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Full Length Research Paper

Amplified fragment length polymorphisms (AFLPs) analysis of species of *Solanum* section *Solanum* (*Solanaceae*) from Uganda

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The taxonomy of species belonging to *Solanum* section *Solanum* (sometimes referred to as the *Solanum nigrum* complex or black nightshades) is known to be difficult and has resulted in extensive synonymy. Yet, these species play a significant role in nutrition and food security, especially in developing countries. The amplified fragment length polymorphisms (AFLPs) technique was used to assess the genetic relationships among 107 accessions representing eight species of the section *Solanum* and to obtain new insight into the taxonomic status of the *S. nigrum* complex. Four primer combinations yielded 510 AFLP bands, only 29 of which were monomorphic. Neighbor-joining and principal coordinates analyses were performed. The AFLP data only partially correlated with earlier classifications based on morphology. We have been able to confirm the presence of only 5 out of eight species of this complex previously recognized from Uganda, that is, *Solanum americanum*, *Solanum scabrum*, *Solanum hirsutum*, *Solanum florulentum*/*Solanum tarderemotum* and *Solanum villosum*. In addition, *S. villosum* did not separate in accordance with previous subspecific ranks. It is likely that neither *Solanum grossidentatum, S. nigrum* nor *Solanum sarrachoides* occurred in Uganda. Also, there was no correlation between geographic localities for the Ugandan material and the AFLP results, indicating that most of the studied species are introduced.

Key words: Amplified fragment length polymorphisms (AFLPs), *Solanum nigum* complex, Africa, genetic variation, taxonomy, Uganda.

INTRODUCTION

Solanum section *Solanum* is a group of roughly 30 non-tuberous species distributed from temperate to tropical regions and from sea level to altitudes over 3500 m (Edmonds, 1978; D'Arcy, 1991; Edmonds and Chweya, 1997). In many developing countries, some species constitute an important food crop. Notable among these species is *Solanum scabrum* Miller, which is by far the most important indigenous leafy vegetable and widely cultivated in Africa and southern Asia (Berinyuy et al., 2002). In southwest Uganda, it is actually a commercial crop. Other species in Uganda whose leaves and/or fruits are eaten include *S. americanum* Miller (*Solanum nodiflorum* Jacq.), *Solanum florulentum* Bitter, *Solanum tarderemotum* Bitter and *Solanum villosum* Miller.

Species of section *Solanum* are known to have potential value in certain crop improvement experiments (Edmonds and Chweya, 1997). They are a source of resistance genes against various diseases (Colon et al., 1993; Dehmer, 2003; Roychoudhury, 1980; Datar and Ashtaputre, 1983) and as an alternative proteinase inhibitor for the production of insect-resistant transgenic plants (Lou et al., 2009). Glycoalkaloid solasodine and the steroidal sapogenins diosgenin and tigogenin in the green berries of some species can be used for commercial steroid hormone synthesis (Eltayeb et al.,

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1997; Máthé et al., 1980). Leaves of *Solanum nigrum* L. *sensu lato* possess antinociceptive, anti-inflammatory and antipyretic effects (Zakaria et al., 2009). Glycoprotein from the fruits has potential for the prevention of colitis (Joo et al., 2009) and the polysaccharides can be used as an immunomodulator and an anticancer agent (Li et al., 2009) The extract of *S. nigrum* has been found to have almost similar cytotoxicity to the extract of *Taxus baccata* on cancer cells (Shokrzadeh et al., 2010).

In Uganda, the name *S. nigrum* has been used in a broad sense prior to the publications of Edmonds (1971, 1972), Bukenya and Hall (1988) and Bukenya and Carasco (1995). In addition to the edible species mentioned, Bukenya and Carasco (1995) also recognized three other Ugandan taxa from the section *Solanum*: *S. nigrum* L. subsp. *nigrum*, *Solanum sarrachoides* Sendtn. and *Solanum grossedentatum* A. Rich. During the fieldwork for this study, *Solanum hirsutum* Dun. was also found. To date, classification of the section *Solanum* in Uganda has been based largely on morphological data of herbarium specimens (Bukenya and Carasco, 1995). However, members of section *Solanum* are morphologically difficult to differentiate for a number of reasons, such as historical factors, phenotypic plasticity, genetic variation, polyploidy, natural hybridization and discordant variation (Edmonds, 1972, 1977, 1979a). Edmonds and Chweya (1997) gave a detailed account of these reasons.

Random amplified polymorphic DNA (RAPD) markers have been used by Stracke et al. (1996) to study *Solanum americanum, S. nigrum and Solanum melanocerasum*. Dehmer and Stracke (1999) and Dehmer (2001, 2003), used simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs) to investigate *S. americanum*, *S. villosum*, *S. nigrum* and *Solanum scabrum*, and Jacoby et al. (2003) used AFLPs to study *S. americanum*, *S. villosum* and *S. scabrum*. No molecular study involving Ugandan material is known.

AFLP fingerprinting was first applied to *Solanum* taxonomy by Kardolus et al. (1998) and Mace et al. (1999) and more recently by Nuez et al. (2004), Lara-Cabrera and Spooner (2004), Olet et al. (2005), Spooner et al. (2005a, b) and Manoko et al. (2007, 2008).

The AFLP technique is known to be highly reproducible and allows for the simultaneous analysis of a large number of loci. Becker et al. (1995) have shown that most AFLP bands are dominantly inherited and cover the whole genome, thereby measuring diversity/similarity along the whole genome. At the intraspecific level, AFLPs have proven to be reliable, whereas interspecific comparisons should be done with caution (Rouppe et al., 1997; El-Rabey et al., 2002; Vekemans et al., 2002). Yet, applied with care, such interspecific AFLP studies in recent publications have proven to represent known taxonomy (Furini and Wunder, 2004; Sasanuma et al., 2004; Drossou et al., 2004). Due to the obvious superiority over other molecular markers in terms of high-volume DNA fingerprinting without prior sequence information, AFLPs were used here to analyze the differences within and between species of the *S. nigrum* complex of Uganda. The results are discussed in light of previous taxonomic treatments.

MATERIALS AND METHODS

Plant material

One hundred seven (107) accessions representing eight species of *Solanum* section *Solanum* were used in this study. Table 1 lists information on the accessions studied (accession numbers, provisional botanical names, number of accessions, country of origin and sources). Ninety (90) of the accessions were collected from localities in Uganda, while the remaining 17 accessions were obtained from the gene bank in Nijmegen (NIJM), Netherlands. These gene bank accessions were grown to mature state in the Makerere University botanical garden for cross-breeding and later checked for true identity. The provisional identification of the Ugandan material was done at Makerere University Herbarium, Uganda and the final identifications at the Royal Botanic Gardens, Kew, England by E. A. Olet; some of them were later confirmed by the world expert on *Solanum* section *Solanum*, Dr. J. E. Edmonds of Leeds University, England. Most of the DNA investigated plant material from Uganda consisted of leaves collected directly from the field and dried in silica gel, except in a few cases in which only the fruits were available. Seeds of the latter, along with those from NIJM, were sown in a greenhouse at the Norwegian University of Life Sciences in order to harvest leaf material. All collections by Olet are preserved with voucher specimens in the Makerere University Herbarium (MHU).

DNA isolation and AFLP fingerprinting

DNA was extracted from 100 to 140 mg of ground leaf material using the CTAB method according to Saghai-Maroof et al. (1984). The AFLP procedure was performed with slight modifications as described by Vos et al. (1995). In short, 500 ng genomic DNA was restricted for 2 h at 37°C using *Eco*RI (BioLabs, New England) and *Mse*I (BioLabs, New England) in a 1× restriction-ligation buffer (10 mM Tris-acetate pH 7.5, 10 mM Mg acetate, 50 mM potassium acetate, 5 mM DTT). The restricted DNA was then ligated for 3 h at 37°C to *Eco*RI and *Mse*I adapters (see Keygene home page for sequences, www.keygene.nl) by adding a mix containing10 mM ATP (http://www.fermentas.com), 1x restriction-ligation buffer and T4 DNA ligase (http://www.fermentas.com). Selective preamplification of the adapter-ligated DNA with E01 and M01 primers (www.keygene.nl) followed using the PCR conditions outlined by Vos et al. (1995). The preamplification products (15 µl) were checked on a 1% agarose gel and the remaining products diluted with sterile water in the ratios 1:20, 1:50 and 1:100 depending on the intensity of the resulting smear on the gel. Selective amplification was done using a [33P] ATP-labeled *Eco*RI +3 primer and an unlabelled MseI +3 primer (www.keygene.nl). The 20 µl hot PCR mix consisted of 5 µl DNA (preamplified and diluted), 5 ng *Eco*RI primer, 30 ng *Mse*I primer, 10 mM dNTP mix (0.2 mM each), 0.5 U Taq polymerase, 2 µl 10 × PCR buffer, 1.2 µl 25 mM MgCl2 and 10.2 µl dd H2O giving a final volume of 50 µl. All the PCRs were run on a GeneAmp® PCR System 2700 version 2.04 (http://www.appliedbiosystems.com/index.cfm) thermocycler according to Vos et al. (1995). Formamide dye (20 µl containing 99% formamide, 10 mM EDTA and 0.1% each of xylene cyanol FF

Table 1. Accessions of *Solanum* section *Solanum* species used in our AFLP analyses.

Accession number	Provisional botanical name	Number of accession	Origin	Source
1-5, 7-10, 16, 51-53, 55-58, 61-67, 84, 91, 94-97, 99-101, 105, 108-109,111	*S. americanum*	38	Uganda	Olet
73 (954750186)	*S. americanum*	1	Brazil	BIRM/NIJM
74 (904759023)	*S. americanum*	1	Unknown	Gottingen - Germany/NIJM
115 (954750174)	*S. americanum*	1	Unknown	BIRM/NIJM
116 (904750026)	*S. americanum*	1	China	NIJM
43, 45, 47, 69	*S. florulentum*	4	Uganda	Olet
44	*S. hirsutum*	1	Uganda	Olet
11, 15, 70, 88	*S. nigrum*	4	Uganda	Olet
117 (884750070)	*S. nigrum*	1	Belgium	NIJM
118 (954750327)	*S. nigrum*	1	Unknown	BIRM/NIJM
48, 49, 50	*S. scabrum*	3	Uganda	Olet
78 (904750213), 79 (954750360), 119 (954750180)	*S. scabrum*	3	Unknown	BIRM/NIJM
80 (954750359)	*S. scabrum*	1	Indonesia	BIRM/NIJM
114 (904750021)	*S. scabrum*	1	Unknown	Cluj Napoca - Romania/NIJM
75 (954750170)	*S. sarrachoides*	1	Unknown	BIRM/NIJM
76 (964750073)	*S. sarrachoides*	1	Unknown	NIJM
46, 68, 106, 124	*S. tarderemotum*	4	Uganda	Olet
6, 12-13, 17-20, 21-42, 54, 59, 83, 93, 103, 110, 112	*S. villosum* subsp. *miniatum*	36	Uganda	Olet
77 (954750336) 113 (954750187)	*S. villosum* subsp. *miniatum* *S. villosum* subsp. *Villosum*	2	Unknown	BIRM/NIJM
71 (934750206)	*S. villosum* subsp. *Villosum*	1	Unknown	Genova - Italy/NIJM
72 (954750162)	*S. villosum* subsp. *Villosum*	1	Unknown	Vacratot - Hungary/NIJM

BIRM and NIJM represent the Birmingham and Nijmegen gene banks, respectively. The gene banks' accession numbers are shown in brackets. All collections by Olet are preserved with voucher specimen in the herbarium MHU.

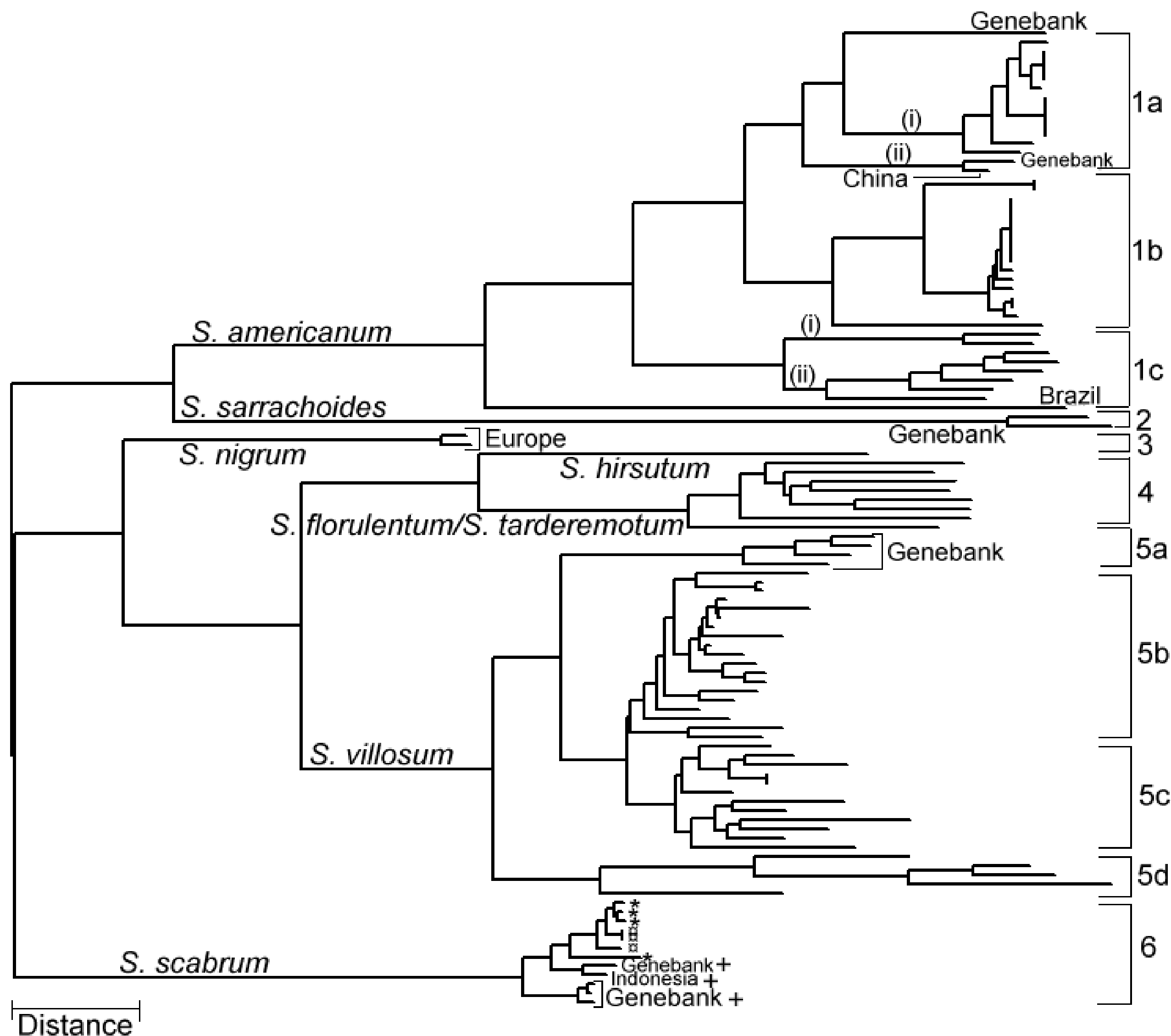

Figure 1. Neighbor-joining tree constructed from a distance matrix of 107 section *Solanum* genotypes of the eight studied species using the total 510 AFLP bands derived from four primer combinations. Clusters 1 to 6 are shown on the right, with the unlabelled genotypes representing collections from Uganda, except for cluster 6. * = accessions previously identified as *S. nigrum* from Uganda, but now named *S. scabrum* subsp. *laevis* Olet. ¤ = *S. scabrum* (Uganda), + = *S. scabrum* (non Ugandan). The scale bar represents 0.04 genetic distance. According to Manoko (2007) the plant named *S. americanum* is *S. nodiflorum*.

and bromophenol blue) was added to each of the amplification products and the DNA was denatured for 10 min at 94°C. The mix (3 µl) was loaded on a 5 % polyacrylamide sequencing gel and electrophoresed in 1 × TBE and 2 × TBE buffer in the lower and upper trays, respectively, at 80 W for 1.5 h. A [33P] ATP-labeled 30 to 330 bp ladder (http://www.invitrogen.com) was used as a size standard. The gel was fixed in 10% acetic acid, washed in dd H_2O and left to dry overnight at room temperature, before exposure to an x-ray film (Kodak BioMax MR) for 3 to 5 days.

Thirty *Eco*RI/*Mse*I primer combinations were screened using eight genotypes in order to find the most informative primer combinations. Based on this screening, four primer combinations were selected because they had distinct clear bands of uniform intensity and lacked disturbing background. The primer combinations were: *Eco*RI –AA G/ *Mse*I –AC G, *Eco*RI –AC A/ *Mse*I –AC G, *Eco*RI –AC C/ *Mse*I –AC G and *Eco*RI –AG T/ *Mse*I- ACG.

Data analysis

Only clear-cut bands were scored as present (1) or absent (0) for each of the four primer combinations. Data matrices from each combination were regarded as replications and entered into the NTSYSpc program version 2.11f (Rohlf, 2000) and analyzed individually (as described further). Since these four data sets gave similar phenograms (see also Mantel test), they were combined into one final data set and are the basis for Figures 1 and 2. Jaccard's similarity coefficient (Jaccard, 1908) using the SimQual option was

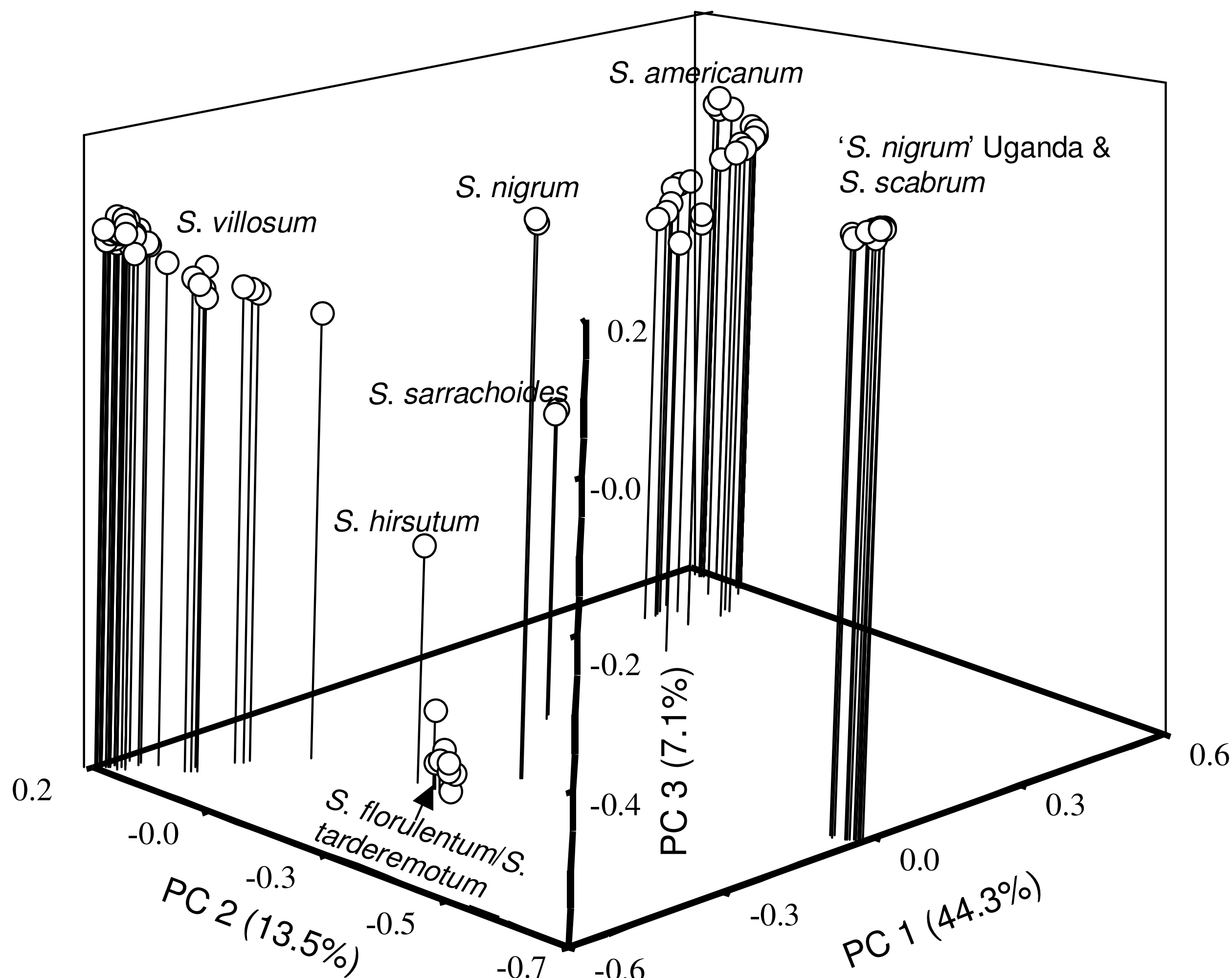

Figure 2. A principal coordinates analysis (PCoA) constructed using 510 AFLP bands derived from four primer combinations. The eight species of the section *Solanum* shown are represented by 107 genotypes. The three dimensions shown represent the first three principal coordinates, accounting for 64.9% of the total variation. Due to identical AFLP banding patterns among many genotypes within some species, there is considerable overlap resulting in the visible genotypes being less than the 107 that were analyzed. What is here named '*S. nigrum*' Uganda is now described as *S. scabrum* subsp. *laevis* (Olet et al. 2006). According to Manoko (2007) the plant named *S. americanum* is *S. nodiflorum*.

calculated from the 1/0 data set and converted to distances by subtracting 1. Neighbor-joining (Saitou and Nei, 1987) and principal coordinates analyses (PCoA, using the DCENTER and EIGEN procedures) were performed with NTSYSpc. Mantel tests (Mantel, 1967) were run with the MxComp option to test the correlations among the four individual data sets and to compare the relationship between the genetic distance of the combined data set (excluding the non-Ugandan genotypes) and the geographic distance. The geographic distance was measured on a map as the shortest distance in kilometers between the different geographic localities.

The selective PCR amplification of restriction fragments from a digest of genomic DNA via the AFLP technique ensures that each primer combination is an independent replication randomly generating (non-overlapping) polymorphisms within a genome. Since each of the four primer combinations yielded more than 100 AFLP bands which gave similar phenograms and since Mantel tests showed very high values, the neighbor-joining and PCoA analysis of the combined data set are robust, and hence bootstrapping was deemed unnecessary.

RESULTS

Each of the four primer combinations yielded over 100 different bands (Table 2), resulting in a total of 510 bands, 29 of which were monomorphic. *S. americanum* and *S. villosum* exhibited the highest levels of polymorphism, influenced by their high number of representatives (Table 3, upper part). Mantel tests showed that the four individual data sets were highly correlated, with all six different combinations of matrices having a product-moment correlation $r > 0.96$.

Three species were found to have species-specific bands (Table 3, lower part). The high number of species-specific bands for *S. sarrachoides* clearly separates this species from the rest of the species. *S. americanum* and *S. hirsutum* can also be distinguished from the rest of the

Table 2. Total number of bands generated by each primer set.

Primer set	Total number of bands
*Eco*RI –AAG/ *Mse*I –ACG	125
*Eco*RI –ACA/ *Mse*I –ACG	140
*Eco*RI –ACC/ *Mse*I –ACG	139
*Eco*RI –AGT/ *Mse*I –ACG	106

species on the basis of their unshared bands. There were only two cases of diagnostic bands shared by two species (Table 3, lower part). These cases are *S. nigrum* and *S. scabrum* and *S. florulentum* and *S. tarderemotum*. The occurrence of AFLP bands in the remaining species is more complicated, so each of the present/absence scores from the four primer combinations and the combined data set (510 AFLP bands) were converted into distances for further analysis.

Overall, the neighbor-joining phenogram showed similar clustering for all four data matrices (not shown, note Mantel test earlier) and were merged; the merged NJ is shown in Figure 1 and the PCoA in Figure 2. Unexpectedly, six clusters (labelled 1 to 6 in Figure 1) were generated instead of eight. We had expected 8 because we believed *S. florulentum* would come out separate from S. *tarderemotum* and that these species would not be in the same main cluster as *S. hirsutum*. There are 3 main clusters, including the two diploids *S. americanum* and *S. sarrachoides* in cluster 1, the hexaploid *S. scabrum* in cluster 6 and all the other species grouped in a joined cluster 3 to 5. The two hexaploids (Table 3) *S. nigrum* and *S. scabrum* are thus, in two different main clusters. All the tetraploids are in one main cluster, where they are well separated from the hexaploid *S. nigrum* in the same cluster. *S. villosum* is well separated from other tetraploid species, while *S. hirsutum* is closely related to *S. florulentum* and *S. tarderemotum*, which in this analysis are intermixed.

S. americanum had three or four subclusters (Figure 1). Subclusters 1a and c are further subdivided to make a total of five groups in the main cluster. Subcluster 1a(i) consists of the Ugandan material and one gene bank accession, while subcluster 1a(ii) consists of one accession from China and another from the genbank. Subclusters 1b, 1c(i) and 1c(ii) consist of only Ugandan material. The accession from Brazil (can be considered as in a fourth subcluster) was the most divergent and joined the rest of the *S. americanum* cluster last.

The *S. hirsutum*, *S. florulentum* and *S. tarderemotum* cluster 4 (Figure 1) exhibited high variability amongst all the genotypes, with no clear subdivision except for *S. hirsutum,* which was always set apart from the rest of the cluster (Figures 1 and 2).

S. villosum had four subclusters, all of which were distinct. Subcluster 5a consisted of non-Ugandan material, while subclusters 5b to d were Ugandan (Figure 1).

S. scabrum had two subclusters (Figure 1) and although, the cluster was mixed, variation within the cluster was more-or-less close-knit compared with the other clusters.

The genotypes in the subclusters within each species were always mixed, irrespective of geographic origin. Also, there was intermixing between Ugandan and non-Ugandan material in *S. americanum*. There was very poor correlation between genetic distance and geographical distance (r = 0.2) detected with a Mantel test (data not shown).

The PCoA, an alternative analysis to neighbor-joining is shown in Figure 2. The sorting of the genotypes is in agreement with the neighbor-joining. The first three co-ordinates explain 44.3, 13.5 and 7.1% of the observed data, resulting in a cumulative value of 64.9%.

DISCUSSION

There is no very good separation of species according to chromosome number. While the two diploid species *S. sarrachoides* and *S. americanum* group nicely together (Figure 1), the hexaploid *S. nigrum* s.str., groups together with the three tetraploids. The other hexaploid, *S. scabrum*, forms its own main cluster.

The diploid *S. sarrachoides* is well separated from *S. americanum* (Figures 1 and 2). It is also previously reported to be morphogenetically completely isolated from all other diploid *Solanums* (Edmonds, 1977). It has not been previously studied using molecular methods. The two lines studied are gene bank accessions. No Ugandan material was found during our fieldwork. The species has been reported to be rare in Uganda (Bukenya and Carasco, 1995). However, Ugandan material previously determined to be *S. sarrachoides* at the Royal Botanic Gardens, Kew, is in fact a different species, perhaps *S. hirsutum* (Olet, 2004). *S. sarrachoides* is a native of South America and relatively rare in Europe and North America and only one African collection from the Eastern Cape is currently accepted (Edmonds and Chweya, 1997).

S. americanum, also a diploid, has been believed to be the most widespread and morphologically variable species of the section *Solanum* (Hunziker, 2001; Edmonds, 1977; Edmonds and Chweya, 1997) and also comes out distinct in our studies. However, the sample from Brazil comes out as rather distinct from all other accessions. Interestingly, Manoko et al. (2007) separates *S. americanum s. lato* in three different species, including S. *nodiflorum* from Africa and Asia, *S. americanum s.str.* from North America and an undescribed species from South America. If Manoko's concept is accepted, all our plants are *S. nodiflorum* except the one from Brazil, which may be the undescribed species. Although, Manoko's work looks convincing, our study neither supports nor contradicts Manoko's concept, since we did not include samples from North America in our analysis.

Table 3. Chromosome number, total number of lines, total number of AFLP bands, percentage polymorphism in each species, species-specific bands (in bold) and shared AFLP bands among species (in normal print).

Species	*S. ame*	*S. nig*	*S. sca*	*S. hir*	*S. flo*	*S. tar*	*S. sar*	*S. vil*
Chromosome number (2n)	24	72	72	48	48	48	24	48
No. of lines	42	6	8	1	4	4	2	40
AFLP bands	206	265	212	166	209	213	127	265
% poly	64	40	10	-	24	32	8	60
Species specific and shared bands								
S. americanum	**9**	0	0	0	0	0	0	0
S. nigrum		**0**	4	0	0	0	0	0
S. scabrum			**0**	0	0	0	0	0
S. hirsutum				**12**	0	0	0	0
S. florulentum					**0**	7	0	0
S. tarderemotum						**0**	0	0
S. sarrachoides							**56**	0
S. villosum								**0**

S. ame = *S. americanum*, *S. nig* = *S. nigrum*, *S. sca* = *S. scabrum*, *S. hir* = *S. hirsutum*, *S. flo* = *S. florulentum*, *S. tar* = *S. tarderemotum*, *S. sar* = *S. sarrachoides*, *S. vil* = *S. villosum*.

The high variability in *S. americanum s. lato* (Figures 1 and 2) is supported by its high polymorphism rate (Table 3). It also agrees with our PCoA and previous studies based on RAPD (Stracke et al., 1996) and AFLP data (Dehmer, 2001, 2003).

The hexaploid *S. scabrum* cluster (Figures 1 and 2) consists of *S. scabrum* and Ugandan material previously identified as *S. nigrum*, now named *S. scabrum* subsp. *laevis* Olet (Olet et al., 2006). This cluster (6) will be referred to as *S. scabrum* for the rest of the discussion. Manoko et al. (2008) includes subsp. *Laevis* in subsp. *Scabrum*.

The *S. nigrum* genotypes (Figures 1 and 2) are well separated from both *S. scabrum* and the tetraploid clusters (4 and 5), that contain *S. hirsutum*, *S. florulentum*, *S. tarderemotum* and *S. villosum*. These genotypes (from Europe) belong to the true *S. nigrum* (Olet et al., 2005) and their position in all the analyses confirms *S. villosum* or another similar tetraploid, as one of the genome donors to *S. nigrum* (Edmonds, 1979a; Edmonds and Chweya, 1997). The position of the *S. nigrum* (cluster 3) relative to the *S. scabrum* cluster in our study (Figures 1 and 2), contradicts Dehmer's (2003) findings, which suggest a recent speciation of these two taxonomic units due to observed small genetic differences between them.

S. hirsutum, *S. florulentum* and *S. tarderemotum* are species that have been listed among the African taxa in need of taxonomic revision, since little is known about them (Edmonds and Chweya, 1997). The high variability among the genotypes in the cluster is supported by morphological data (Olet, 2004). The consistent differentiated position of *S. hirsutum* in all the analyses (Figures 1 and 2) implies that it is a distinct species. The unclear separation of *S. florulentum* and *S. tarderemotum* (Figures 1 and 2) could be an indication that they form a hybrid swarm and they probably share at least one of their diploid parents. However, analyses based on the morphological data clearly separate these two species (Olet, 2004). Further studies involving more accessions are needed in order to make reliable conclusions about the taxonomic status of *S. florulentum* and *S. tarderemotum*.

The high variability in the remaining cluster of *S. villosum*, further supported by a high polymorphism rate (Table 3), suggests greater diversity than was previously known. Some authors have separated *S. villosum* into two subspecies on the basis of pubescence type and stem shape, that is, *S. villosum* subsp. *villosum* with patent, glandular hairs and smooth round stems and *S. villosum* subsp. *miniatum* with eglandular headed, appressed hairs and angled dentate stems (Hawkes and Edmonds, 1972; Edmonds, 1979a, b, 1984). This two-way classification does not agree with our AFLP data, which led to the generation of four subclusters (Figure 1), with cluster 5a consisting of both subspecies and containing all four genotypes from the gene bank and the remaining three subclusters (5b to 5d) consisting of the African lines with appressed, eglandular headed hair type and dentate stems. Furthermore, subcluster 5b had genotypes that were light green with mostly lax solitary cymes and subcluster 5c had genotypes that were dark green with mostly simple umbellate cymes. Subcluster 5d was morphologically similar to subcluster 5b, and it differed greatly from the other subclusters. The PCoA (Figure 2) is also in agreement with this variability. This

complex differentiation in *S. villosum* possibly merits redefining the subspecies and perhaps further intraspecific ranking.

S. villosum, considered to be a Eurasian taxon (Edmonds and Chweya, 1997; Hunziker, 2001), was most probably introduced to Uganda in recent years. Its sole Ugandan locality as a weed in an agricultural area near the capital Kampala is a characteristic habitat for recent introductions. The position of *S. americanum* (*S. nodiflorum*) is more confusing. If *S. americanum* is an originally American plant, which later spread throughout most parts of the tropical and subtropical world agreeing with Edmonds (1977), Edmonds and Chweya (1997) and Hunziker (2001), our results with lack of correlation between molecular data and Ugandan geographic localities must be interpreted as multiple introductions of *S. americanum* to Uganda. If another concept is accepted that the African plants are *S. nodiflorum* following Manoko et al. (2007), then the distribution of *S. nodiflorum* in Uganda could be the result of native populations mixing with introduced populations from neighbouring countries or from elsewhere. Environmental interactions may have had little impact following the introduction(s) to the different Ugandan geographic localities. However, the stated explanations do not hold for *S. florulentum* and *S. tarderemotum,* which are considered to be of East African origin. These two species are sympatric. This could imply that they are hybrids segregating and/or both species have been around long enough to exhibit ecogeographical variation, as shown by numerical analysis (Olet, 2004). The intermixing of the Ugandan with the non-Ugandan material in the *S. americanum* and *S. scabrum* clusters (Figures 1 and 2) suggests multiple introductions of these species. The intermixing of genotypes in our study, irrespective of geographic locality, further points to the predominantly autogamous nature of members of the section *Solanum*, although, natural outbreeding can occur (Edmonds, 1979a).

On the other hand, the non-Ugandan species were generally highly differentiated from their Ugandan counterparts (except for *S. scabrum*), suggesting that molecular data correlates with geographic origin on a wider spatial scale for these species. Earlier molecular DNA studies (Stracke et al., 1996; Dehmer and Stracke, 1999; Dehmer, 2001) have differentiated *S. americanum* and *S. villosum* on the basis of geographic origin but, again, this is not the case for *S. nigrum* and *S. scabrum,* just as in our study. In particular, Dehmer (2003) separated *S. americanum* (Cuba, Central America) and *S. villosum* (Europe, Kenya, Canary Islands).

In conclusion, molecular DNA data only partially correlate with earlier taxonomic treatments based on morphology for some of the species. Our data do not justify the separation of the Ugandan material *S. florulentum*/*S. tarderemotum* into distinct species, although, these species exhibit considerable phenotypic distinctions (Olet, 2004). *S. sarrachoides* and *S. americanum* were the most distant species, while *S. florulentum*/*S. tarderemotum* and *S. hirsutum* were the most related. Also, there was no correlation between the AFLP data and geographic locality for the Ugandan material, suggesting that most of the studied species are introduced. Our AFLP analysis sheds light on the taxonomic resolution of this otherwise complex section and gave an insight into the origins/introductions of some of the species studied.

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