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Studies on the *Rosaceae* II- SDS-PAGE Seed Protein Electrophoresis and its Significance in the Taxonomy of the Family

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Abstract: In the present work SDS–PAGE criteria of seed protein was performed on 47 taxa of the *Rosaceae senu lato*. Taxa were selected so as to represent the accepted four subfamilies in the family (*Maloideae, Prunoideae*, *Rosoideae* and *Spiraeoideae*) and most of the tribes included in them. The obtained data were numerically analyzed by the UPGMA cluster analysis using the NTsys-pc. The results revealed that although the family as a whole represents a clearly monophyletic lineage. The studied taxa were distributed across the constructed phenograms independent of the previous suprageneric classification, particularly in members of the subfamilies *Rosoideae* and *Spiraeoideae*. A revision of the suprageneric classification of the family was suggested (particularly in the *Spiraeoideae* and *Rosoideae*).

Key words: *Rosaceae*, SDS-PAGE, electrophoresis, seeds proteins, numerical analysis

INTRODUCTION

The *Rosaceae* is a large sub-cosmopolitan family of about 95 genera and 2825 species. It is located mainly in the temperate and warm areas of the Northern Hemisphere (Mabberley, 1997). However, Heywood, 1993 stated that the family consists of 122 genera and 3370 species.

The classification of the *Rosaceae* itself raises many problems. Opinions differ as to the relation of the family with other families, the delimitation of its subfamilies, tribes, genera and even species (especially in genera where sub sexual or asexual reproduction is the normal). Limits cannot be drawn sharply between many of the apparent genera or species. Some have splitted the family to more than 27 separate families. Many of the controversies regarding the family are of long standing and are not near solution today more than they were at the time of Linnaeus in the Seventeenths Century (Lawrence, 1951; Hutchinson, 1973; Heywood, 1993; Mabberley, 1997 and Judd *et al*., 1999).

In all the systems of classification, the *Rosaceae* belong to the order *Rosales*, yet the families included in this order differs greatly either in number or in identity or both depending on the author opinion on the reliability of the used characters. The *Rosaceae* was classified into its minor categories on the basis of few characters as fruit type and basic chromosome number (Focke, 1894 and Heywood, 1993). Four subfamilies are currently recognized viz. *Maloideae*, *Prunoideae*, *Rosoideae* and *Spireaoideae* (Judd *et al*., 1999). However, when data sets from molecular, chemical or micromorphological criteria were used, the suprageneric classification of the family appeared to be in need of revision (Challice, 1973; 1974 and 1981; Morgan *et al*., 1994; Uzunova and Mladenova, 2000; Evans and Dickinson, 2001 and Eriksson *et al*., 2001).

Seed protein banding patterns as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyle sulphate, (SDS–PAGE) have provided a valid source of taxonomic evidence for addressing taxonomic relationships at the generic and specific levels. This is mainly due to the fact that seed proteins are highly stable, being unaffected by environmental conditions (Ladizinsky and Hymowitz, 1979; Cooke, 1984; Harborne and Turner, 1984; Quicke, 1993 and Badr, 1995). Quicke (1993) gave a detailed account of seed protein electrophoretic methods and its application in systematic and identification of taxa and showed that it could be a powerful tool on below the specific level.

SDS–PAGE has been used successfully in clarifying the relations between certain taxa and also in the delimitation of species, genera, sections and tribes in various families as the *Asteraceae* (Fischer and Jensen, 1992 and Kamel, 1996), the *Chenopodiaceae* (Safa, 1992) the *Lauraceae* (Kamel and Loutfy 2001), the *Leguminosae* (Badr, 1995; Schmit *et al*., 1996; Badr *et al*., 1998; Abou El-Enain and Loutfy, 1999; Kamel and El-Mashad, 1999 and Badr *et al*., 2000), the *Ranunculaceae* (Jensen, 1984 and Aboel-Atta *et al*., 1999) and the members of the *Solanaceae* have been also subjected to seed protein electrophoresis studies dealing with the taxonomic and phylogenetic relationships (Lester and Hasan, 1991 and Abou El-Enain, 1995).

The present work was carried out to study seed protein profiles in the *Rosaceae* on 47 samples of seed collected from different parts of the world. The resulted

data were used to throw some light on the suprageneric classification of the *Rosaceae*.

MATERIALS AND METHODS

Seeds of the examined species and their sources are listed in Table 1. The seed proteins were analyzed using continuous Polyacrylamide Gel Electrophoresis method in the presence of Sodium Dodecyle Sulphate (i.e. Cont.–SDS–PAGE).

Extraction procedures: 0.1 g of seed powder were mixed with 1 ml of Tris-EDTA buffer (pH 8) for 2 hours at room temperature with gentle agitation. The slurry was boiled for 8 minutes and centrifuged at 12.000 rpm for 20 minutes.

Table 1: Names of the studied species of the *Rosaceae* and its sources

Species	Subfamily	Source
Aruncus dioicus (Walt.) Fern.	*Spiraeoideae*	Japan
Gillenia trifoliata (L.) Moench.	"	Germany
Spiraea albiflora (Miq.) Zabel.	"	Italy
Spiraea betulifolia Pall.	"	Italy
Spiraea chamaedryfolia L.	"	Italy
Spiraea nipponica Maxim.	"	Italy
Spiraea salicifolia L.	"	Italy
Spiraea sargentiana K. Koch.	"	Italy
Exochorda korolkowii Lav.	"	Switzerland
Exochorda racemosa Lindl.	"	France
Filipendula ulmaria (L.) Maxim.	*Rosoideae*	Switzerland
Filipendula vulgaris Moench.	"	Austria
Rhodotypos scandens (Thunb.) Makino.	"	France
Fragaria nipponica Makino.	"	Germany
Fragaria vesca L.	"	France
Geum rivale L.	"	Switzerland
Geum urbanum L.	"	Switzerland
Potentilla argyrophylla Wall. ex. Lehm.	"	Germany
Potentilla concinna A. Gray.	"	Germany
Rubus grayanus Maxim.	"	Japan
Rubus parvifolius L.	"	Japan
Acaena saccaticupula Bitter	"	Germany
Alchemilla fissa Gunth. and Schumm.	"	Germany
Sanguisorba minor Scop.	"	Germany
Rosa canina L.	"	Italy
Rosa gallica L.	"	Italy
Rhaphiolepis ovata Briot.	*Maloideae*	Austria
Rhaphiolepis umbellata (Thunb.) Makino.	"	Austria
Cotoneaster salicifolius Franch.	"	England
Cotoneaster simonsii Baker.	"	England
Crataegus cuneata Siebold. and Zucc.	"	Japan
Crataegus monogyna Jacq.	"	England
Pyracantha angustifolia Franch.	"	England
Pyracantha coccinea M. Roe	"	England
Pyracantha crenulata D. Don Roem.	"	England
Sorbus commixta. Hedl.	"	Japan
Sorbus sorbifolia (Poir.) Hedl.	"	Japan
Photinia wrightiana Maxim.	"	Japan
Mespilus germanica L.	"	England
Malus sylvestris Mill.	"	Egypt
Amelanchier ovales Medik.	"	France
Amelanchier rotundifolia Dum. Cours.	"	Spain
Prunus laurocerasus L.	*Prunoideae*	Egypt
Prunus domestica L. C. K. Schneid.	"	Egypt
Prunus amygdalus Batsch.	"	England
Prunus armeniaca L.	"	France
Pyrus communis L.	*Maloideae*	Egypt

The supernatant was kept at-20°C until use. For Tris-EDTA extracted proteins, electrophoresis was run in 15% gel concentration and in Tris-Glycine running buffer (pH=8.3) at 150 Volts for about 3-4 hours. The banding profile of the examined species was photographed. The number of bands was scored and the recording data was computerized and analyzed by Gel Work I/D advanced soft ware, UVP Corbiration England at the Molecular Cytogentic Lab., Department of Genetic Faculty of Agriculture, Ain Shams University.

Numerical analysis: The use of 50 seed protein electrophoretic attributes for all species have made it possible to produce a phenetic classification based on the dissimilarity between species. The result produced by the analysis was compared with the current taxonomic classifications of the family. The presence or the absence of each of 50 attributes was treated as a binary character in a data matrix i.e. coded 1 and 0 respectively. For the numerical analysis the NTsys-pc., version 1.50 program (Rohlf, 1993) was used.

RESULTS

Seed protein electrophoresis: An electrophoretic study was carried out on the studied taxa of the *Rosaceae* (47 taxa) by using Tris-EDTA as a sample buffer. The electrophoretic protein patterns are illustrated in Fig. 1. The distributions of protein bands revealed by the patterns are given in Tables 2, 3, 4 and 5. These results are a brief description of the banding profiles for all the family and each of the subfamilies and species used in the study.

The electrophoretic protein patterns of Tris-EDTA buffer revealed the presence of 50 different bands within the studied taxa. The highest number of bands recorded in all species studied was 23 bands recorded in the genus *Gillenia* Moench., while the lowest number of bands recorded was two bands only and it was recorded in *Rhaphiolepis umbellata* (Thunb.) Makino. The highest molecular weight band was 74.5 KDa recorded in *Rosa gallica* L., while the lowest molecular weight band recorded was 12.1 KDa in *Pyracantha crenulata* D. Don. Roem. The difference between higher and lower molecular weights ranged between 74.5 to 12.1 KDa.

Subfamily: *Maloideae* (*Pomoideae*): In this subfamily, 17 species were studied. The electropherograms of those species are illustrated in Fig. 1 and the distributions of bands recorded are shown in Table 2. The highest number of bands recorded in these species was 18 bands in *Pyrus communis* L., while the lowest number of bands was two bands recorded in *Rhaphiolepis umbellata* (Thunb.) Makino. The highest M.Wt. band recorded was 70.8 KDa

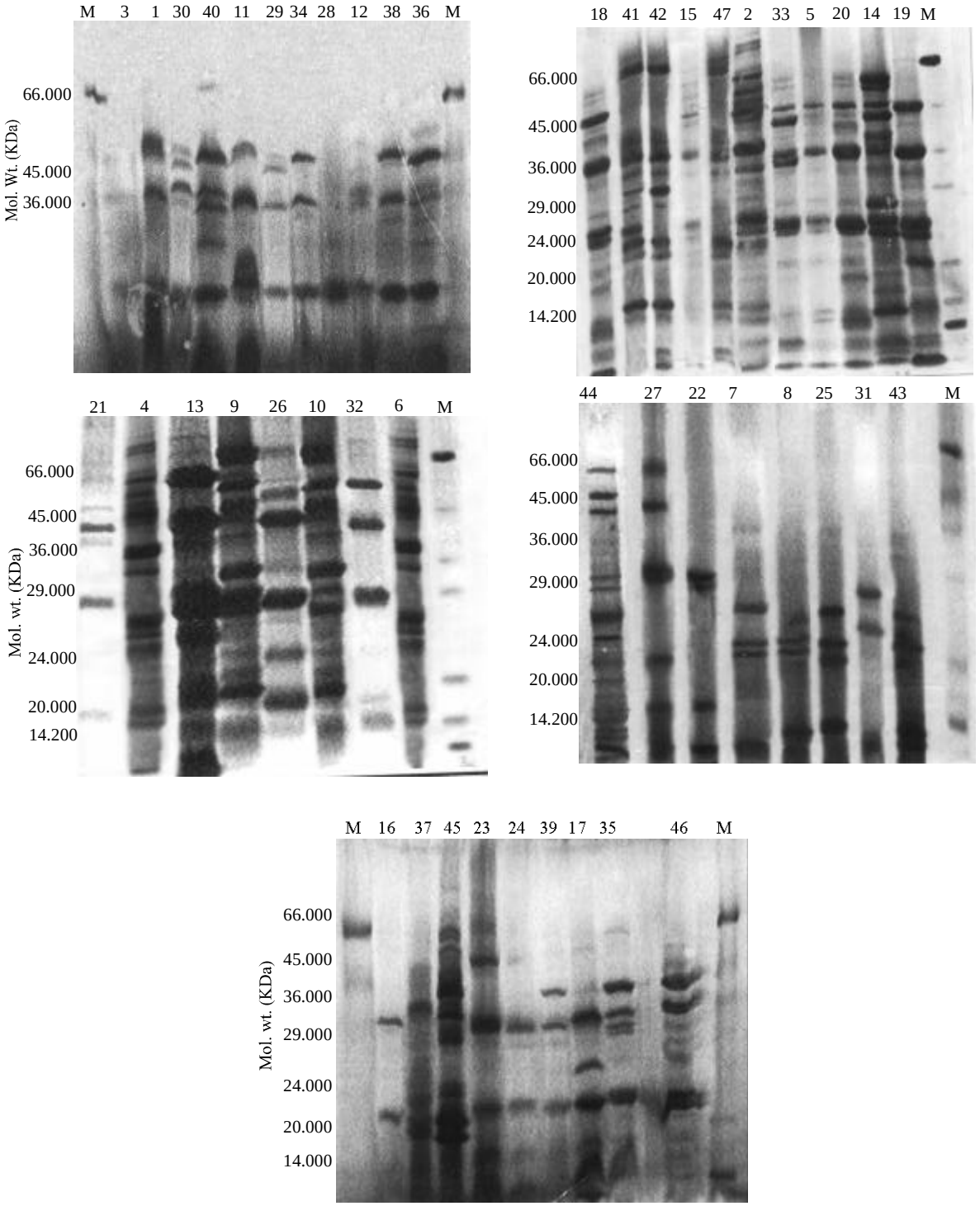

Fig. 1: Electrophoretic-banding profiles of seed proteins extracted in Tris-EDTA buffer of the studied species of the Rosaceae (Numbering as in Table 1).

Table 2: The molecular weights of protein band extracted in Tris–EDTA buffer in studied species of subfamily *Maloideae*

	Species								
	27	28	29	30	31	32	33	34	35
Molecular	55.0	20.5	45.9	48.1	29.8	65.6	54.1	45.6	56.3
weights	45.5	13.2	42.9	43.8	24.9	53.6	51.9	35.6	41.2
(KDa)	35.0	--	34.5	37.9	14.4	44.4	46.9	20.9	35.5
	32.7	--	20.8	35.5	--	31.7	43.6	14.3	32.9
	22.6	--	13.7	19.7	--	--	37.2	--	31.6
	17.9	--	--	--	--	--	35.6	--	21.5
	14.6	--	--	--	--	--	29.2	--	16.2
	14.0	--	--	--	--	--	21.6	--	12.1
	--	--	--	--	--	--	16.7	--	--
	--	--	--	--	--	--	14.4	--	--
	--	--	--	--	--	--	12.9	--	--
	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--	--
Total no. of bands	8	2	5	5	3	6	14	4	8

	Species							
	36	37	38	39	40	41	42	47
Molecular	53.2	45.8	45.9	39.9	69.2	62.5	63.0	70.8
weights	45.3	36.3	35.6	32.9	46.1	59.0	59.5	61.1
(KDa)	36.2	27.3	31.3	20.6	37.5	42.3	42.0	59.0
	31.5	24.2	20.7	12.6	34.3	37.6	37.7	57.3
	27.5	21.6	13.5	--	27.7	31.7	34.7	41.3
	20.9	17.8	--	--	20.2	29.3	31.7	37.1
	16.5	14.8	--	--	13.1	25.9	29.3	34.2
	13.4	12.7	--	--	--	24.1	24.4	24.0
	--	--	--	--	--	22.8	22.6	22.6
	--	--	--	--	--	21.0	21.3	20.8
	--	--	--	--	--	19.6	19.9	19.4
	--	--	--	--	--	17.7	17.6	17.5
	--	--	--	--	--	16.6	16.5	16.4
	--	--	--	--	--	--	13.8	13.8
	--	--	--	--	--	--	12.8	13.2
Total no. of bands	8	8	5	4	7	16	17	18

Table 3: The molecular weights of protein bands extracted in Tris–EDTA buffer in the studied species of subfamily *Prunoideae*

	Species			
	43	44	45	46
Molecular	38.9	55.0	72.6	52.7
weights	35.4	48.7	62.1	45.9
(KDa)	26.9	45.5	56.9	42.6
	24.8	32.7	54.4	37.1
	23.2	31.4	50.6	32.9
	21.9	27.3	44.9	30.1
	15.3	25.1	39.9	27.3
	14.5	22.6	35.0	22.2
	13.7	20.3	33.0	20.9
	--	18.8	30.6	19.8
	--	17.9	28.4	17.2
	--	16.9	23.5	15.4
	--	16.2	22.4	13.9
	--	15.6	21.0	12.4
	--	14.6	19.3	--
	--	14.0	17.3	--
	--	--	14.7	--
	--	--	12.3	--
Total no. of bands	9	16	18	14

in *Pyrus communis* L., while the lowest M.Wt. band recorded was 12.1 KDa in *Pyracantha crenulata* D. Don. Roem. The difference between higher and lower molecular weights ranged between 70.8 to 12.1 KDa.

Subfamily: *Prunoideae*: Of this subfamily, only four species of the genus *Prunus* were studied (Fig. 1 and Table 3). The highest number of bands recorded in these species was 18 bands in *Prunus amygdalus* Batsch., while the lowest number of bands recorded was nine bands, which was recorded in *Prunus laurocerasus* L. The highest M.Wt. band recorded was 72.6 KDa in *Prunus amygdalus* Batsch., while the lowest M.Wt. band recorded was 12.3 KDa in the same species. The difference between higher and lower M.Wt. ranged between 72.6 to 12.3 KDa.

Subfamily: *Rosoideae*: Of the subfamily *Rosoideae* 16 species were studied (Fig. 1 and Table 4). The highest number of bands recorded in these species was 20 bands in *Fragaria nipponica* Makino., while the lowest number of bands was three bands, which was recorded in *Geum urbanum* L. The highest M.Wt. band recorded in these species was 74.5 KDa in *Rosa gallica* L., while the lowest M.Wt. band recorded in these species was 12.2 KDa in *Sanguisorba minor* Scop. The difference between higher and lower molecular weights ranged between 74.5 to 12.2 KDa.

Table 4: The molecular weights of protein bands extracted in Tris-EDTA buffer in the studied species of subfamily *Rosoideae*

	Species							
	11	12	13	14	15	16	17	18
Molecular	66.9	36.6	67.9	54.3	61.7	33.7	50.9	54.3
weights	47.8	43.5	63.3	51.9	58.4	19.9	40.6	52.3
(KDa)	35.4	21.4	55.9	47.7	54.6	12.4	34.4	47.5
	12.7	13.5	52.9	44.9	51.2	--	26.2	42.5
	13.9	--	46.9	40.5	46.4	--	20.9	41.3
	--	--	40.8	37.7	44.1	--	16.9	36.9
	--	--	32.0	34.5	37.7	--	14.5	33.7
	--	--	30.1	31.9	34.2	--	12.5	29.1
	--	--	27.2	28.9	28.7	--	--	26.2
	--	--	21.6	26.6	26.3	--	--	25.1
	--	--	20.4	25.2	24.7	--	--	23.7
	--	--	19.6	24.0	22.9	--	--	21.6
	--	--	17.8	21.9	21.5	--	--	19.7
	--	--	15.1	20.5	17.2	--	--	15.9
	--	--	14.0	18.7	16.4	--	--	13.9
	--	--	--	16.9	12.9	--	--	12.6
	--	--	--	14.7	--	--	--	--
	--	--	--	14.4	--	--	--	--
	--	--	--	13.8	--	--	--	--
	--	--	--	13.2	--	--	--	--
Total no. of bands	5	4	15	20	16	3	8	16

	Species							
	19	20	21	22	23	24	25	26
Molecular	60.2	54.9	72.9	32.7	62.1	47.9	40.3	74.5
weights	47.5	53.3	64.7	31.4	58.6	32.7	35.9	64.4
(KDa)	37.6	47.7	56.7	22.6	47.6	29.4	27.3	56.4
	34.2	43.1	53.3	17.9	44.6	20.9	23.5	52.6
	31.9	42.0	49.7	14.6	32.8	16.1	21.9	46.6
	29.3	37.6	45.1	13.5	21.8	12.2	15.8	43.5
	26.6	34.7	42.3	--	20.7	--	14.5	37.3
	24.8	32.3	32.0	--	16.0	--	13.5	31.8
	21.6	29.4	19.1	--	12.4	--	--	29.4
	19.4	25.9	--	--	--	--	--	24.7
	17.3	23.8	--	--	--	--	--	19.8
	16.4	220.0	--	--	--	--	--	17.5
	14.8	20.0	--	--	--	--	--	--
	14.4	15.6	--	--	--	--	--	--
	13.3	14.4	--	--	--	--	--	--
	--	12.9	--	--	--	--	--	--
	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--
	--	--	--	--	--	--	--	--
Total no. of bands	15	16	9	6	9	6	8	12

Subfamily: *Spiraeoideae*: In the present work, ten species were studied of subfamily *Spiraeoideae* (Fig. 1 and Table 5). The highest number of bands recorded in these species was 23 bands which was recorded in *Gillenia* Moench., while the lowest number of bands three were recorded in *Spiraea albiflora* (Miq.) Zabel. The highest M.Wt. band recorded in these species was 70.7 KDa in genus *Spiraea nipponica* Maxim., while the lowest M.Wt. band recorded was 12.9 KDa in *Gillenia trifoliata* Moench. The difference in molecular weights ranged between 70.7 to 12.9 KDa.

Numerical analysis based on seed protein attributes: The phenogram produced by cluster analysis based on 50 attributes observed from seed protein analysis of the studied species of the *Rosaceae* (47 species) is shown in Fig. 2. This phenogram show that all species have a highest average taxonomic distance of 1.80 level. At this level, *Gillenia trifoliata* (2) of the *Spiraeoideae* is split off from all species of the family. Then at the level of 1.50, six species (four species of the *Spiraeoideae* and two species of the *Rosoideae*) are separated as a group. Within this group two species of the *Spiraeoideae* (*Exochorda korolkowii* - 9 and *E. racemosa* - 10) are separated at 1.50 level and then distinguished from each other at 0.80 level. The other four species are divided at the 1.33 level. Two species of the *Rosoideae* (*Rhodotypos scandens* - 13 and *Rosa gallica* - 26), which are

Table 5: The molecular weights of protein bands extracted in Tris-EDTA buffer in the studied species of subfamily *Spiraeoideae*

	Species									
	1	2	3	4	5	6	7	8	9	10
	49.3	68.2	35.8	72.0	47.5	70.7	41.7	24.1	62.8	63.8
	36.9	65.2	19.2	69.2	45.7	68.3	35.4	22.7	60.7	61.3
	19.4	63.1	13.0	66.8	43.8	65.4	28.3	21.6	56.6	54.2
	16.4	57.2	--	64.2	37.8	63.7	23.9	18.5	54.2	50.1
	12.9	55.2	--	58.2	29.4	57.6	22.7	15.6	49.6	46.9
	--	51.5	--	55.9	26.8	55.6	18.7	14.2	46.3	42.9
	--	48.6	--	52.3	25.3	51.8	14.5	13.6	42.4	36.7
	--	46.6	--	49.8	21.7	49.6	13.4	--	36.8	33.0
	--	45.2	--	47.7	16.9	46.1	--	--	32.5	30.1
	--	43.3	--	45.8	16.2	39.0	--	--	30.2	27.1
	--	38.0	--	40.3	14.3	36.2	--	--	27.2	24.9
	--	34.6	--	36.4	12.9	28.5	--	--	25.0	22.8
	--	32.4	--	28.9	--	27.0	--	--	22.9	20.7
	--	30.2	--	27.2	--	25.3	--	--	20.9	18.1
	--	26.7	--	25.3	--	23.1	--	--	18.4	17.3
	--	25.3	--	22.9	--	21.5	--	--	17.5	15.0
	--	23.5	--	21.9	--	18.9	--	--	15.0	--
	--	21.3	--	18.9	--	17.8	--	--	--	--
	--	19.9	--	17.9	--	15.7	--	--	--	--
	--	17.2	--	14.4	--	14.4	--	--	--	--
	--	16.2	--	--	--	--	--	--	--	--
	--	14.4	--	--	--	--	--	--	--	--
	--	12.9	--	--	--	--	--	--	--	--
Total no. of bands	5	23	3	20	12	21	8	7	17	16

distinguished from each other at level 1.18 and the other two species of the *Spiraeoideae* (*Spiraea betulifolia* - 4 and *S. nipponica* - 6) which are distinguished from each other at 0.37 level.

At the levels 1.63, 1.53 and 1.47, *Prunus amygdalus* (45) of the *Prunoideae*, *Rubus parviflolius* (21) of the *Rosoideae, Prunus domestica* (44) and *P. laurocerasus* (44) of the *Prunoideae* are split off from the remaining species respectively. At the level of 1.37, two groups are formed, the first group consists of three species of the *Maloideae; Pyrus communis* (47) which is separated from this group at the 1.37 level and clustered with two varieties of *Amelanchier* (41 and 42) at the 1.20 level. The later two species are distinguished from each other at the 0.83 level.

The second group consists of seven species and divided into two subgroups at the 1.18 level. The first one consists of three species of sub family *Rosoideae*. Of those species; *Rubus grayanus* (20) is separated from the other species at the 1.03 level and *Fragaria nipponica* (14) and *Potentilla argyrophylla* (18) are distinguished from each other at the 0.93 level. The second subgroup consists of four species separated from each other at the 1.13 level. Within this subgroup *Fragaria vesca* (15) of the *Rosoideae* and *Pyracantha angustifolia* (33) of the *Maloideae* are distinguished from each other at 0.87 level. On the other hand, *Spiraea chamaedryfolia* (5) of the *Spiraeoideae* and *Potentilla concinna* (19) of the *Rosoideae* are distinguished from each other at the 0.93 level. At the level 1.24 and 1.18, *Prunus armeniaca* (46) of the *Prunoideae* and *Geum urbanum* (17) of the *Rosoideae* are split off from the remaining species respectively.

At the 1.01 level, three species are separated as a group; *Pyrachantha crenulata* (35) of the *Maloideae* is separated from other species at 0.97 level and then the two species of the *Rosoideae* (*Alchemilla fissa* - 23 and *Sanguisorba minor* - 24) are distinguished from each other at the 0.87 level.

At the 1.07 level, *Crataegus monogyna* (32) of the *Maloideae* is split off from the rest of the species. *Acaena saccaticupula* (22) of the *Rosoideae* and *Rhaphiolepis ovata* (27) of the *Maloideae* are separated together from the other species at the 1.03 level and distinguished from each other at the 0.90 level. At the 1.03 level four species are separated as a group. Within this group; *Sorbus sorbifolia* (37) of the *Maloideae* is separated from other species of the group at 0.97 level, *Spiraea sargentiana* (8) of the *Spiraeoideae* is separated from *Rosa canina* (25) of the *Rosoideae* and *Spiraea salicfolia* (7) of the *Spiraeoideae* at the level 0.73 and the later two species are distinguished from each other at the 0.63 level.

At the levels 0.93 and 0.87, the two species of the *Maloideae* (*Sorbus commixta* - 36 and *Crataegus cuneata* - 31) are split off from the remaining species respectively. From the remaining species, another two groups are formed at the average taxonomic distance of 0.83. Within the first group which consists of nine species; *Malus sylvestris* (40) of the *Maloideae* is split off

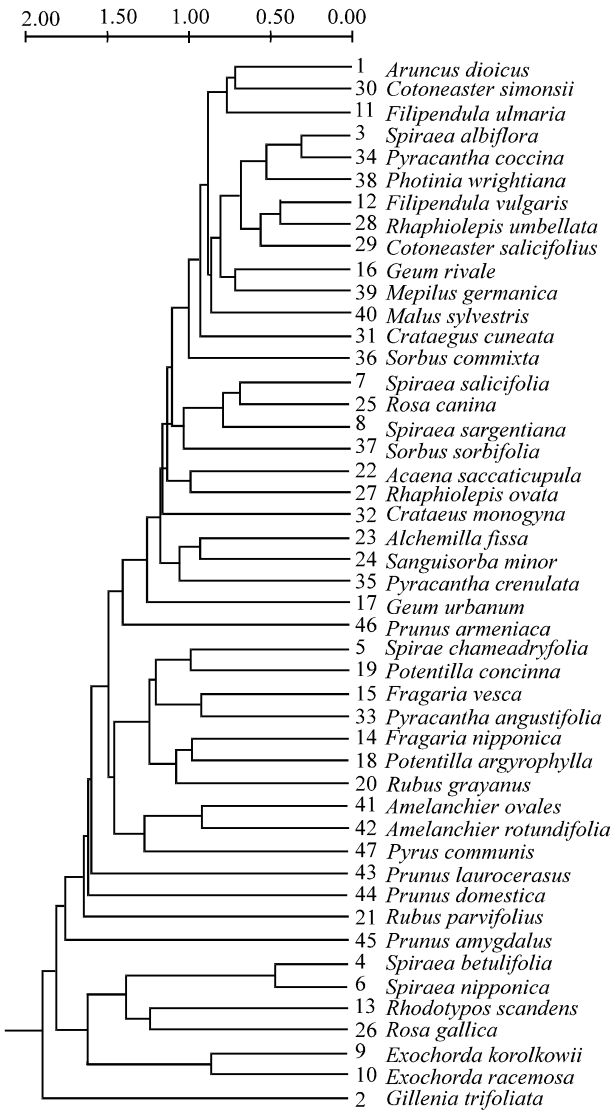

Fig. 2: UPGMA-phenogram based on coding of 50 attributes obtained from SDS-PAGE profiles of seed proteins extracted in Tris-EDTA buffer illustrated the average taxonomic distance (dissimilarity) between the studied species of the *Rosaceae*

and then *Geum rivale* (16) of the *Rosoideae* and *Mespilus germanica* (39) of the *Maloideae* are separated together from other species at the 0.75 level and then distinguished from each other at the 0.68 level.

At the level of 0.63, the remaining species are divided into two subgroups. The first one consists of three species, *Cotoneaster salicifolius* (29) of the *Maloideae,* which is separated from the other species at the 0.53 level and *Filipendula vulgaris* (12) of the *Rosoideae* and *Rhaphiolepis umbellata* (28) of the *Maloideae* which are separated from each other at the 0.4 level. The second subgroup consists of three species; *Photinia wrightiana* (38) of the *Maloideae* which is separated from the other species, *Pyracantha coccinea* (34) of the *Maloideae* and *Spiraea albiflora* (3) of the *Spiraeoideae* at the 0.5 level and the later two species are distinguished from each other at the 0.30 level.

The second group consists of three species; *Filipendula ulmaria* (11) of the *Rosoideae* is separated from *Aruncus dioicus* (1) of the *Spiraeoideae* and *Cotoneaster simonsii* (36) of the *Maloideae* at the level 0.73 and then the later two species are distinguished from each other at the 0.68 level.

DISCUSSION

The suprageneric classification of the *Rosaceae* into its minor categories was first done by Focke (1894) based on fruit morphology. He classified the *Rosaceae* into six subfamilies. However, only four of Focke's subfamilies are recognized today, as the other two are now placed in separate families (Mabberley, 1997 and Judd *et al.,* 1999). The accepted four subfamilies of the *Rosaceae* are respectively: *Maloideae, Prunoideae, Rosoideae* and *Spiraeoideae* (Judd *et al.,* 1999).

Yet, as regarding a diverse family as the *Rosaceae,* dating back at least to the Cretaceous (Doyle and Donoghue, 1986 and Stewart and Rothwell, 1993), the old history of the family has rendered the generic and specific delimitation more problematic and a more or less a formidable task (Evans and Dickinson, 2001). This is mainly because many phylogenetic lineage linking between its taxa are either lost completely or need to be clarified, as many taxa are now extinct, while new taxa are discovered annually (Hafez, 2002).

Artificial boundaries between the taxa of the *Rosaceae* are widespread (Heywood, 1993). The relationships between its species, genera and even subfamilies are still unclear. The problems are aggravated by a relatively high percentage of hybridization, polyploidy and agamospermy among the family taxa (Eriksson and Donoghue, 1995; Eriksson *et al.,* 1998 and 2001; Morgan *et al.,* 1994; Heywood, 1993 and Judd *et al.,* 1999).

Numerous studies were made on the *Rosaceae sensu lato* to clarify the phylogenetic relationships and evolution within either certain genera, tribes, subfamilies or the family on a broad sense, utilizing data sets from entirely different attributes as chemotaxonomy (Challice, 1973; 1974 and 1981), floral development and morphology

(Evans, 1994; Rohrer *et al.*, 1994; Sattler, 1973 and Steeves *et al.*, 1991); wood anatomy (Zhang, 1992), petal ultrastructure (Evans, 1999); pollen morphology (Li *et al.*, 2001); leaf epidermal criteria (Uzunova and Mladenova, 2000) molecular criteria (Eriksson and Donghue, 1995; Eriksson *et al.*, 1998 and Morgan *et al.*, 1994). However, the obtained results were very different. None of these studies have reached a conclusive result in determining the intergeneric or specific relationships between the taxa of the *Rosaceae* (Evans and Dickinson, 2001).

In the present work, SDS-PAGE of seed protein profiles of 47 of the *Rosaceae senu lato* was investigated. These taxa were chosen so as to represent the accepted four subfamilies of the family (*Maloideae, Prunoideae, Rosoideae* and *Spiraeoideae*).

The results of SDS-PAGE in general, did not fit with the accepted four subfamilies of the *Rosaceae* based mainly on the type of the fruit. The same conclusion was stated by Mourad and Al-Nowaihi, 2001 who reported that recent studies on the phylogeny of *Rosaceae* using other criteria as molecular systematic segmented this family into several infra-familial taxa and the number is so fluctuating that no clear consensus can be cited.

In subfamily *Maloideae*: The following relationships were recorded with members of the other subfamilies:

- *Cotoneaster simonsii* with *Aruncus dioicus* (*Spiraeoideae*) at 0.68 due to their possessing similar characteristic bands having the molecular weights of 48, 37 and 20 KDa, which were recorded in the protein profile.
- *Pyracantha coccinea* with *Spiraea albiflora* at dissimilarity level (0.30) due to the similar characteristic bands (35 and 20 KDa), which was recorded in the protein profile.
- *Mespilus germanica* with *Geum rivale* (*Rosoideae*) at 0.68 due to the similar characteristic bands (12 and 20 KDa), which were recorded in the protein profile.
- *Sorbus sorbifolia* with *Spiraea sargentiana*, *S. salicifolia* (*Spiraeoideae*) and *Rosa canina* (*Rosoideae*) at 0.97 due to the similar characteristic band (14 KDa), which was recorded in the protein profile.
- *Pyracantha crenulata* with *Alchemilla fissa* and *Sanguisorba minor* (*Rosoideae*) at 0.97 due to similar characteristic band (32 KDa), which was recorded in the protein profile and the later two species clustered at 0.97 due to similar characteristic bands (32 and 20 KDa), which were recorded in the protein profile.
- *Pyracantha anguistifolia* and *Fragaria vesca* (*Rosoideae*) at 0.87 due to similar characteristic bands (54, 51, 47, 43, 37, 34, 29, 21 and 16 KDa), which were recorded in the protein profile.
- *Rhaphiolepis umbellata* and *Filipendula vulgaris* (*Rosoideae*) at 0.4 due to similar characteristic band (20 KDa), which was recorded in the protein profile.

Evans and Dickinson (1999) reported that several authors have suggested that the origin of the *Maloideae* be within the *Spiraoideae*. In their opinion, the fleshy pome fruit was derived from the expansion of the hypanthium (floral cup). They also stated that the incorporation of the ovaries by the enlarged hypanthium resulted in the inferior ovaries present in the majority of *Maloideae* genera. Data from seed protein electrophoresis SDS-PAGE in the present study give some support to the previous hypothesis about the *Maloideae* origin as many studied taxa had relationships with members of the *Spiraeoideae*.

Regarding the relationships of some *Maloideae* taxa with members of the *Rosoideae,* Heywood (1993), stated that the *Maloideae* origin can be within members of the *Rosoideae* with basic chromosome numbers of 8 and 9, i.e. any of the other three subfamilies and not within the *Spiraeoideae* in particular, as mentioned above.

In subfamily *Prunoideae*: The SDS-PAGE data clearly showed that the four studied species of *Prunus* had relationships with most taxa of the other three subfamilies. However, *Prunus armeniaca* is here widely separated from the other three species of *Prunus* studied. This may agree with Bailey (1949), who stated that *Prunus armeniaca* possess some features that distinguish it clearly from the remaining three species as its cordate to ovate leaf blade and its glandular petiole.

On the other hand, the SDS-PAGE data does not agree with Rehder (1940) and Lee and Wen (2001) classification of *Prunus,* particularly the grouping of *P. laurocerasus* with *P. domestica* and *P. amygdalus.*

In subfamily *Rosoideae*: The relations of certain *Rosoideae* with members of the other subfamilies were discussed former. However, the following relations were observed:

- *Rubus grayanus* with both *Fragaria nipponica* and *Potentilla argyrophylla* at 1.03 due to similar characteristic bands which having the molecular weights 47, 41, 37, 34, 29, 26, 23, 21 and 19 KDa, which were recorded in protein profile.

In subfamily *Spiraeoideae*: The relationships of the studied taxa of the *Spiraeoideae* with members of the *Rosoideae* were as follows:

- A relation between *Spiraea salicifolia* and *Rosa canina* at 0.63 due to the similar characteristic bands (40, 36, 27, 24 and 14 KDa) which were recorded in the protein profiles.

- A relation between *Spiraea chamaedryfolia* and *Potentilla concinna* at 0.93 due to the similar characteristic bands (47, 37, 29, 26, 24, 21, 16 and 14 KDa), which were recorded in the protein profiles.
- However, the most remarkable observation was that, *Gillenia trifoliata* was separated from all the studied taxa at 1.80 mainly because it differed from them in possessing the band (69 and 67 KDa) in the protein profile.

The wide distribution of the *Spiraeoideae* across the constructed dendrograms (either from seed morphology or SDS-PAGE data) shows that this subfamily may be a polyphyletic assemblage as suggested by Morgan *et al.* (1994), Evans (1999), Evans and Dickinson, 1999 and 2001 and Judd *et al.* (1999).

Finally, the study showed that the present suprageneric classification of the family and also the relations between the *Rosaceae* taxa may be in need of revision and so gives support to previous studies urging on the same issue (Morgan *et al.*, 1994; Evans, 1999; judd *et al.*, 1999; Eriksson *et al.*, 2001 and Evans and Dickinson, 2001).

A more comprehensive view on the *Rosaceae* can be achieved in the future, through the investigation and study of more cosmopolitan material and also by utilizing other criteria that serve as good phylogenetic and/or taxonomic markers.

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