

RAPD Based Genetic Diversity Analysis Within The Genus *Solanum*

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SOLANUM L., the largest genus of the Solanaceae family, varies morphologically and is ecogeographically distributed. In Saudi Arabia, previous studies for characterization and genetic variability of *Solanum* had focused mainly on chromosome morphology, genome description and medicinal values, which are insufficient for genetic affinities. This investigation assessed molecular diversity of some species of *Solanum* from Taif highlands based on random amplified polymorphic DNA markers and proteins by SDS-PAGE. From 25 random primers investigated, 11 primers gave reproducible amplification banding patterns of 208 polymorphic bands scoring 100% polymorphism across the genotypes. Primer OPA-09 generated maximum polymorphic pattern, whereas primers OPP-10, OPN-13 and OPN-05 produced minimum polymorphism. The size of the amplified products varied from 265 to 2003bp. SDS denatured protein gels resolved 51 bands most of them were not shared and some were unique and can be correlated with the DNA polymorphism. Based on these markers, genetic similarity coefficient was calculated and a dendrogram was constructed.

Keywords: *Solanum*, RAPD, Electrophoresis, Genetic relationships.

Solanum L., a complex and large genus of the family Solanaceae contains roughly between 1,500 and 2,000 species (Bohs, 2001). In Saudi Arabia, the genus is represented by about 16 species, mainly in West and Southwest side of the country (Chaudhary, 2001 and Collenette, 1999).

Limited work has been done on the nature of genetic diversity and characterization of wild and cultivated *Solanum*, especially using molecular methods. Taxonomic studies on *Solanum* species have been based on chromosome morphology (Al-Wadi & Lashin 2007), medicinal and food values (Al-Oqail *et al.*, 2012). These have not resolved the problems of synonymy and taxa misidentification common to the genus in Saudi Arabia. Molecular biology has revolutionized the field of plant systematics and has been used successfully in phylogenetic relationships at all taxonomic levels (Bohs, 2005) as well as in genetic diversity studies (Isshiki *et al.*, 2008). Random amplified polymorphic DNA (RAPD), when compared with other molecular markers, is more effective in this regard as it is simple, rapid, requires only a small quantity of DNA and it is well adapted for nonradioactive DNA fingerprinting of genotypes (Cao *et al.*, 1999). It is also able to generate numerous polymorphisms

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(Williams *et al.*, 1990). Karihaloo *et al.* (1995) focused directly on nuclear genomic diversity of *Solanum* by undertaking RAPD analysis. Karihaloo and Gottlieb (1995) also reported that greater DNA polymorphism exists in weedy *Solanum incanum* than in advanced cultivars of eggplants. RAPD data of *Solanum* was used in several other studies such as Miller & Spooner (1999), Stedje & Bukenya-Ziraba (2003) and Singh *et al.* (2006) to clarify phylogenetic relationships.

Therefore, RAPD-PCR and protein electrophoresis (SDS-PAGE) techniques will be utilized for better characterization of some *Solanum* species of Taif highlands as well as studying the genetic relationships among them.

Material and Methods

Fresh leaves (young and matured) of one cultivar; *Solanum lycopersicum* L., two wild accessions of *S. incanum* L. (from two localities; Alshafa & Sasied) and five wild species; *S. nigrum* L., *S. schimperianum* Hochst., *S. glabratum* var. *sepicula* Dunal, *S. albicaule* Kotschy ex Dunal and *Solanum* sp., were collected from Taif highlands of Saudi Arabia (Longitude 40°18'270"-40°29'820"E and Latitude 21°5'290"- 21°17'750"N) as shown in Fig.1. The collected materials were identified according to Collenette (1999) and Chaudhary (2001).

Total genomic DNA was extracted from young fresh leaves using Wizard genomic DNA purification kit (Promega Corporation, USA). Verification of the quality of the purified DNA samples was achieved by electrophoresis on a 1% Agarose gel. Twenty five random primers (Oligo MacroGen Inc., South Korea) were screened. Only 11 that are highly polymorphic and gave reproducible bands were selected and used in the analysis of all *Solanum* genotypes. Total reaction volume for PCR was 25 µl containing 0.1 µl of primer, 1 µl of 10 mg/µl sample DNA, 12.5 µl Green Master Mix (Promega corporation, USA) and 11.4 µl of distilled water. Amplification was accomplished on the Techne TC- 3000 thermal cycler (Model FTC41H2D, Barloworld Scientific Ltd, Staffordshire, UK) using the following temperature profile: Initial strand separation step of 5 min at 94°C followed by 40 cycles each consisting of a denaturing step of 1 min. at 94°C, annealing step of 30 sec. at 36°C and an extension step of 2 min. at 72°C. The last cycle was followed by 7 min. extension at 72°C to allow complete extension of the PCR products with a final hold at 4°C till electrophoresis. 7 µl of each of PCR product (amplicon) were mixed with 3 µl of 10X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a micro centrifuge before loading on a 1.2% agarose gel which has been previously stained with safe view. This was run for 37 min. at 110 V/cm. Thereafter, the gel was viewed (with the aid of eye protector) and photographed in the Gel Documentation and Analysis Systems (Gel Doc-It™ 300, Ultra-Violet Products Ltd., Cambridge, UK).

SDS-PAGE was performed in 12% acrylamide slab gels (C.B.S. Scientific, USA) following the system of Laemmli (1970). Protein extraction was conducted by grinding 1g of leaves of each sample in 1 ml of 1.5 M Tris-HCl buffer, pH 8.8 using a mortar and pestle. The homogenate was centrifuged at 1000 rpm for 10 min. The clear supernatant was transferred to clean Eppendorf tube and kept at -20°C until use. For electrophoresis, 20 μl of supernatant was mixed with 10 μl of loading dye and 20 μl of mixture was loaded in the gel for each sample. After the run completed, the gel was stained, destained and photographed.

Differences in bands intensity among profiles of the different samples were not considered. The produced clear well defined bands are used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Then the presence or absence of each RAPD and protein band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram among the 8 samples. Data generated by RAPD-PCR and SDS-PAGE was used to compile a binary matrix for cluster analysis. Genetic similarity among species was calculated according to Dice similarity coefficient (Dice, 1945) and used to construct a dendrogram using unweighted pair group method with arithmetic average (UPGMA) using SPSS-20 program (SPSS, 2011).

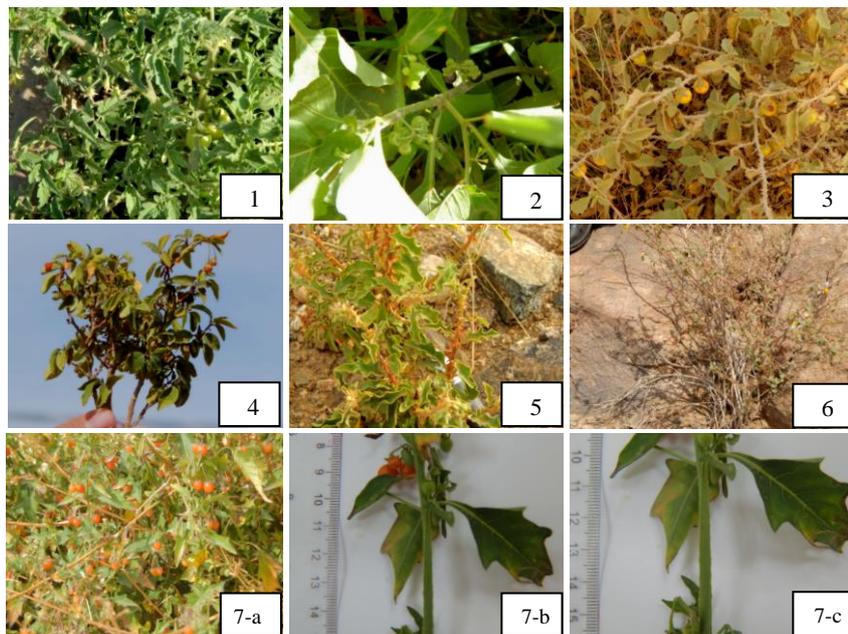


Fig. 1. Collected *Solanum* species from Taif highlands of Saudi Arabia. 1: *S. lycopersicum*, 2: *S. nigrum*, 3: *S. incanum* 4: *S. schimperianum*, 5: *S. glabratum* var. *sepicula*, 6: *S. albicaule*, 7-a,b&c: *Solanum* sp.

Results

Table 1. and Fig. 2 revealed that the selected 11 RAPD primers allowed us to obtain 208 DNA fragments, an average 18.9 per primer. OPP-10, OPN-13 and OPN-05 primers gave the fewest bands (14 fragments) and OPA-09 the most (29 fragments). All detected DNA fragments were polymorphic with polymorphism percentage 100% for all investigated primers. RAPD analysis revealed no band in *S. lycopersicum* in primer OPP-10, *S. incanum* (Alshafa) in primers OPP-10, OPN-13, OPN-10 & OPN-08, *Solanum sp.* in primer OPN-13 and *S. albicaule* in primer OPA-02 (Fig. 3). Each species showed specific characteristic RAPD fingerprinting through all studied primers. RAPD profiles recorded 135 unique bands; 19 in *S. lycopersicum*, 15 in *S. nigrum*, 3 in *S. incanum* (Alshafa), 14 in *S. incanum* (Sasied), 23 in *S. schimperianum*, 26 in *S. glabratum var. sepicula*, 11 in *Solanum sp.* and 24 in *S. albicaule*, that could be used to discriminate the respective species.

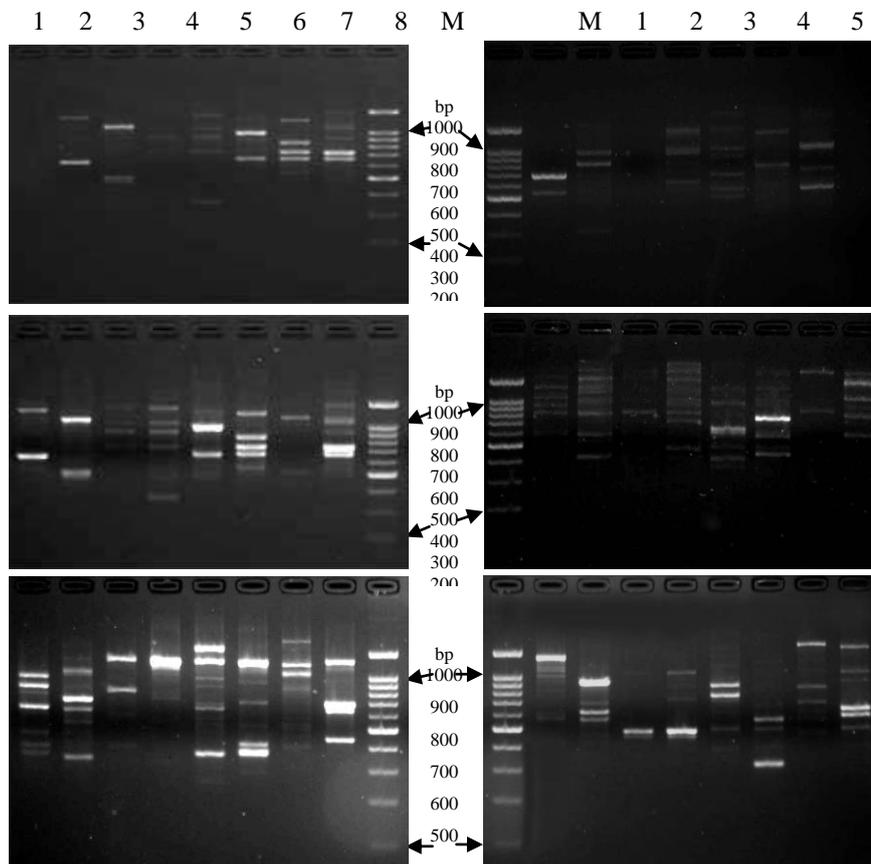


Fig. 3. RAPD profiles of *Solanum* species generated by the 1:OPP-10, 2:OPA-02, 3:OPP-09, 4:OPA-01, 5:OPN-16 and 6:OPA-09 primers; M = Marker. 1: *S. lycopersicum*, 2: *S. nigrum*, 3: *S. incanum* (Alshafa), 4: *S. incanum* (Sasied), 5: *S. schimperianum*, 6: *S. glabratum var. sepicula*, 7: *Solanum sp.*, 8: *S. albicaule*.

TABLE 1. Operon primers selected with their nucleotide sequence, number and types of the amplified DNA bands as well as the percentage of the total polymorphism generated by 11 RAPD primers in *Solanum* species.

Primer code	Primer sequence (5'- 3')	Polymorphic band		Total band	Polymorphism (%)
		Unique	Not shared		
OPA-01	CAGGCCCTTC	15	10	25	100
OPA-02	TGCCGAGCTG	9	7	16	100
OPP-10	TCCCGCCTAC	9	5	14	100
OPP-09	GTGGTCCGCA	11	7	18	100
OPN-13	AGCGTCACTC	10	4	14	100
OPN-10	ACAACGGGG	16	3	19	100
OPN-09	TGCCGGCTTG	8	7	15	100
OPN-08	ACCTCAGCTC	12	4	16	100
OPN-05	ACTGAACGCC	10	4	14	100
OPN-16	AAGCGACCTG	17	11	28	100
OPA-09	GGGTAACGCC	18	11	29	100

The *Solanum* genotypes exhibited substantial changes in protein levels as shown in Fig. 3. SDS denatured protein gels resolved a total of 51 bands. Highest number of protein bands was observed in *S. incanum* (Sasied). Using gel analyzer 3 software the molecular weight and the Rf value of each band was calculated. These SDS protein bands had different molecular weights ranging from 155.2kDa to 13.9kDa. Two bands at 56.3 and 40.8kDa were shared by all species. The profile revealed 49 polymorphic bands with polymorphism percentage 96%. From them 23 unique bands; one in *S. schimperianum* & *S. albicaule*, two in *S. nigrum*, *S. incanum* (Alshafa) & *Solanum sp.*, four in *S. lycopersicum* & *S. glabratum var. sepicula* and seven in *S. Incanum* (Sasied).

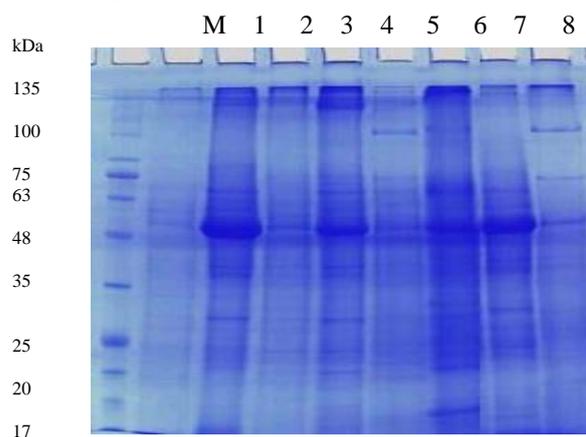


Fig. 2. The produced seed protein profile of *Solanum* species using SDS-PAGE technique. M: Standard protein marker, kDa: kilo Dalton. 1: *S. lycopersicum* , 2: *S. nigrum*, 3: *S. incanum* (Alshafa), 4: *S. incanum* (Sasied), 5: *S. schimperianum*, 6: *S. glabratum var. sepicula*, 7: *Solanum sp.*, 8: *S. albicaule*.

Genetic similarity was calculated from the dice similarity index value for all species of *Solanum*. Based on RAPD and protein markers, The maximum genetic similarity was 0.46 between the two accessions of *S. incanum*. while the lowest genetic similarity of 0.13 was between *S. lycopersicum* and *S. glabratum* var. *sepicula* (Table 2). The phylogenetic relationships among *Solanum* species were analyzed by UPGMA method (Fig. 4). The cluster result indicated that all species could be distinguished by RAPD and protein markers, respectively. A dendrogram based on UPGMA analysis grouped the 8 taxa into one main cluster and the cultivar *S. lycopersicum* formed a separate operational taxonomic units (OTU) in cluster showing less similarity coefficient with the other wild taxa. Species within main cluster were further divided into three subclusters (IIa, IIb and IIc). Subcluster IIa comprised the two accessions of *S. incanum*. Subcluster IIb comprised *S. glabratum* var. *sepicula* with *S. albicaule*. Cluster IIc grouped *Solanum* sp. with *S. nigrum* and *S. schimperianum*.

TABLE 2: Dice similarity coefficient of *Solanum* species based on RAPD and SDS-PAGE data analysis.

	<i>S. lyco.</i>	<i>S. nig.</i>	<i>S. inc. (Al)</i>	<i>S. inc. (Sa)</i>	<i>S. schim.</i>	<i>S. glab.</i>	<i>S. sp.</i>	<i>S. alb.</i>
<i>S. lyco.</i>	1.000							
<i>S. nig</i>	.189	1.000						
<i>S. inc. (Al)</i>	.139	.200	1.000					
<i>S. Inc. (Sa)</i>	.131	.256	.462	1.000				
<i>S. schim.</i>	.150	.320	.198	.238	1.000			
<i>S. glab.</i>	.127	.266	.191	.202	.233	1.000		
<i>S. sp.</i>	.146	.281	.200	.296	.261	.237	1.000	
<i>S. alb.</i>	.154	.252	.158	.250	.235	.288	.256	1.000

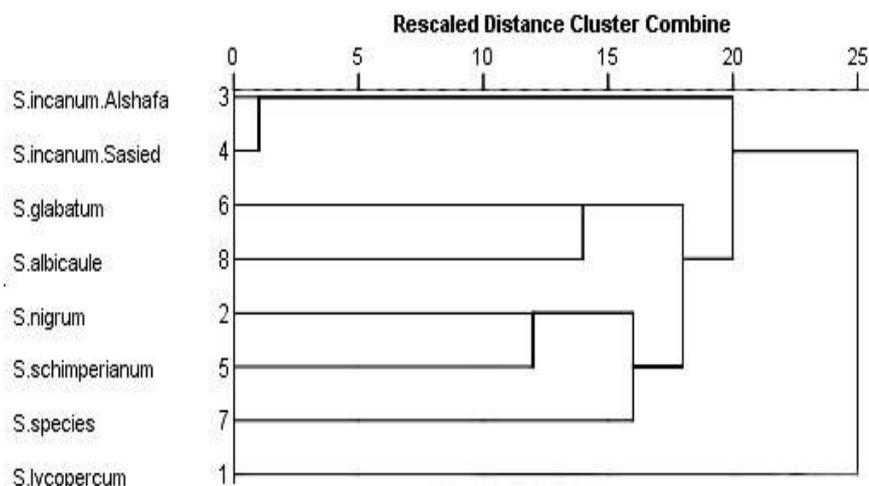


Fig. 4. UPGMA phenogram showing genetic diversity of *Solanum* species based on combination of RAPD and SDS-PAGE bands.

Discussion

RAPD analysis provided insight into the genetic diversity of some species of *Solanum* in Taif. RAPD markers had the greatest advantage of its capability to scan across all regions of the genome hence highly suited for phylogeny studies at species level (Viorel *et al.*, 2009; Zabihollah *et al.*, 2012). These markers revealed considerable genetic diversity among the species through a minimal profile for each species allowing them to be differentiated from each other, a finding which strongly agrees with the great morphological variability observed. These findings agreed with those of Li *et al.* (2010) and Singh *et al.* (2006). The High degree of diversity of species belonging to *Solanum* may be attributable in part to the use of different RAPD primers, ecology, difference in their reproductive biology as the species was insect pollinated, or to the fact that it is an ancient plant (Whalen, 1979). Furthermore, RAPD analysis revealed no band in some species, due to the absence of those sequences in these species. Similar finding has been reached by Alam *et al.* (2012).

During protein profiling of total proteins of experimental species, 51 seed protein bands including 23 unique bands with polymorphism percentage of 96% were observed. This revealed a characteristic variability among the *Solanum* taxa that may be due to environmental factors which affected the qualitative and quantitative attributes of leaves proteins. This was in accordance with the data of Hasan and Isa (1998) who reported a great variability in seed protein profiles in eggplant cultivar (*Solanum melongena* L.) and its nearest wild species; *S. cumingii* Dun., *S. insanum* L. and *S. incanum* L. Finding out intergeneric or interspecific correlation among members of family Solanaceae using SDS-PAGE had been done by several earlier workers *e.g.* Sheadai *et al.* (2002) , Karihaloo *et al.* (2004), Bhat & Kudesia (2011) and Florina (2012).

Using cluster analysis, *solanum* species are categorized into one main cluster including wild species and one ungrouped cultivar (*Solanum lycopersicum* L. var. *lycopersicum*) formed a separate OTU in cluster. Similar results were obtained by Hasan & Isa (1998) and Singh *et al.* (2006). Sandra *et al.* (2014) revealed that Bayesian analysis clustered all individuals of genus *Corylus* into three groups showing a good separation among wild genotypes, landraces and cultivars. Our data revealed the close relationship between *S. glabratum* var. *sepicula* and *S. albicaule*. On the other hand, the unknown wild species was more closely related to *S. nigrum* and *S. schimperianum* than other species. The previous data was in accordance with Jaeger (1985) who reported that *Solanum lycopersicum* L. var. *lycopersicum* is belonging to subgenus *Potatoe* section *Petota* subsection *Lycopersicon* ser. *Neolycopersicon*, while *S. albicaule* Dunal and *Solanum glabratum* var. *sepicula* (Dunal) J.R.I. Wood are belonging to subgenus *Leptostemonum* section *Oliganthes*, *Solanum incanum* L. is also belonging to subgenus *Leptostemonum* section *Melongena*, and disagreed in case of *Solanum schimperianum* Hochst. ex Dunal that belonged to subgenus *Leptostemonum* section *Giganteiformia*.

The present study suggested a new species of *Solanum* in Taif highlands, that needed more investigation to be identified. The distinguishing morphological characters were shrub up to 1.5 m unarmed with irregular tree like hairs (dendritic hairs); stem striated with small dents on the ribs; leaves petiolate (up to 1.5cm), ovate-hastate lobed with entire margin, 3.5-4.5 x 2.5-3cm, leaf apex acute-acuminate, veins green, flowers have white petals with violet midrib, 4-5 stamens, yellow colour anthers, fruits aggregates on umbellate clusters of 4-5 fruits, fruiting peduncle up to 2cm and directed upward, pedicels reflexed of 1cm long, sepals persist and reflexed; fruits are dull orange of 0.8-0.9cm diameter, seeds dull yellow striated, 0.7-9mm diameter. The morphological and molecular characters of unknown species showed marked relation to subgenus *Solanum*. High level of polymorphism was observed going by the coefficient of variation which exhibited a good separation from a conserved region of the genome. This revealed a wide and diverse genetic base in Saudi Arabian *Solanum*. Knowledge of molecular relationships between plant species is very useful in planning effective breeding strategies designed to transfer desirable genes or gene clusters from one species into another, thereby producing fruitful genomic reconstructions and disease free plants.

In conclusion, species database of some wild species of *Solanum* was provided in Taif of Saudi Arabia with emphasis on variation patterns which was a major contribution to global biodiversity information system. It was evident that RAPD and other discontinuous markers could be used as a means of genetic distances to establish *Solanum* taxonomy as well as phylogenetic relationships among taxa.

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التباين الوراثى داخل جنس السولانم فى مرتفعات الطائف اعتمادا على تقنية قطع الدنا المكثرة عشوائيا

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جنس السولانم جنس كبير ينتمى للعائلة الباذنجانية ويشتمل على اثنين من المحاصيل الغذائية الاقتصادية الهامة هما البطاطا والطماطم، كما أنه يحتوي على العديد من النباتات المزروعة بغرض الزينة والفاكهة. و لاختلاف انواع هذا الجنس من ناحية الشكل والتوزيع الجغرافى داخل المملكة العربية السعودية اختلافا كبيرا، فقد اهتمت الدراسات السابقة القليلة جدا بالتوصيف والتفريق بين الأنواع داخل الجنس بناءا على شكل الكروموسومات والأهمية الطبية، لها وذلك لم يكن كافيا من الناحية الوراثية. لذا اهتم هذا البحث بتقييم التنوع الجزيئى لبعض أنواع من جنس السولانم البرية والمنزرعة فى مرتفعات الطائف بناء على الكاشفات المستنبطة من قطع النا المكثرة عشوائيا وبروتينات الأوراق المفردة كهربيا. ولقد سجلت احدى عشرة بادئة من بين خمسة وعشرين تم استخدامها نسب تباين عالية (١٠٠٪) بين الانواع محل الدراسة. وسجلت البادئة OPA-09 النسب الأعلى فى حين سجلت البادئات OPP-10 و OPN-13 و OPN-05 النسب الأقل. وأعطى الفصل الكهربى لبروتينات الأوراق ٥١ حزمة أغلبها لم يكن مشتركا بين الأنواع وبعضها كان متفردا فى كل نوع على حدة مدعما التباين الكبير المسجل فى تقنية تفاعلات انزيم البلمرة. ولقد تم توصيف الشكل الظاهرى لنوع برى يعتقد أنه جديد حيث يحتاج لمزيد من الدراسات لتعريفه وتسجيله كنوع جديد فى بيئة الفلورة السعودية. وكذلك تمت دراسة علاقات القرابة والتشابه الجينى بين بعض الأنواع البرية والمنزرعة داخل جنس السولانم.