International Journal of Plant Breeding ©2009 Global Science Books

Genetic Structure in Two African Vegetable Nightshade Species

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ABSTRACT

Fifty one accessions from two African vegetable nightshade species, 29 accessions of *Solanum scabrum* and 22 accessions of *S. villosum* were collected in Tanzania and examined for the extent and structure of genetic variation within and among accessions and between species using random amplified polymorphic DNA (RAPD) markers. The two species are important leafy vegetables and are important contributors to the nutritional well being of small-scale subsistence farmers in much of sub-Saharan Africa. The two main research questions were: 1) can RAPDs efficiently distinguish two morphologically distinct species of *Solanum*? and 2) what is the spatial structure of the genetic variation in these two species? In this study the efficiency of RAPDs in clustering accessions by species was very high, further encouraging the use of RAPDs for inter- and intraspecific comparative studies. The spatial structure of genetic variation in the two species was distinctly different. *S. scabrum* accessions were genetically similar for a distance up to 50 km and regionally structured, possibly indicating an exchange of seeds among neighboring farms combined with genetic differentiation due to selection for distinct ecological niches. In *S. villosum* no spatial structure was found even at a scale of a few kilometers.

Keywords: African leafy vegetable, genetic diversity, *Solanum*

INTRODUCTION

African indigenous vegetables, especially leafy vegetables, play an important role in food security and nutritional balance of both urban and rural populations of sub-Saharan Africa (Schippers 1997). Two species of the nightshade family, *Solanum scabrum* (Mill.) and *S. villosum* (Mill.), are important leafy vegetables in many parts of Eastern, Western, Central and Southern Africa and play a significant role in providing vitamins and minerals in the diet of people, especially subsistence farmers, living in these areas (Edmonds and Chweya 1997; Fontem and Schippers 2004; Olet *et al*. 2005; Jacoby and Labuschagne 2006; Mendlinger *et al*. 2006; Keding *et al*. 2007; Mwai *et al*. 2007; Ojiewo *et al*. 2007; Manoko *et al*. 2008). Traditionally, they are grown by small-scale farmers for subsistence with any surplus being sold in local and urban markets. However, recently farmers have begun cultivating these species specifically for sale in markets (Edmonds and Chweya 1997; Olet *et al*. 2005; Mendlinger *et al*. 2006; Keding *et al*. 2007). As in many if not most African indigenous vegetables, these species have not had the extent of genetic, breeding and agromanagement research that their importance requires and few systematic collections of landraces or primitive varieties have been conducted. To date little is known about the extent and structure of intra- and interspecific genetic variation in these two species. This is important not only for selection, breeding and agromanagement programs but also if we are to develop efficient *in situ* and *ex situ* conservation strategies. Unfortunately, as in may other indigenous African vegetables, genetic erosion is most likely occurring.

Edmonds and Chweya (1997) and Mwai *et al*. (2007) described morphological variation both within and between these two species, but did not know how much of this variation may be due to selection by local farmers. Dehmer (2003) used AFLP analysis to examine the potential of molecular markers for *ex situ* conservation and found that five species of the nightshade group were distinguishable from one another. Dehmer and Hammer (2004) employed AFLP analysis of 44 accessions over 5 nightshade species including 3 accessions of *S. scabrum* from unknown origins and 18 of *S. villosum* from Kenya, Italy and unknown origins. They found that the accessions of each species tended to clump together but could provide little information of the extent of intra- and inter-specific genetic variation. Olet *et al*. (2005) used AFLP to examine eight *S. scabrum* accessions, one *S. villosum* and four *S. nigrum* from around the world and found that *S. scabrum* and *S. nigrum* were distinct from *S. villosum*. Manoko *et al*. (2008), employing AFLP primers on *S. scabrum* accessions, found that genetic variation was higher within than among accessions and found no clustering according to geographic provenance.

In this study we analyzed genetic variation in accessions of S. *scabrum* and *S. villosum* collected in Tanzania using random amplified polymorphic DNA (RAPD) markers. We collected 29 accessions of *S. scabrum* and 22 accessions of *S. villosum* in Tanzania as a first step in building a systematic germplasm collection in these species for future selection and breeding programs (Mendlinger *et al*. 2006).

In plant systematics, resolving phylogenies among closely related taxonomic units remains a perplexing problem. Such markers as cpDNA and mtDNA have insufficient resolution due to low variability while AFLP and SSRs are often too variable for reliable inferences about species phylogeny. Therefore we decided to use RAPD markers. While the usefulness of RADPs for assessment of intra-specific genetic variation has been questioned (Rieseberg 1996; Adams and Rieseberg 1998; Harris 1999), it has been successfully used in several studies (cited in Weising *et al.* 2005).

We were interested in: 1) the capacity of RAPDs to efficiently distinguish between two morphologically distinct

Received: 30 May, 2009. Accepted: 7 July, 2009.

Original Research Paper

species of *Solanum*; and 2) the spatial structure of genetic variation in these two species in Tanzania.

MATERIALS AND METHODS

Plant material, DNA extraction and PCR amplification

All 29 and 22 accessions of *S. scabrum* and *S. villosum*, respectively, plus one unidentified accession were analyzed. *S. scabrum* is an allogamous hexaploid and *S. villosum* an allogamous tetrapolid species (Ojiewo *et al*. 2007b). The accessions were collected during 2003-2006 in Tanzania (see Mendlinger *et al*. 2006 for collection protocols, GPS coordinates and details on each accession). Several accessions were bought in local markets or seed stores and are classified as unknown origin. One accession of S. *americanum* was added to the analysis (provided by AVRDC – origin unknown). Both species are predominately autogamous (Edmonds and Chweya 1997).

RAPD analysis was performed using DNA from 5 plants per accession. DNA was purified from freshly harvested leaves ground to a powder in microfuge tubes under liquid nitrogen. For extraction, we used the modified method of Rogers and Bendich (1985).

Each amplification was performed in a reaction volume of 25 µl containing 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 5 pmols of the primer, 0.5 units of *Taq* DNA polymerase and 25 ng of wild barley DNA. Nine primers of 10 bp each (**Table 1**) were selected from Operon Technologies kits A, B, C and F and from 10 primers from University of British Columbia. The main criterion for selection was production of clear amplified polymorphic bands that were replicable in two test reactions.

The RAPD reactions were carried out in Eppendorf Thermo Cycler using the following protocol: 94°C for 3 min, followed by forty-five cycles of 94°C for 1 min, 35°C for 1 min, 72°C for 2 min and final extension 72°C for 7 min. Amplified DNA was immediately analyzed or stored at –20°C. The amplification products were analyzed by electrophoresis in 1.4% TBE agarose gel (Hispanagar, Spain), stained with ethidium bromide and photographed under UV light. The molecular weight of all bands was calculated by comparison with the øX174/*Hae*III marker run in three lanes. All reactions were repeated at least two times and only reproducible bands were scored for statistical analysis.

Table 1 Primers used in the RAPD analysis, their sequence, number of bands and size range. "OP" series are primers from Operon Technologies Inc.

Primer	Sequence	Number of scorable bands	Size range of scorable bands
OPC-02	5'GTGAGGCGTC3'	5	270-1400
OPC-13	5'AAGCCTCGTC3'	3	500-1100
OPC-17	5'TTCCCCCCAG3'	2	550-1400
OPC-19	5'GTTGCCAGCC3'	6	400-2035
OPH-4	5'GGAAGTCGCC3'	4	1300-1700
Total		20	

Data analysis

Bands were scored as present (1) or absent (0) by independent observers using SYNGENE gel imaging system. Bands of identical size amplified with the same primer were considered to be the same allele/locus.

Matrix of genetic similarity among accessions was computed using simple matching coefficient and subjected to a cluster analysis with UPGMA (unweighted pair-group method, arithmetic average) clustering algorithm and principal coordinates analysis as implemented in NTSYSpc version 2.0 (Rohlf 1998).

The effect of geographic distance between accession locations on their genetic similarity was analyzed by Mantel test and spatial autocorrelation analysis. In the latter analysis the autocorrelation coefficient measured the genetic similarity between pairs of individuals whose geographic separation fell within the specified distance classes (from 1 to 1000 km). Statistical significance of the autocorrelation coefficient was estimated using 1000 random permutations. For these analyses genetic similarity was calculated using binary genetic distance coefficient (Huff *et al.* 1993):

$$D = n\left[1 - \frac{2n_{xy}}{2n}\right]$$

where $2n_{xy}$ = the number of shared character states and n = the total number of binary characters.

Analysis of molecular variance (AMOVA) (Excoffier *et al*, 1992) was done to estimate variance components due to differences: 1) among regions and accessions/within regions; and 2) among species and accessions/within species. In addition, variation components among regions and accessions/within regions were calculated for each species separately. Only regions with more than one accession were used in these analyses.

All the above analyses were done with GeneAlEx (Peakall and Smouse 2006).

RESULTS

Genetic diversity

While we did not find genetic differences among individual plants within accessions in both species, we found considerable genetic diversity among accessions. All loci were found to be polymorphic. The number of bands in *S. scabrum* and *S. villosum* with frequency ≥ 5% was 18 and 19, and mean H_e was 0.340 ± 0.032 and 0.311 ± 0.038, respectively.

Genetic differentiation

Analysis of the structure of genetic variation by AMOVA revealed high species segregation (Φ_{PT} = 0.530) (**Table 2**). AMOVA, conducted for each species separately, revealed strong and very significant regional structuring in *S. scabrum* accessions (Φ_{PT} = 0.556) but not in *S. villosum* accessions (Φ_{PT} = 0.044). When both species were pooled we had significant regional segregation (Φ_{PT} = 0.096) albeit relatively low.

Principal coordinates analysis and cluster analysis demonstrated distinct patterns of RAPD variation in the two

Table 2 Results of AMOVA. The p-value is derived from 1000 permutations and denotes the probability of observing a larger component of variance by chance alone.

	Source of variation	df	SSD	MSD	Variance component	Φ_{PT}	p-value
(a)	Among regions	5	37.6	7.5	0.47	0.096	0.040
	Accessions within regions	35	153.8	4.4	4.39		
(b)	Among species	1	91.3	91.3	3.53	0.530	0.001
	Accessions within species	49	153.0	3.1	3.12		
(c)	Among regions (*S. scabrum*)	4	41.1	10.3	1.79	0.556	0.001
	Accessions within regions	20	28.6	1.4	1.43		
(d)	Among regions (*S. villosum*)	3	7.1	2.4	0.09	0.044	0.307
	Accessions within regions	10	20.5	2.1	2.05		

Notes: (b) two accessions (*S. americanum* and an unidentified species) were excluded; (c) and (d) regions with only one accession were excluded.

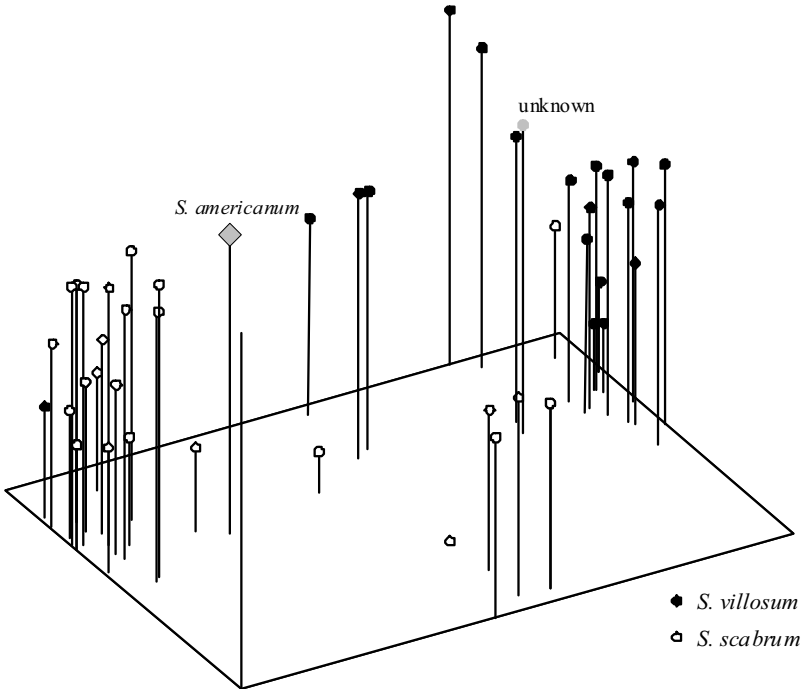

Fig. 1 A PCoA plot. The first two axes accounted for 55.5 and 9.58%, respectively, of the total variation.

species (**Fig. 1**). The first axis accounted for over 55% of the total variation but the second axis less than 10%. All but two accession of each species clustered together by cluster analysis, and separated in three-dimensional space by PCoA (**Fig. 2**). The lone accession of *S. americanum* was found to be closer to *S. scabrum* than *S. villosum*.

Genetic distance between accessions correlated with geographic distance in *S. scabrum* (r = 0.335, p = 0.003, Mantel *t*-test) but not in *S. villosum* (r = 0.133, p = 0.106, Mantel *t*-test).

Spatial autocorrelation analysis of *S. scabrum* found a significant spatial structure for a distance between accessions of up to 50 km, but not at distance above that (**Fig. 3**). No spatial structure was found in *S. villosum*.

DISCUSSION

Our collection of accessions from Tanzania is, to the best of our knowledge, the most comprehensive collection in both species in respect to the number of accessions combined

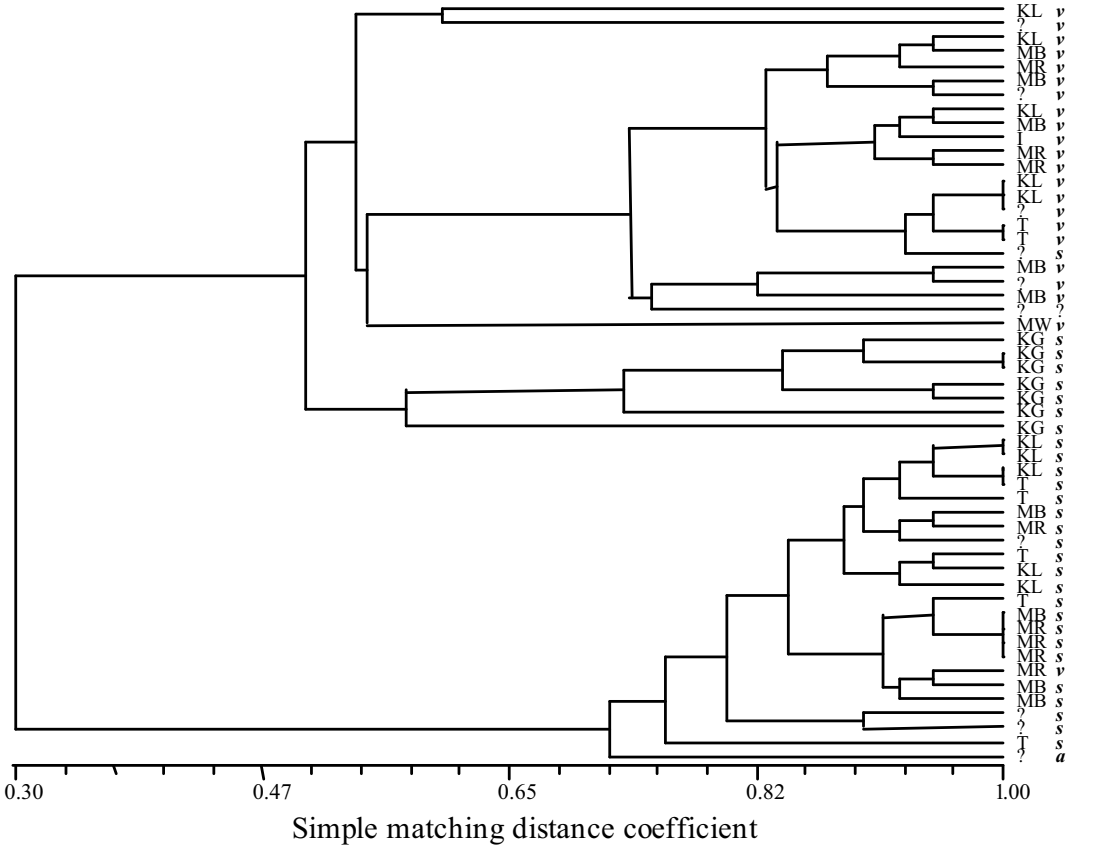

Fig. 2 UPGMA dendrogram of analyzed accessions classified by regional identity and species. Abbreviations: KL – Kilimanjaro, T – Tanga, I – Iringa, MB – Mbeya, MR – Mara, KG – Kagera, ? – not known, *v* – *S. villosum*, *s* – *S. scabrum*, *a* – *S. americanum*.

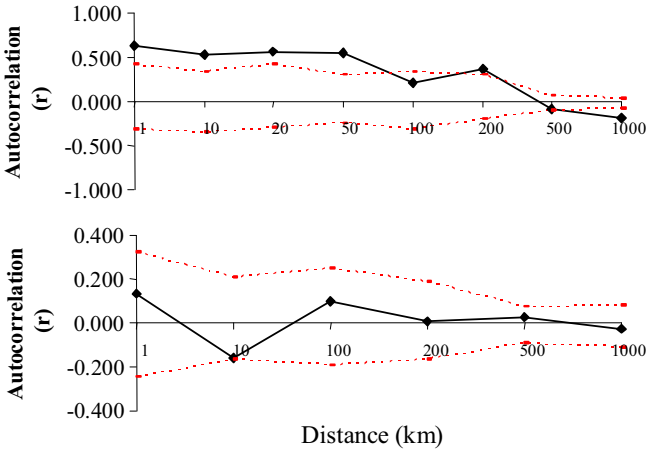

Fig. 3 The autocorrelograms with 95% confidence interval (dotted lines) for autocorrelation coefficient *r* (solid line) for *S. scabrum* (above) and *S. villosum* (below).

with knowledge of each accession's origin which has been analyzed for molecular genetic variation.

We found considerable genetic variation between both species and among accessions of each species, but not within accessions of either species. The spatial structure of genetic variation in the two species was distinctly different. Accessions of *S. scabrum* were genetically similar for a distance up to 50 km. In *S. villosum*, however, no spatial structure was found even at a scale of a few kilometers. The regionally structured genetic variation found in *S. scabrum* may indicate a deliberate dissemination or exchange of seeds among neighboring farms, friends and relatives in this species but not in *S. villosum*. However, Mendlinger *et al*. (2006) and Keding *et al*. (2007) reported considerable exchange and sale of seed in both *S. scabrum* and *S. villosum*, as well as in other vegetable species, among friends and family members who can live quite a distance apart. Thus we should have a similar spatial pattern in both species. Significantly, we found that the ranges of these two species differed in Tanzania with *S. villosum* primarily being cultivated in fairly uniform cool elevated regions of Tanzania whereas *S. scabrum* was found being cultivated over a wider range of environments from cool, moist highlands, to more arid and sub-tropical lowlands (Mendlinger *et al*. 2006). Thus the regional differences found in *S. scabrum* may correspond to the larger number of ecological niches that it occupies in Tanzania than *S. villosum* with selection occurring due to ecological niche differences. Edmonds and Chweya (1997) reported that *S. scabrum* tends to be more widespread and occupies more ecological niches than *S. villosum* in sub-Saharan Africa. In addition, *S. villosum* tends to be more susceptible to diseases such as *Fusarium* and insects, especially red spider mites than *S. scabrum*.

Our results using RAPDs differ from previously reported molecular analysis using AFLPs. Dehmer and Hammer (2004) and Manoko *et al*. (2008) reported within accession variation in both species which we did not find. Dehmer and Hammer (2004) found that *S. villosum* clustered according to geographic locations and we did not. However their clustering represented geographic distances of thousands of kilometers with no seed exchange (Kenya vs. Italy) not hundreds of kilometers with seed exchange as ours does. Manoko *et al*. (2008) reported that *S. scabrum* accessions did not cluster according to geographic provenance whereas we found significant clustering.

While RAPDs were found to be less effective than other molecular marker methods (RFLP, SSR and AFLP) in separating subspecies from one another in some studies (Powell *et al.* 1996), they were found to effectively separate accessions into logical clusters in several *Brassica* species (Thormann *et al.* 1994), two melon (*Cucumis melo*) subspecies (Garcia-Mas *et al.* 2000) and several *Leucaena* species (Bailey *et al* 2004). In our study, the effectiveness of RAPDs in clustering accessions by species identity was high. Only two out of 51 accessions clustered with the other species. These results encourage further use of RAPDs for interspecific comparative studies. However precautions should include checking for repeatability and within-accession variability. If the latter two conditions are met, i.e. results are highly repeatable and no within-accession variation is found, RAPDs can provide very accurate assessment of genetic variability among- and in some cases within-species.

Our results indicate that different strategies may need to be employed for *in situ* and *ex situ* conservation and for selection and breeding programs in the two species. With *S. scabrum* it may be better to divide its range into as many ecological zones as possible and conserve or utilize a few accessions per zone. With *S. villosum* it may be more efficient to utilize only a few geographically different areas with more accessions per area.

ACKNOWLEDGEMENTS

This study was supported by a grant from the United States Agency for International Development: Bureau for Global Programs, Field Support and Research project number TA-MOU-C21-054. We would like to thank Hassan Mndiga for help in collecting the accessions.

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