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GENETIC RELATEDNESS AMONG *SOLANUM* L. SPECIES ASSAYED BY SEED MORPHOLOGY AND ISOZYME MARKERS

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Abstract

In spite of their economic and medicinal value, no adequate attention has been paid to the diversity, characterization and taxonomical identification of *Solanum* L. species in Saudi Arabia. In this study, Scanning Electron Microscopy (SEM) of seed coat morphology and isozyme electrophoresis were employed for studying the genetic variability and relationships among seven *Solanum* L. species namely; *S. incanum* L., *S. nigrum* L., *S. villosum* L., S. *schemprianum* Hochst, *S. galabratum* Dunal, *S. lycopersicum* L. and *S. melongena* L. collected from Taif highlands. Scanning Electron Microscope (SEM) investigation of seed coat sculpturing showed three basic patterns namely; rugulate, reticulate and levigate. The analyses on six enzymes were coded by 19 loci. The number of alleles ranged from one to three with a mean of 1.58 alleles per locus. The proportion of polymorphic loci for *Solanum* L.species ranged from 0.87 for *S. nigrum* L.and *S. villosum* L.to 0.80 for *S. lycopersicum* L. The mean observed heterozygosity varied from 0.00 to 1.00, while mean expected heterozygosity ranged between 0.00 and 0.5. The UPGMA phenogram confirmed the extensive genetic diversity existed in the studied *Solanum* L. species and showed the close relationship between *S. incanum* L. and *S. melongena* L.

Key words: *Solanum,* SEM, Isozyme, Heterozygosity, Genetic variability.

Introduction

Solanum L., a complex and large genus of the family Solanaceae, contains roughly between 1,500 and 2,000 species (Tepe *et al*., 2012). Species of *Solanum* are useful in the headache, heart burning and heat of stomach (Yousaf *et al*., 2006). In Saudi Arabia, the genus is represented by about 16 species, mainly in West and Southwest sides of the country (Collenette, 1999; Chaudhary, 2001). Limited work has been made on the nature of genetic diversity and characterization of wild and cultivated *Solanum*, especially using molecular methods. Taxonomic studies on *Solanum* L. 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.

Due to gross morphology of seeds, sculpturing details of outer seed coat under the SEM are quite variable between different species and has been well recognized as a reliable approach for assessing phenetic relationship and identification of species or the other taxa in Solanaceae (Koul *et al*., 2000; Zhang *et al*., 2005; Bohs *et al*., 2007; Upadhye *et al*., 2012; Anilkumar *et al*., 2014).

Despite the use of DNA markers such as RAPDs, AFLPs and RFLPs, isozymes, as biochemical markers, are still widely employed in species delimitation and conservation (Ferguson & Robertson, 1996; Shinwari *et al.,* 2014; Jan *et al.,* 2016), assessment of genetic variability in species and populations (Harris *et al*., 1994; Shinwari *et al.,* 2013), cultivar identification (Samec *et al*., 1998; Jan *et al.,* 2016) and evolutionary studies (Testolin & Ferguson, 1997). Isozymes are especially useful when several taxa, accessions and individuals are to be compared, as the assumption of homology is more accurate than with some DNA markers (Klaas, 1998; Iqbal *et al.,* 2014). They have been used for the identification of cultivars and lines of solanaceous groups (Rocha *et al*., 2001; Toppino *et al*., 2008).

Therefore, in the present study SEM and isozyme electrophoresis were used to evaluate genetic relatedness among two cultivars; *Solanum lycopersicum* L., *S. melongena* L. and five related wild species native to Taif of Saudi Arabia.

Materials and Methods

Seeds and fresh leaves (young and matured) of 26 individuals, varying from 3 to 6 per species and belonging to 2 cultivars; *Solanum lycopersicum* L. (3) & *S. melongena* L. (3) and 5 wild species; *S. incanum* L. (6), *S. nigrum* L. (3), *S. villosum* L. (3), *S. schimperianum* Hochst (4) and *S. glabratum* Dunal (4), 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). The collected wild materials were identified according to Collenette (1999) and Chaudhary (2001).

The finer morphological details were examined using the Scanning Electron Microscope (SEM) Model JEOL JSM-5600 at the Electron Microscope Unit, Taif University. The SEM-micrographs were taken after the mature seeds were coated with a thin layer of gold in JEOL JFC-1200 Fine Coater and examined in different positions using different magnifications. The morphological characters of seeds; shape, color, texture, surface feature, and hilum shape were recorded. All terminology used for the description of the testa sculpturing patterns are that of Lersten (1981).

The examined isozymes were: α-and β-esterases (*EST*); (E.C.3.1.1.1), alcohol dehydrogenase (*ADH*); (E.C. 1.1.1.1), aldehyde oxidase (*ALO*); (E.C.1.2.3.1), malate dehydrogenase (*MDH*); (E.C.1.1.1.37) and peroxidase (*PRX*); (E.C.1.11.1.7). For their extraction, 1 g of fresh leaves was homogenized in 1 ml extraction buffer (1 M Tris-HCl, pH 8.8) using a mortar and pestle; centrifuged at 10000 rpm for 5 minutes; the supernatant was kept at –20ºC until use. For their separation, 10% (w/v) native-polyacrylamide gel electrophoresis method was used (Stegemann *et al*., 1985). For electrophoresis, 50 µl of extract was mixed with 20 µl of treatment buffer and 35 µl of this mixture was applied to the well. In gels staining, protocols of Scandalios (1964) were used for *α*-and *β-EST*.; Wendel and Weeden (1989) for *ALO*; Weeden and Wendel (1990) for *ADH*; Jonathan and Wendell (1990) for *MDH* and Heldt (1997) for *PRX*. After run finished, gels were washed 2 or 3 times with tap water; fixed in ethanol: 20% glacial acetic acid (9:11 v/v) for 24 hours and photographed.

Seed multistate characters were transformed to two-state characters in coding (Sneath & Sokal, 1973; Crisci & Lópezarmengol, 1983). The parameters including allelic frequencies mean number of alleles per locus, the proportion of polymorphic loci and the observed expected heterozygosities were calculated according to Nei (1978). The presence or absence of each seed character (coded 1 and 0) and allelic frequencies of isozymes were pooled together to obtain unweighted pair group method (UPGMA) phenogram using SPSS-20 software program (Anon., 2011).

Results

SEM micrograph of seeds of *Solanum* species are shown in Figure 1. Some differences in shape, colour, texture, surface features and hilum shape were recorded. The seed surface as viewed under SEM showed three basic sculpturing patterns namely; rugulate (i.e., irregular Wrinkled), reticulate (i.e., hexagonal areas with undulating walls) and levigate (i.e., smooth). Rugulate pattern with undulating walls occurred in *S. lycopersicum*, *S. melongena*, *S. incanum* and *S. schemprianum*. Reticulate pattern occurred in *S. nigrum* and *S. villosum*. levigate pattern recorded only in *S. galabratum* with scattered feebly pits. On the other hand, hilum shape showed four patterns; oval shape in *S. lycopersicum*, *S. incanum*, *S. nigrum* and *S. villosum*, round in *S. schemprianum*, triangle in *S. galabratum* and oblong in *S. melongena*. The shape of seed was usually ovate to reniform, while seed colour ranged between yellow to brown. Seed texture was rough in all species with exception of *S. galabratum*.

Table 1. Allele frequencies of different gene loci influencing isozyme patterns detected in seven *Solanum* species, observed (H_{obs}), expected (H_{exp}) mean heterozygosities and percentage of polymorphic loci (%). (N) represents the number of individuals examined.

Locus	Allele	*S. incanum*		*S. nigrum*		*S. villosum*		*S. schimperianum*		*S. glabratum*		*S. lycopersicum*		*S. melongena*	
		N	Freq.	N	Freq.	N	Freq.	N	Freq.	N	Freq.	N	Freq.	N	Freq.
ADH1	*a*	6	0	3	1	3	1	4	1	4	0	3	1	3	1
	b		1		0		0		0		0		0		0
ADH2	*a*	6	1	3	1	3	1	0	0	0	0	0	0	0	0
ALO1	*a*	6	1	3	1	3	1	4	1	4	0	3	0	3	0
	b		0		0		0		0		1		1		1
ALO2	*a*	4	1	1	0.5	3	0.5	4	1	4	1	1	1	0	0
	b		0		0.5		0.5		0		0		0		0
α-EST1	*a*	6	1	3	1	3	1	4	1	4	1	3	1	3	1
α-EST2	*a*	6	1	0	0	0	0	0	0	0	0	0	0	3	1
α-EST3	*a*	0	0	1	0	3	0	4	0	3	0	3	1	0	0
	b		0		0		0		1		0		0		0
	c		0		1		0		0		1		0		0
β-EST1	*a*	6	0	1	1	3	1	4	1	4	0	3	0	3	0
	b		1		0		0		0		1		1		1
β-EST2	*a*	0	0	0	0	0	0	4	1	4	0	0	0	3	0.5
	b		0		0		0		0		1		0		0
	c		0		0		0		0		0		0		0.5
β-EST3	*a*	0	0	1	1	3	1	0	0	0	0	0	0	0	0
β-EST4	*a*	3	1	1	0	1	1	0	0	0	0	0	0	0	0
	b		0		1		0		0		0		0		0
MDH1	*a*	6	1	3	1	3	1	4	1	4	1	3	1	3	1
MDH2	*a*	2	1	3	1	3	1	0	0	0	0	0	0	0	0
MDH3	*a*	0	0	1	1	3	1	0	0	0	0	0	0	0	0
MDH4	*a*	3	0	0	0	0	0	4	1	4	0.88	3	0.83	3	0.5
	b		1		0		0		0		0.12		0.17		0.5
PRX1	*a*	6	1	0	0	0	0	0	0	4	1	3	1	3	1
PRX2	*a*	6	1	3	1	2	1	4	1	4	1	0	0	3	1
PRX3	*a*	3	1	1	1	1	1	4	1	4	1	0	0	3	1
PRX4	*a*	0	0	2	0.5	3	0.5	0	0	0	0	2	0.5	0	0
	b		0		0.5		0.5		0		0		0.5		0
H_{exp}		0.00		0.5		0.5		0.00		0.21		0.39		0.5	
H_{obs}		0.00		0.5		1		0.00		1		0.85		1	
Poly. loci (%)		0.86		0.87		0.87		0.82		0.83		0.80		0.82	

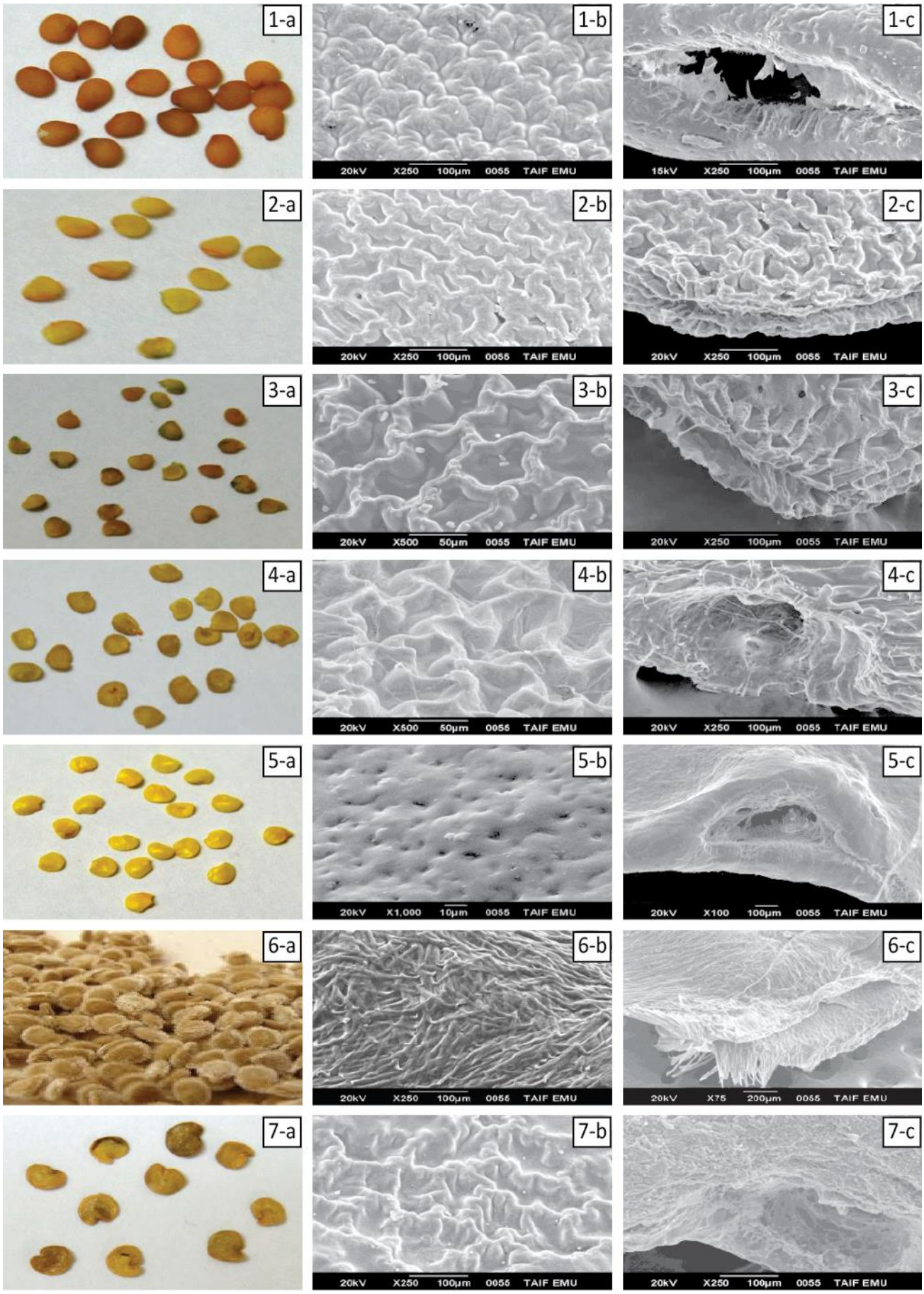

Fig. 1. Scanning electron microscopy study of *Solanum* species. 1- *S. incanum.* 2- *S. nigrum.* 3- *S.villosum.* 4- *S. schimperianum.* 5- *S. glabratum.* 6- *S. lycopersicum.* 7- *S. melongena*. a) seeds. b) micrographs of seed surface. c) micrographs of hilum.

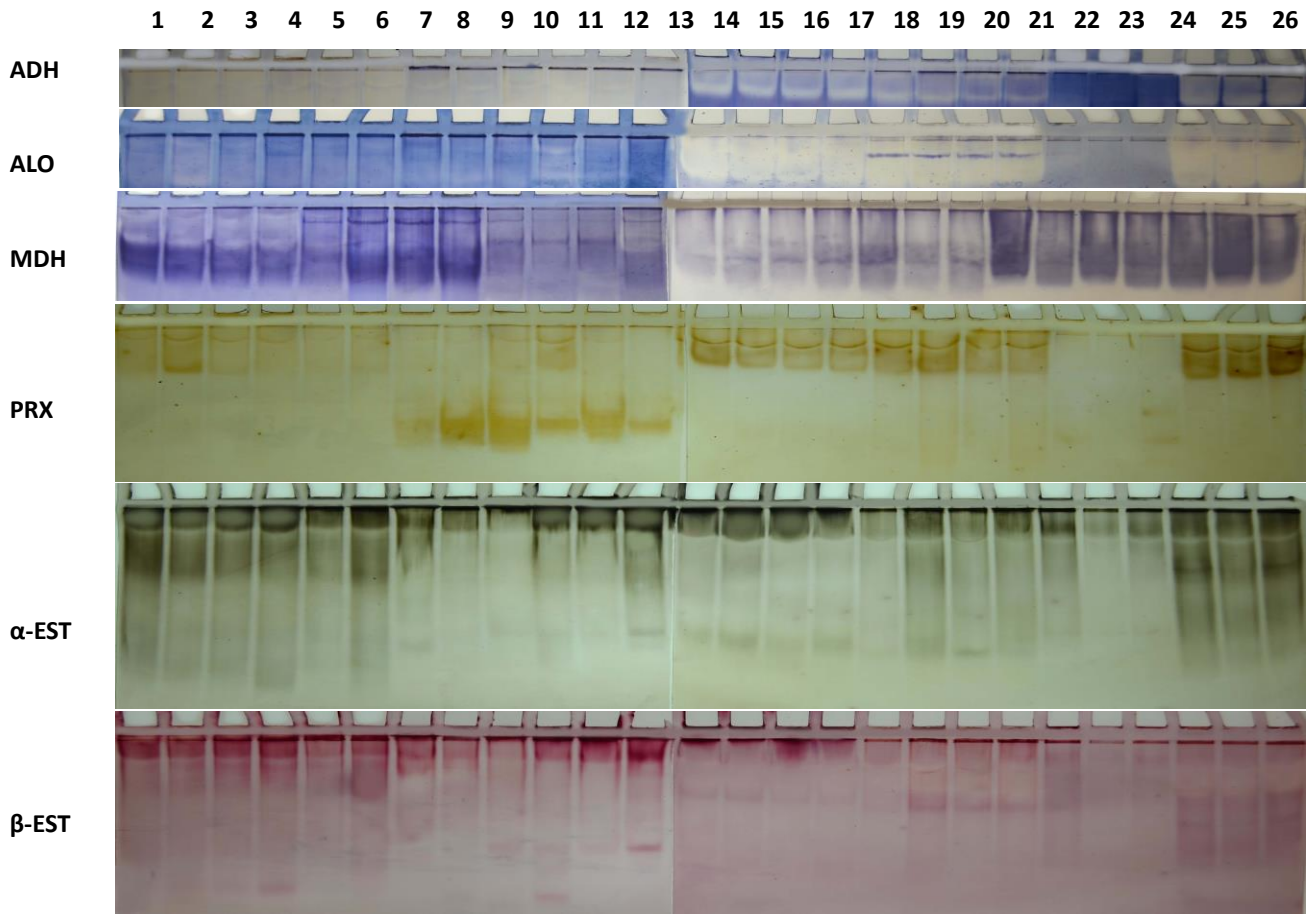

Fig. 2. Zymograms of seven *Solanum* species using six enzyme techniques. (1-6) *S. incanum*. (7-9) *S. nigrum.* (10-12*) S.villosum*. (13-16) *S. schimperianum*. (17-20) *S. glabratum*. (21-23) *S. lycopersicum*. (24-26) *S. melongena.*

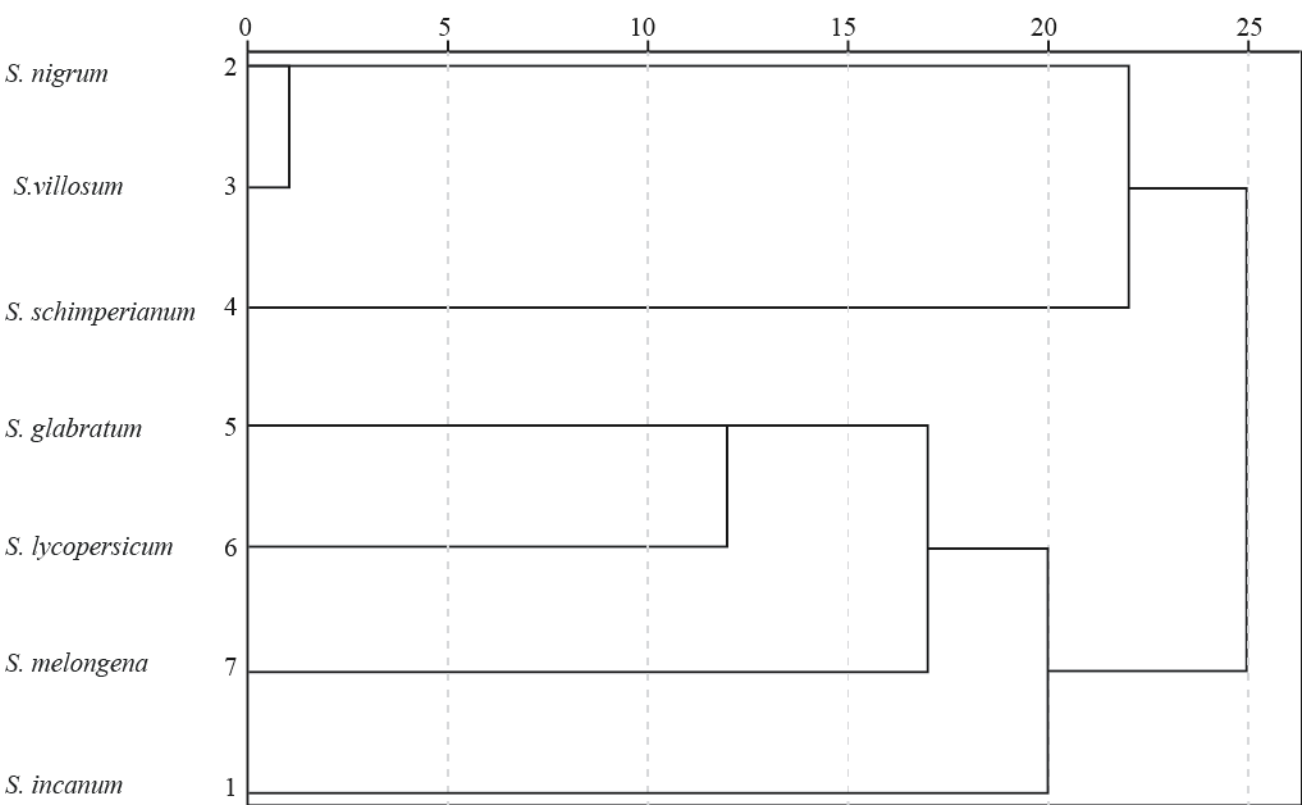

Fig. 3. UPGMA phenogram showing genetic relatedness of seven *Solanum* species based on combination of seed morphology and allozyme characters.

The six enzyme systems showed high levels of genetic variability (Fig. 2). 30 presumptive alleles, encoded by 19 enzyme loci, were identified. The mean number of alleles per locus was 1.58. The proportion of polymorphic loci for *Solanum*species ranged from 0.87 for *S. nigrum* and *S. villosum* to 0.80 for *S. lycopersicum*. The observed and expected heterozygosities ranged from 0.00 to 1.00 and 0.00 to 0.5 respectively (Table 1). 2 loci (*α-EST–1, MDH-1*) were invariant in all taxa examined and considered as monomorphic in genus *Solanum*. *α-* and *β- EST*s were expressed as 5 and 8 different alleles at 3 and 4 monomeric loci respectively. All of the alleles did not express simultaneously in one sample except for *α-EST–1* and they showed different isozyme patterns. Locus *α-EST–2* distinguished *S. incanum*and *S. melongena*from other species. Locus *α-EST–3a* characterized *S. villosum and S. lycopersicum. β-est-1a* scored in *S. nigrum, S.villosum*and *S. schimperianum*, while *β-est-1b* recorded in *S. incanum, S. glabratum, S. lycopersicum*and *S. melongena. β-est-2a* occurred in *S. schimperianum*and *S. melongena*. The *α-EST–3b, α-EST–3c, β-est-2b, β-est-2c* and *β-est-3*allozymes were unique and characterized *S. schimperianum, S. glabratum, S. melongena*and *S. villosum*respectively. Each of *ADH* and *ALO* appeared to be controlled by 2 polymorphic loci that were monomeric except for locus *ALO-2*. Loci *ADH-1b*, *ALO-2b* was unique in *S. incanum*and *S. villosum* respectively. *MDH* and *PRX* were coded by 4 loci for each. Three of them were monomeric, while loci *MDH-4* and *PRX-4* appeared as dimeric. All loci were polymorphic except for locus *MDH-1*. Allozyme *MDH-4b* distinguished *S. incanum*and *S. melongena*from other species. Using cluster analysis, *Solanum* species were categorized into two clusters as shown in Fig. 3.

Discussion

Although, some experimental taxonomic techniques studied relationships among *Solanum* species, high genetic variability and taxonomic confusion in identification and classification of these species had occurred due to their distribution over a wide area in southern Asia and eastern Africa (Jaeger & Hepper, 1986). In this investigation, SEM revealed considerable differences in the seed coat morphology among *Solanum* species. The variability in seed surface patterns was seemingly useful in the recognition of some species studied. This agreed with the results reported by Hasan & Lester (1990); Al-Wadi & Lashin (2007); Junlakitjawat *et al*. (2010) and Upadhye *et al*. (2012).

Isozymes as direct gene products are becoming biochemical markers of choice for initiating or advancing genetic studies of plants (Karaca, 2013). Results revealed that *ALO*, *MDH* and *PRX* as a mixture of monomeric and dimeric enzymes, while, *EST*s and *ADH* occurred as monomeric, were in accordance with Weeden & Wendel (1990). Although the investigation was restricted to only 3 to 6 individuals of each species, high interspecific variability was detected, both in number and frequency of bands and the complexity of the patterns, due to the number and deferent type of bands, was very informative and useful for discrimination. These high levels of genetic variability may be due to the interaction of several evolutionary factors: (1) natural and artificial selection; (2) sexual polyploidization, allowing transmission of heterozygosity of the diploid parents to the polyploids; (3) introgression from the related wild or weedy species via 2 n gametes (Oliver &Zapater, 1984). Based on the interspecific variation of the 6 enzymes used, the number of alleles ranged from 1 to 3 with a mean of 1.58 alleles per locus. The proportion of polymorphic loci for the *Solanum* species ranged from 0.87 to 0.80. The mean observed heterozygosity varied from 0.00 to 1.00 with a mean of 0.6, while mean expected heterozygosity ranged between 0.00 and 0.5 with a mean of 0.3. These values were accordant with the range of heterozygosity reported on *Solanum* spp. i.e., 0.6 to 0.7 by Freyre & Douches (1994) and were found to be higher than those reported on *Solanum* sect. Petota (0.36-0.83) (Oliver & Zapater, 1984) and (0.00-0.04) (Spooner *et al*., 1992). Heterozygosity in isozyme data has been reported to be influenced by factors such as the number and types of enzyme gene loci studied. The enzymes used in this study i.e., *EST, ADH, MDH, ALO* and *PRX* were polymorphic thus made the estimation of the variation to be relatively high. Furthermore, discrepancies in diversity estimation among different studies, even on the same species, could also occur if the number of loci controlling an enzyme were interpreted differently by different investigators. In addition, the number of populations screened could also influence the estimation, especially when the population size differed among studies.

Cluster analysis categorized *Solanum* species into two clusters. Cluster I grouped *S. nigrum* with *S. villosum*. This agreed with Edmonds & Chweya (1997) who mentioned that *S. villosum* was of the major species within the *Solanumnigrum* complex that can be taxonomically confused more so by intermediate forms and hybridization between the species. On the other hand, cluster II included *S. incanum*with *S. melogena* reflecting the closeness between them and supporting the earlier finding of Sakata & Lester (1994); Karihaloo *et al*. (1995); Furini & Wunder (2004); Singh *et al*. (2006) and Spooner *et al.* (2014). The closeness between *S. lycopersicum*and *S. glabratum* var. *sepicula* disagreed with Jeager & Hepper (1986) who reported that the two species belonged to two different subgenera; Potatoe section Petota and Leptostemonum section Oliganthes respectively.

Conclusion

In conclusion, from the small sample examined, seed coat microstructure may be useful in the identification and classification of the sectional and generic levels in the *Solanaceae.* The general species groupings by isozyme banding patterns were consistent with the traditional taxonomic species delimitation especially when several systems were employed. Therefore, isozyme patterns were useful and reliable biochemical markers for the genetic relatedness of genus *Solanum*.

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