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Assessing molecular biodiversity within the *Solanum* genus collected from Saudi Arabia

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Abstract
Ten RAPD primers and two isozymes were used to determine genetic biodiversity for thirteen genotypes representing six different species of *Solanum* (*S. villosum, S. nigrum, S. incanum, S. glabratum, S. torvum,* and *S. dulcamara*). The plant samples were collected from different five areas in Aseer province, south-west of Saudi Arabia. We aim, fill in some of the present gaps in the *Solanum* species taxonomy in Saudi Arabia. The RAPD used primers amplified 159 bands, 143 of them were polymorphic (89.94%). Alcohol dehydrogenase isozyme showed a low level of variation with four polymorphic enzymatic bands. While, malate dehydrogenase isozyme showed a relatively higher level of polymorphism in which ten polymorphic out of total twelve enzymatic bands were scored. Our results indicated that *S. nigrum* and *S. villosum* can be divided into two or three subspecies. We suggest that *S. nigrum* was originated from *S. villosum*. *Solanum torvum* may be related to *S. nigrum*. *Solanum incanum* had different genetic origin from the other species under study. We decided some specific markers for *Solanum* species and genotypes.

Keywords: genetic biodiversity, isozymes, RAPD primers, *Solanum*

1. Introduction
Economically, *Solanaceae* is one of the most essential and serving to mankind in Saudi Arabia and all over the world. It contains familiar food plants such as eggplant (*Solanum melongena*), potato (*S. tuberosum*), tomato (*S. Lycopercicum*), and pepper (*Capsicum frutescens*) El-Shaboury (2016) [14]. The importance of *Solanum* species cannot be overestimated. Species of this genus are considered one of the biggest groups of vegetables, and are a significant source of income for farmers around the world (Manoko and van der Weerden, 2004) [22]. Moreover, some species are used as a source of drugs in medicine pharmacology and drug therapy. But the raw materials of these drugs are poisonous (Jainu and Devi, 2005) [18].
Genus *Solanum* species still need moretaxonomic study. The main concern about *Solanum* taxonomy is that in spite of the economic significance and prospect of this genus in the world, its taxonomy still understood. El-Shaboury *et al*. (2018) [13] recent taxonomical studies identified three new species of *Solanum* as new addition for Saudi Arabia flora. These new additions related tofamily *Solanacaeae* and have been defined as *Solanum sisymbriifolium* Lam., *Solanum torvum* Swartz and *Solanum dulcamara* L., and they stated that the report of these new records shows that this region needs extra floristic exploration.
Classification of *S. villosum* and *S. nigrum* as distinct species or varieties have a long taxonomic argument on the taxonomic identity of these two species from Linnaeus, who classified *S. villosum* as varieties of *S. nigrum*, contrary to that proposed by Miller, who recommended *S. nigrum* and *S. villosum* as distinct species (Edmonds and Chweya, 1997) [11]. A restricted effort has been done on the nature of genetic diversity and classification of wild and cultivated *Solanum* is Saudi Arabia. Haroun and Al-Wadi (1999) [17]; Al-Wadi and Lashin (2007) [4] they studied cytological characters of limited *Solanum* species from Aseer province, south-west Saudi Arabia and their taxonomic significance. Ahmed and Fadl (2015) [2] study the molecular diversity of seven *Solanum* species collected from Taif highlands using RAPD and SDS-PAGE. El-Shaboury *et al*. (2017) [15] they investigated the diversity of some *Solanum* species, Aseer province, south-west Saudi Arabia established on alterations in the secondary metabolites by GC-MS analysis, they revealed that *S. villosum* and *S. nigrum* can be considered as one species. Their results also showed a relationship between *S. schimperianum* and *S. incanum.* The objective of the present work is to study the taxonomic information of some species of *Solanum* using a considerable number of samples and molecular techniques as analysis of isozymes and RAPD analysis also assessment their genetic relationship to the recently new recorded species *S. torvum* and *S. dulcamara*.

2. Materials and Methods
2.1 Plant Samples
Plant materials of thirteen genotypes representing six different species of *Solanum* were collected as a mature flowering plants from different locations representing different areas in Aseer region, south west of Saudi Arabia. The collected samples were identified rendering to Collenette (1999) [8] and Chaudhary (2001) [7]. The all information of the area and the site of the collection of the examined species are given in Table 1. Herbarium specimens of each species have been deposited in the herbarium of the College of Science and Humanities, Shaqra University, Riyadh, Saudi Arabia.

2.2 Isozymes Analysis
Isozymes analysis were applied using one gram of the young leaves of the collected plant samples were crushed in a mortar with 5ml of extraction buffer (0.25M Sodium phosphate (pH 7.3) and 20% Sucrose) The suspension was left at 4°C for 1 h, then clarified by centrifugation at 10000 rpm in a microcentrifuge at 4°C for 5 min. and 30 µ1 of each sample

were applied to the gel (Agarwal *et al*., 2001) [1]. The electrophoretic analysis was approved using native polyacrylamide gel electrophoresis as described by Vladova and Petkolicheva (1996) [29]. Electrophoresis was approved under cooling conditions at constant currant of 50 mA. Staining assays of different enzymes were carried out according to Weeden and Wendel (1989) [30]. Alcohol dehydrogenase (ADH) 50mg NAD, 30mg nitroblue tetrazolium, 2 mg phenazine mesosulfate, 3 ml ethanol (95%) dissolved in 0.05 M Tris- HCl buffer, pH 7.1, to a final volume of 100 ml. Malate dehydrogenase (MDH) 50 NAD, 30 mg Nitroblue tetrazolium, 2 mg phenazine methosulfate, 10 ml 1 M Na L-malate dissolved in 0.05 M Tris-HCl buffer, pH7 to a final volume 100 ml.

2.3 RAPD Analysis

2.3.1 Extraction Genomic DNA

From the young seedling leaves of the collected *Solanum* samples genomic DNA was extracted using saleable kit, "DNeasy plant mini kit product by QIAGEN-COMPANY". All the steps were indicated in the Kit.

2.3.2 Primers

Ten RAPD markers were used to investigate any alteration in DNA finger prints pattern collected *Solanum* samples, names and sequences for each primer described in Table 2.

2.3.3 PCR Conditions

PCR (Polymerase chain reaction) for RAPD markers were did by using Bio-Rad. thermal cycler in a ultimate volume of 25 μL, {25 ng of template genomic DNA, 12.5 μL of Taq DNA polymerase (2.5 mMMgCl2, 2.5 mMof each dNTPs) and 7 μL from each primer} and completed to desired volume by free nuclease water (Sushant *et al*., 2013) [28]. Amplification process scheme as follows: DNA Initial denaturation of genome at 94°C for 5 min., followed by repetitive 49 denaturation cycles at 94°C for 1 min., then annealing temperature 30°C for 1 min, 72°C for 2 min for extension and 72°C for 7 min for final extension.

2.3.4 Gel Electrophoresis

1%Agarose gel was organized for imagining of resulted genomic DNA from the collected *Solanum* samples. 1.45% agarose gel was used to visualize the resulted RAPD-PCR amplified fragments of DNA. Ethidium bromide with 0.5 µg/mL concentration was used to stain the Agarose gels. The used running buffer was 0.5X Tris-borate-EDTA (TBE) buffer. The running time was for 60 min at 90 voltages, ProXima AQ-5 gel documentation system was used for imagining (Marsafari and Mehrabi 2013) [23]

2.4 Data Analysis

The relationship among the examined *Solanum* species was estimated based on differences in isozymes analysis and analysis of ten RAPD markers. In the meantime the clear, unambiguous and reproducible isozymes and DNA bands were considered for scoring. Each band was considered a single locus and scored as 1 for the presence and 0 for the absence, for all examined *Solanum* species/populations. A dendrogram was constructed from the distance by the unweighted paired-group method, arithmetic mean (UPGMA) algorithm contained in the computer program package NTSYS 1.5 (Rohlf, 2002) [27].

3. Results

3.1 Variation of isozymes

Polyacrylamide gel electrophoresis was used for the separation and characterization of two isozyme systems in collected *Solanum* samples. These isozymes were alchol dehydrogenase (ADH) and malate dehydrgenase (MDH). They showed a degree of polymorphism within the studied *Solanum* genotypes. Alcohol dehydrogenase isozyme showed a low level of variation with four polymorphic enzymatic bands (Adh1, Adh2, Adh3 and Adh4) as shown in Figure (1-A). The two genotypes representing *S. glabratum* have the same banding pattern (Adh 2, Adh 3 and Adh 4). *Solanum nigrum* genotype (1), *S. villosum* genotype (5), *S. incanum* genotypes (7 and 8) and genotypes representing *S. tovum* have only one enzymatic band (Adh 2).Genotypes 2 and 3 of *S. nigrum*, genotype 4 of *S. villosum* and genotype 6 of *S. incanum* have three enzymatic bands (Adh 1, Adh 3 and Adh 4). Malate dehydrogenase isozyme showed a relatively high level of polymorphism in which 10 polymorphic out of total 12 enzymatic bands were scored across different *Solanum* genotypes as shown in Figure (1-B). The lowest number of enzymatic bands (4) was found in *S. nigrum* genotype (2). While *S. incanum* genotype (8) showed the highest number (8) of enzymatic bands.

3.2 Genetic diversity based on isozymes analysis

Based on the results obtained from isozymes (Figure 1), the phylogenetic relationships among *Solanum* species were analyzed by UPGMA method. A dendrogram generated using isozyme markers separates the analyzed samples into two main clusters Figure (2). The first one included the three genotypes of *S. incanum*, in which accession (6) which collected from Abha (El-Soda) was separated from the other two genotypes (accessions 7 and 8 which collected from Abha- Alarin and Bisha respectively). The other main cluster included the other five studied *Solanum* species. This cluster divided into two subclusters, the first cluster included *S. dulcamara* and the two genotypes of *S. glabratum*. While, the second subcluster contained the two genotypes of S. *torvum*, the two genotypes of *S. villosum* and the three genotypes of *S. nigrum*.

3.4 RAPD Analysis

In this study, ten random 10-mer primers were used and amplified 159 bands, 143 of them were polymorphic (89.94%). The distribution of the polymorphic DNA bands reflects the variability among the studied genotypes. The minimum band number amplified per primer was only one band in primer OPB-05 and primer OPB-09. The size of amplified fragments ranged from 225 bp for primer OPB-07 to 2500 bp for primers OPB-10. (Figure 3).

The dendrogram demonstrating the relationships among the studied *Solanum* genotypes generated by DICE computer package based on RAPD markers is shown in Figure (4). The thirteen *Solanum* genotypes were separated by a wide range of differences. The studied genotypes were clustered into two main clusters. The three genotypes representing *S. incanum* were grouped in one cluster. Moreover, genotype (6) of *S. incanum* was separated from the other two genotypes, similar to the result obtained based on isozymes analysis. The other *Solanum* species (*S. glabratum*, *S. torvum*, *S. dulcamara*, *S. nigrum* and *S. villosum*) were grouped in the second main cluster. The two genotypes representing *S. torvum* and the two genotypes of *S. glabratum* were grouped in separate

subcluster for each group. *Solanum dulcamara* was included in one subcluster with *S. nigrum* and *S. villosum*. Genotype (3) of *S. nigrum* was separated from the other two genotypes representing this species.

3.5 Specific markers for *Solanum* genotypes

In the present study, seventeen specific markers were scored as shown in Table 3. Three specific markers were scored for malate dehydrogenase isozyme (MDH). I-mdh-3 isozyme marker was specific for *S. glabratum*. I-mdh-10 and I-mdh-12 isozyme markers were negatively specific for *S. dulcamara*. Fourteen RAPD markers scored as unique bands, out of 143 polymorphic bands which amplified by ten primers. One of these markers was specific for *S. dulcamara* (R-B09-100). No specific markers were detected for the primers B-06, B-08 and B-10. However, primers B-01and B-05 detected only one markers. While, the highest number of specific markers was detected by primers B-02 and B-09. (Figure 3 and Table 3).

Table 1: The elevation, area, GPS location and sites from which the studied *Solanum* Samples were collected.

No	Collected accessions	Area	Site	GPS location	Elevation (m asl*)
1	*Solanum nigrum* L.	Abha	El-Soda	N 18º-16´-18.235" E 42 º - 23´- 2.406"	2784
2	*Solanum nigrum* L.	Bisha	King Saud Road	N 19 º -59´-0.791" E 42 º -36´-14.866"	1166
3	*Solanum nigrum* L.	Jazan	Jazan University	N 16 º -58´-25.954" E 42 º -32´-36.98"	0
4	*Solanum villosum* Mill.	Abha	El-Soda	N 18-16´-18.235" E 42- 23´- 2.406"	2784
5	*Solanum villosum* Mill.	Bisha	King Saud Road	N 19 º -59´-0.791" E 42 º -36´-14.866"	1166
6	*Solanum incanum* L.	Abha	El-Soda	N 18 º -16´-18.235" E 42 º - 23´- 2.406"	2784
7	*Solanum incanum* L.	Abha	Abha- Al-Arin	N 18 º -14´-3.86" E 42 º -32´-12.697"	2155
8	*Solanum incanum* L.	Bisha	King Saud Road	N 19 º -59´-0.791" E 42 º -36´-14.866"	1166
9	*Solanum torvum* Swartz.	Jazan	Airport Road	N 16 º -53´-51.698" E 42 º -34´-18.765"	3
10	*Solanum torvum* Swartz.	Wadi El-Dawaser	El-Fraa Road	N 20 º -29´-14.663" E 44 º -45´-40.132"	680
11	*Solanum glabratum var. sepicula* Dun.	Jazan	Jazan University	N 16 º -58´-25.954" E 42 º -32´-36.98"	0
12	*Solanum glabratum var. sepicula* Dun.	Najran	King Abdulaziz Road	N 17 º -29´-25.7" E 44 º -8´-12.509"	1309
13	*Solanum dulcamara* L.	Bisha	King Saud Road	N 19 º -59´-0.791" E 42 º -36´-14.866"	1166

* Metre above sea level

Table 2: Codes and sequences of Operon B arbitrary 10-mer primers.

Code	Sequence (5´→3´)	Code	Sequence (5´→3´)
OPB-01	5´-GTTTCGCTCC-3´	OPB-06	5´-TGCTCTGCCC-3´
OPB-02	5´-TGATCCCTGG-3´	OPB-07	5´-GGTGACGCAG-3´
OPB-03	5´-CATCCCCCTG-3´	OPB-08	5´-GTAGACCCGT-3´
OPB-04	5´-GGACTGGAGT-3´	OPB-09	5´-CCTTGACGCA-3´
OPB-05	5´-TGCGCCCTTC-3´	OPB-10	5´-TTCCCCCGCT-3´

Table 3: Specific markers for *Solanum* species and genotypes across different levels of analysis

Species-specific markers	Specific markers
Solanum glabratum var. sepicula Dun.	I-mdh-3
Solanum dulcamara L.	R-B09-100
Genotype-specific markers	Specific markers
Solanum nigrum L. (genotype 1)	R-B03-160, 200
Solanum nigrum L.(genotype 2)	R-B07-1620, R-B09-1382, R-B04-393
Solanum nigrum L.(genotype 3)	R-B04-1270
Solanum dulcamara L. (genotype 13)	I-mdh-10*, I-mdh-12*
Solanum villosum Mill(genotype 5)	R-B07-1140
Solanum glabratum (genotype 11)	R-B02-583
Solanum incanum L. (genotype 6)	R-B02-953, R-B09-44
*Solanum torvum*Swartz. (genotype 9)	R-B01-1220, R-B02-866, R-B05-1385

Negative marker band: (*)

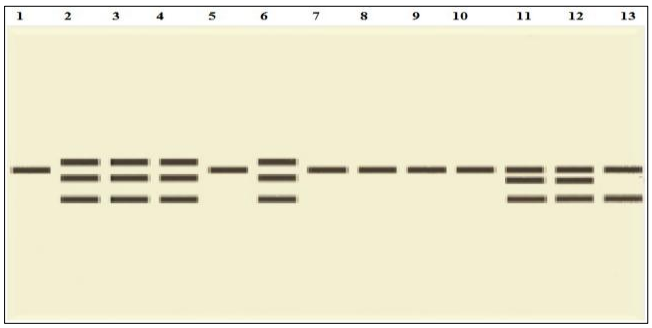

Fig (1A): Zymogram of alchol dehydrogenase (ADH) of studied *Solanum* genotypes as numbered in Table 1.

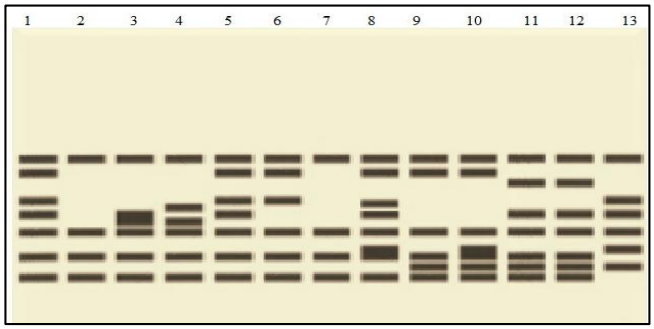

Fig (1B): Zymogram of malate dehydrogenase (MDH) of studied *Solanum* genotypes as numbered in Table 1.

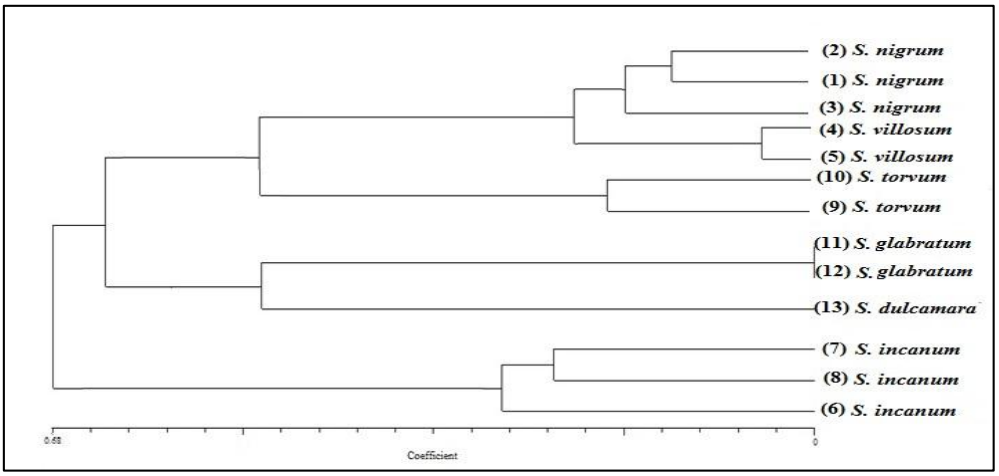

Fig 2: UPGMA distance tree computed using the NTSYS-pc showing the relationships among the *Solanum* species/populations based on the analysis of isozymes.

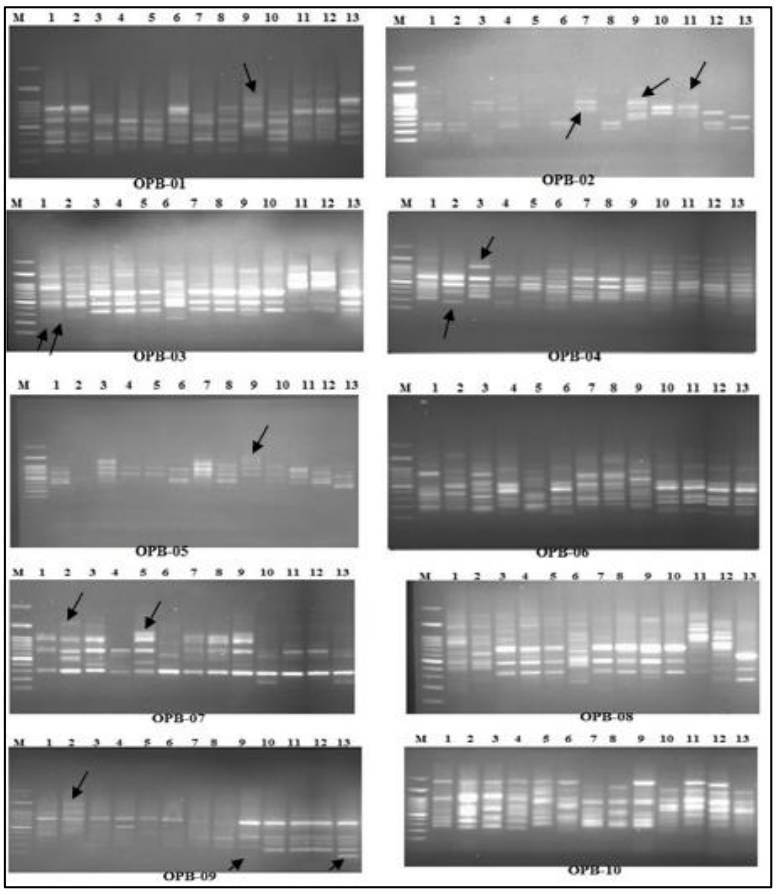

Fig 3: RAPD fingerprinting profile produced by primer OPB-01 to OPB-10 for *Solanum* species/populations as numbered in Table 1. (Unique bands is indicated by arrows). *M: 100 bp marker DNA ladder.

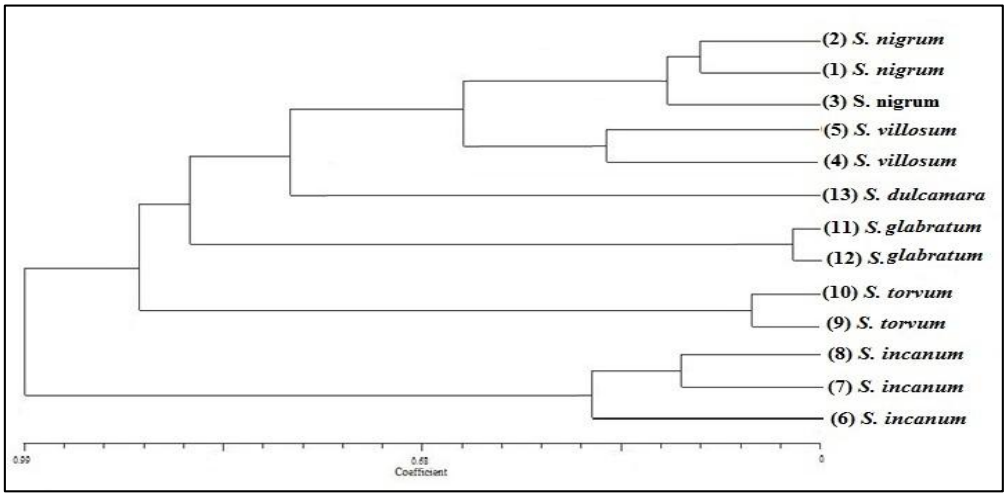

Fig 4: UPGMA distance tree computed using the NTSYS-pc showing the relationships among the *Solanum* species/populations based on the analysis of RAPD markers.

4. Discussion

In our study thirteen genotypes representing six different *Solanum* species were collected from different locations of the study area Table (1). Molecular analysis using isozymes and RAPD analysis were performed for all collected samples to assess their genetic biodiversity. Molecular markers (DNA finger printing and protein bands) represent a powerful and potentially rapid method for the characterization of plant diversity (Ford-Lloyd, 2001) [16].

Isozymes had been used in biodiversity by many workers e.g. (Arulsekar *et al*., 1986a, 1986b; Mowrey *et al*., 1990) [5, 6, 25]. In our study, alcohol dehydrogenase (ADH) isozyme showed a low level of variation with four polymorphic enzymatic bands. While, malate dehydrgenase (MDH) isozyme showed a relatively higher level of polymorphism in which ten polymorphic out of total twelve enzymatic bands were scored across different *Solanum* genotypes. These results agree with Karihaloo *et al.* (1995) [20] they carried out RAPD analysis and leaves isozyme survey on 52 *Solanum melongena* accessions (eggplant) and *S. incanum*, the results of electrophoretic isozyme study indications that *S. melongena* and *S. incanum* were highly diverse.

The dendrogram generated based on isozymes analysis, could not separate the two genotypes of *S. glabratum* Figure (2). In addition, the three genotypes representing *S. nigrum* were not grouped in separate cluster, where accession 1 and 2 clustered in one group and accession 3 separated individually these results may reflected due to the site of collection because accession 1 and 2 collected from Abha and Bisha at high elevation of see level than the accession 3 which collected from Jazan at zero elevation of see level. Also the separation of *S. incanum* (6) from the other two genotypes 7 and 8 may be related to the site of collection from different elevations. Moreover, the dendrogram did not show a clear separation between the two genotypes representing *S. villosum* due to the high similarity level which recorded between the two genotypes.

Our results based on isozymes analysis, did not support the separation of *S. nigrum* and *S. villosum* into different species due to the high similarity level between the two species so we confirm that *S. villosum* is related to *S. nigrum*. Based on this results, *S. villosum* may be considered as sub species of *S. nigrum*. Edmonds and Chweya (1997) [11] stated that, there are morphological difference between *S. nigrum* and *S. villosum* with consideration that the former has black berries while the other has orange/orange-red berries. However Mohy-UD-Din (2008) [24] defined *S. nigrum* and *S. villosum* as distinct species based on analysis of morphological traits and secondary metabolites. Our data also agree with Linnaeus, who classified *S. villosum* as varieties of *S. nigrum*, contrary to that proposed by Miller, who recommended *S. nigrum* and *S. villosum* as distinct species (Edmonds and Chweya, 1997) [11]. Moreover the recently introduced species *S. torvum* may be also related to *S. nigrum* because it also clustered with *S. nigrum* in one group. Also the two accessions of *S. glabratum* were clustered with the recently introduced species (*S. dulcamara*) in one cluster this may indicate that the two species may be related to each other Figure (2). This results in accordance with (El-Shaboury *et al*., 2017) [15] where they study the biodiversity of some species of *Solanum* based on alterations in the secondary metabolites using GC-MS analysis the generated denderogram showed that *S.torvum*, and *S. nigrum* were found in one group and also the used marker could not separate the two genotypes of *S. glabratum*.

In the present study, RAPD-PCR analysis for DNA of thirteen *Solanum* genotypes involved ten random 10-mer primers. These primers showed high degree of polymorphism (89.94%) Figure (3). The dendrogram demonstrating based on RAPD-PCR analysis showed the relationships among the studied *Solanum* genotypes and indicated that the thirteen *Solanum* genotypes were separated by a wide range of differences (Figure4). The separation of genotype (6) of *S. incanum* from the other two genotypes, and the separation of genotype (3) of *S. nigrum* from the other two genotypes representing this species was supported by the results recorded from isozymes analysis. Also the two genotypes of *S. glabratum* were successfully separated as individual subcluster. Our results are in agreement with (Alam *et al.*, 2012) [3] on their study on five varieties of potato (*S. tuberosum*), they indicated that each variety showed specific characteristic RAPD markers. Similar results were also obtained by (Mace *et al*., 1999) [21] who studied the genetic variations between *S. melongena* L. and wild relatives using amplified fragment length polymorphism (AFLP).

Based on the results obtained from RAPD markers; the genetic distances recorded between *S. nigrum* genotype (3) and genotype (1).In addition, genotype (3) of *S. nigrum* was separated from the other two genotypes representing this species as shown in the dendrogram (Figure 4), this provide

a strong evidence to separate these genotypes in different subspecies. Each one of the genotypes representing *S. nigrum* had its own specific numbers of RAPD markers, except *S. nigrum* genotype (3) has no specific marker **(**Table 3**)**. Division of *S. nigrum* into two subspecies may be attributed to its occurrence in different ploidy levels (tetraploids and hexaploids). This idea is in agreement with the results of (Edmonds, 1979; Edmonds and Chweya, 1997) [9, 11] in their study on the hexaploid *S. nigrum*. However, some authors as Olet (2004) [26] revealed that no evidence for subspecies in *S. nigrum* accessions from Europe, Uganda and Australia.

Similarly, we indicated that the two genotypes of *S. villosum* can be divided into two subspecies, where RAPD analysis successfully separated the two genotypes with a relatively high value of genetic distance. This is in agreement with Edmonds (1979)[9] and Edmonds and Chweya (1997) [11].The occurrence of *S. nigrum* and *S. villosum* in the same cluster as shown in the dendrogram (Figure 4), reflects their close relationship, this may lead to suggest that *S. nigrum* was originated from *S. villosum*. Jardine and Edmonds (1974) [19]; Edmonds and Glidwell (1977) [12]; Edmonds (2005) [10] revealed that *S. villosum* was one of the parents of *S. nigrum*. This was also evident from earlier studies based on morphological data (Edmonds, 1979) [9].

Solanum incanum which was represented in our study by three genotypes was isolated in a separate cluster in the dendrogram, suggesting different genetic origin from the other five species under study (*S. glabratum, S. torvum, S. dulcamara, S. nigrum* and *S. villosum*). Moreover, genotype (6) of *S. incanum* was separated from the other two genotypes, suggesting two subspecies. Data from our study indicated the genetic stability of the diploid species *S. glabratum, S. torvum* and *S. dulcamara* as indicated from the dendrogram (Figure 4), and supported by previous studies as El-shaboury *et al*. (2017) [15]. Our results revealed that the two new introduced species (*S. torvum* and *S. dulcamara*) have high degree of relationship with the other common *Solanum* species in addition we decided some specific markers for the all studied *Solanum* species.The study revealed that species database of some wild species of *Solanum* was provided in south-west of Saudi Arabia with emphasis on variation patterns which was a major contribution to global biodiversity information system. It was evident that isozymes and RAPD analysis could be used as a means to establish the genetic distances as well as phylogenetic relations among *Solanum* species.

Conflict of Interest

The author declare that they have no conflict of interest.

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