity indices of five Rose variety based on SDS-PAGE

	wild	yellow	white	pink	red
wild	1.00				
yellow	0.38	1.00			
white	0.63	0.81	1.00		
pink	0.52	0.66	0.72	1.00	
red	0.47	0.72	0.60	0.50	1.00

The phylogenetic analysis based on similarity coefficients was done using NTSYS pc software and a UPGMA dendrogram was constructed (Fig. 1), which closely placed wild variety and white coloured hybrid variety with 75% similarity and pink and red coloured hybrid variety with 90% similarity. The dendrogram showed that the studied five different varieties of roses grouped into two main clades, UC and LC with 72% similarity. The UC is further subdivided into two subclades USC_1 and USC_2, holding the wild variety and yellow colured variety with a similarity of 75%. The LC was further subdivided into LSC_1 and LSC_2, the LSC_1 was further subdivided into two divisions, LSC_1A holding pink and red coloured variety with a similarity of 90 % and LSC_2B forming a separate clade holding white coloured variety with 82% similarity (Figure. 1).



Wl - wild variety; Y - yellow; P - pink; R - red; Wt - white

Figure. 1. Cladistical analysis of Protein samples of five different varieties of Roses

The results suggest that characteristic electrophoretic pattern was with respect to thickness, intensity and rate of mobility of bands. Occurrence of such protein polymorphism is a reflection of the complex genetic nature of the species. In the present study, the electrophoretic banding profile of total soluble leaf proteins of five variedly coloured varieties of roses, showed divergence among the varieties. These specific variations were analyzed to assess the protein polymorphism between them and clarify the genetic nature of polymorphic bands [11]. Similar study was done [13] in *Elaeagnus umbellata* (Thunb.) that belongs to the family Elaeagnaceae. The seeds of eight ecotypes from Azad, Jammu and Kashmir, Pakistan, were analyzed to study their relationship and evolution based on SDS-PAGE of total seed proteins. Phylogenetic relationship and similarity indices of some *Acacia* species using SDS-PAGE gel electrophoresis were done [14]; Phylogenetic diversity of some tomato varieties was done [11]. The protein analysis by SDS-PAGE reveals distinct differences between the varieties at the molecular level. The dendrogram constructed from the protein data, helps to understand the similarity and dissimilarity between the varieties. There is considerable amount of variability observed among the five varieties of rose; further analysis using molecular markers will help to understand the genetic variability of these varieties.

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References

[1] Rose, available from: www.en. Wikipedia.org/wiki/rose, as accessed on 23-02-2012.

[2] Medicinal value of Rose plant, available from:www.gardernguides.com/89951-medicinal values-rose-plants assessed on 23-02-2012.

[3] T. Isemura, N. Shiyo, and M. Shigeyuki, Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins, Breeding Sci., 51 (2001) 225-230.

[4] W.J. Goodrich, R.J. Cook, and A.G. Morgan, The application of electrophoresis to the characterization of cultivars of *Vicia faba* L. Fabis, Newsletter, 13 (1985) 8.

[5] N. Garcia Jacas, A. Susanna, Y. Aarnatje, and R. Vilatersanna, Generic delimitation and phylogeny of the subtribe *Centaureinae* (Asteraceae): a combined nuclear and chloroplast DNA analysis, Ann.Bot.,87 (2001) 503-515.

[6] M.A. Khan, Seed protein electrophoretic pattern in *Brachypodium* P. Beavu. Species. Ann. Bot., 70 (1992) 61-68.

[7] Rabbani MA, Quershi AS, Azfal M, Anwar R and Komatsu. 2001. Characterization of mustard (*Brassica juncea* (L.) Czern. & Coss.) germplasm by SDS-PAGE of total seed proteins, *Pak. J. Bot*. 33(2): 173-179.

[8] E.M. Bartke, D.D. Watt, and T. Tu, Electrophoretic pattern of venoms from species of crotalidae and elapidae snakes, Toxicol., 4 (1966) 73-76.

[9] S.D. Tanksley, and R.A. Jones, Application of alcohol dehydrogenase allozymes in testing the genetic purity of F1 hybrids of tomato, Hort. Sci., 16 (1981), 179-181.

[10] V.O.C. Thanh, and Y. Hirata. Seed storage protein diversity of three rice species in the Mekong Delta, Biosphere Conserv. 4 (2002) 59-67.

[11] A.A. Elham, Abd El-Hady, A.A. Atef Haiba, R. Nagwa, Abd El-Hamid, and A. Aida Rizkalla, Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis, J. Amer. Sci., 6 (2010) 434 - 441.

[12] U.K. Laemmli, Cleavage of structural proteins during the assembly of head of bacteriophage T4, Nature, 227 (1970) 680-685.

[13] D.A. Syed, M. Syed, D. Sabir, M.S. Halimi, and S. Yousaf. Evolutionary Relationship and Divergence Based on SDS-PAGE of *Elaeagnus umbellata* Thunb. Populations, a Multipurpose Plant from the Himalayas, Turk. J. Biol., 32 (2008) 31-35.

[14] S. Somia El-akkad, Phylogenetic Relationship and Similarity Indices of Some *Acacia* Species Using Seed Protein Analysis, Int. J. Agricult. Biol, 3 (2004) 435-439.