Genetic diversity and morphological characterization of African nightshade entries (section *Solanum* L.)

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Abstract

African nightshade is an indigenous leafy vegetable in sub-Saharan Africa valued for its high nutrient content and therefore provide nutritional security and also potential to generate income especially to small scale farmers. Production is however below potential due to lack of superior varieties that are high yielding and tolerant to environmental stresses. Development of varieties through breeding programs has been initiated in order to supply farmers with quality seeds. The efforts to improve this crop however have been hampered by limited information available on intra- and interspecific genetic diversity. In this study new SSR markers developed using next generation sequencing and AFLP markers were used to assess genetic diversity and differentiation in 54 African nightshade entries. The genotypes comprised entries of two species, *Solanum scabrum* (Mill.) and *S. villosum* (Mill.) including developed lines and farmer cultivars. Morphological traits relevant for agronomic performance of the entries were also analysed. In addition to the diversity studies, the genome size and pollen viability parameters were determined. The molecular markers clearly distinguished the two species and *S. scabrum* was found to be less diverse as compared to *S. villosum.* Farmer cultivars had higher allelic richness and a larger number of unique alleles than developed lines. Analysis of molecular variance showed higher variation within than between entries. Cluster analysis grouped the entries into two clusters representing the two species. The analysis of agronomic traits revealed significant differences between entries. All entries of *S. scabrum* were found to be hexaploid whereas entries of *S. villosum* were tetraploid with a haploid genome size of all samples slightly varying around 1pg. Pollen viability parameters significantly differed between the two species but did not correlate to any of the other parameters measured in this study.

Key words: AFLP, indigenous leafy vegetable, *S. scabrum*, SSR, *S. villosum*.

Zusammenfassung

"African nightshades" umfassen indigene, traditionelle Blattgemüse, die in Afrika südlich der Sahara wegen ihres Nährstoffgehalts geschätzt werden und die ein großes Potential für zusätzliches Einkommen kleiner Farmen darstellen. Durch die zu geringe Verfügbarkeit von Sorten mit ausreichendem Ertrag und Toleranz gegen Stressfaktoren bleibt die Produktivität dieser Gemüsearten jedoch hinter den Möglichkeiten zurück. Es wurden jedoch bereits Zuchtprogramme gestartet, die zum Ziel haben der Landwirtschaft verbessertes Saatgut zur Verfügung zu stellen. Diese Zuchtprogramme sind jedoch in ihrer Effektivität durch fehlende Informationen über die verfügbare inter- und intraspezifische genetische Variabilität der Gemüsearten begrenzt. In der hier vorgelegten Dissertation wurden neue SSR-Marker durch das so genannte „Next-Generation-Sequencing" entwickelt und zusammen mit AFLP Markern dazu verwendet, die genetische Diversität innerhalb und zwischen 54 Herkünften von „African nightshades" zu untersuchen. Die untersuchten Genotypen umfassten Herkünfte zweier Arten, *Solanum scabrum* (Mill.) und *S. villosum* (Mill.) wobei sowohl sogenannte „developed lines" (Zuchtlinien) als auch Landrassen lokaler Farmen untersucht wurden. Zusätzlich zu den molekularen Markern wurden auch morphologische Merkmale mit Bedeutung für die landwirtschaftliche Nutzung untersucht. Außerdem wurden die Genomgröße und die Pollenvitalität bestimmt. Die Analysen mit molekularen Markern resultierten in einer klaren Trennung der beiden Arten, wobei *S. scabrum* eine generell geringere genetische Diversität aufwies als *S. villosum*. Die Landrassen wiesen eine höhere Alleldiversität sowie eine größere Zahl an herkunftsspezifischen Allelen auf als die sogenannten „developed lines". Die Analyse der molekularen Varianz zeigte, dass es eine höhere genetische Variabilität innerhalb als zwischen den Herkünften gab. Eine Clusteranalyse gruppierte die Herkünfte entsprechend ihrer Artzugehörigkeit. Auch die Untersuchung der morphologischen Merkmale zeigte signifikante Unterschiede zwischen den

Herkünften auf. Alle Herkünfte von *S. scabrum* wurden als hexaploid charakterisiert, während alle Herkünfte von *S. villosum* tetraploid waren, wobei die Genomgröße des haploiden Genoms bei allen Herkünften im Bereich um 1pg lag. Die Werte für die Vitalität des Pollens unterschieden sich signifikant zwischen den beiden Arten, waren aber mit keinem anderen untersuchten Merkmal korreliert.

Schlagwörter: AFLP, traditionelle Blattgemüse, *Solanum scabrum*, SSR, *S. villosum.*

Abbreviations

AFLP	Amplified Fragment Length Polymorphisms
ANOVA	Analysis of variance
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PIC	Polymorphism information content
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SD	Standard deviation
SSR	Simple sequence repeats

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1 General introduction

1.1 Description of African nightshades

African nightshades comprise several species within the section *Solanum*. There are 30 species reported in the section and about 12 species are found in Africa (Shackleton et al., 2009). African nightshades are utilized mostly as leafy vegetables in sub-Saharan Africa (Berinyuy et al., 2002; Chweya and Eyaguirre, 1999; Edmonds and Chyweya, 1997; Ojiewo et al., 2013a). They can also be used as herbal medicine (Schippers, 2000) and also as source of dye (Lehmann et al., 2007). In spite of the big diversity only a few species have widespread use. The most popular species utilized as vegetables are *Solanum scabrum, S. villosum, S. retroflexum, S. americanum* and *S. sarrachodes* (Mwai et al., 2007; Ojiewo et al., 2013b). Some species are specific in distribution and a large number are distributed widely across different geographical locations (Maundu et al., 2009). Historically, African nightshades were conserved and utilized by farmers in home gardens or harvested from the wild (Dinssa et al., 2014). The area under cultivation is still low since they are produced at subsistence level (Tuwei et al., 2013). Empirical data have shown that African nightshades are rich in micronutrients (Kamga et al., 2013; Luoh et al., 2014) and easy to cultivate and therefore have a potential to provide nutritional security and a source of livelihood for the resource poor rural communities. The realization of its high nutrient level has led to increase in consumer demand which currently surpasses the production. Low productivity may be due to environmental factors and poor agronomic techniques. But the major production bottleneck though is access to quality seed stock (Dinssa et al., 2013).

1.1.1 Genetic resources and breeding in African nightshades

African nightshades are propagated from seeds. They are generally autogamous although low levels of outcrossing do occur. The absence of a self-incompatibility system is useful in stabilizing any crossings that are made, whereby the new populations will be sufficiently uniform after only 2-3 generations. Cultivars can thus be created within a short period. Farmers prefer cultivars which are high yielding with long seasonality of picking, uniformity and resistance to biotic and abiotic stresses (Afari-Sefa et al., 2012). Consumers seek for quality and nutritional value. Seed companies select for improved cultivars with both high leaf and seed yield (Dinssa et al., 2015). Unfortunately there are still gaps in the knowledge of taxonomy of this section (Ronoh et al., 2017). Moreover little information about breeding potential is available.

1.1.2 Origin and description of African nightshades

African nightshade is an annual dicot with erect or decumbent growth habit growing between 0.5 to 1.0 m high (Edmonds and Chyweya, 1997). Leaves maybe oval or lanceolate and sizes vary considerably between species ranging between 8-15 cm long, entire or angularly lobed and slightly hairy. The inflorescence is a simple cyme and flowers may be purple or white. Berries are 5 to 12 mm in diameter, green when unripe and turn purple, orange or yellow when ripe depending on species. There is much diversity in African nightshades related to growth patterns, leaf sizes, tastes (bitterness), flowering time, colour, (Mwai et al., 2007) as well as nutritional and nutraceutical value, along with quantities and composition of anti-nutrient factors (Jared et al., 2016; Mohyuddin et al., 2010). The origin of the majority of *Solanum* species is within South America. The most popular African representatives of *Solanum* can also be found in areas of Europe, Asia, but the most valued nightshade vegetable species *S. scabrum,* is native to Australia (http/www.pfaf.org/database/plants).

1.1.3 Growing conditions

African nightshade is propagated from seeds. It performs well in a varying degree of climatic conditions, but grows best within cool, high-moisture environments in both medium and high altitudes (Ondieki et al., 2011a). Shady conditions cause a reduction in total plant weight, as well as leaf yield. Though African nightshade can tolerate shade, growth is better when the plant is exposed to full sunlight (Ojiewo et al., 2013a). For adequate growth of African nightshades annual rainfall of approximately 500–1200 mm is necessary (Masinde et al., 2006). African nightshades grow in a variety of soils but require large amounts of nutrients and are best adjusted to soils with high nitrogen, phosphorous and are rich in organic matter (Khan et al., 2000; Ondieki et al., 2011b). Nitrogen fertilizer increases leaf yields 1.5-2.5 folds when applied at 52 kg/ha (Ayua et al., 2016; Masinde et al., 2009; Opiyo, 2004). Sandy loam to friable clay soils with a pH of 6.0-6.5 are appropriate. Plant heights, leaf numbers and area, as well as leaf yields, increase when a higher volume of phosphorus fertilizer is used (Tuwei et al., 2013). African nightshade is ready for harvest 4-6 weeks past transplanting. Picking of leaves is done in weekly intervals and the African nightshade can be sun-dried post-harvest as a means of preservation (Ojiewo et al., 2013b).

1.1.4 Pests and diseases

Pests and diseases of African nightshade are similar to those of other representatives of the *Solanaceae* family and include aphids, spider mites, early and late blight (Boydston et al., 2008). Beetles (*Lagria spp*.) and bacterial wilt have also been reported. Aphids (*Aphis fabae*) feed by sucking plant sap, causing the leaves to curl. The feeding of spider mites may cause a decrease in plant growth, flowering, and number of berries and seeds (Mureithi et al., 2017). Early blight caused by *Alternaria solani* mostly found in lowland regions causes brown leaf spots, up to 1 cm in diameter in a circular pattern. It often occurs under warm conditions, beginning on the oldest leaves, making its way up the plant. However, major infestations are

due to late blight caused by *Phytophthora infestans* during wet seasons in tropical highlands (www.infonet-biovision.org/planthealth/crops/African-nightshade). Dumping-off disease caused by *Pythium spp*. results in significant losses at seedling stage (Juma et al., 2017).

1.2 Assessing diversity using molecular markers

Genetic diversity is a critical component of biodiversity. The conservation and sustainable use of plant genetic resources require accurate identification of their accessions. Classical methods of estimating the genetic diversity or relatedness among groups of plants have relied upon morphological characters. However, these characters can be influenced by environmental factors. Molecular markers avoid complications of environmental effects acting upon characters and represent a reliable and potentially rapid method for characterizing diversity for *in situ* and *ex situ* conservation (Costa et al., 2016; Idrees and Irshad, 2014). Molecular markers are commonly used to identify genetic variations and similarities among species, varieties, or accessions and therefore provide important input in screening and selection of germplasm within crop improvement programs and conservation (Govindaraj et al., 2015). Molecular markers also provide easy and more effective tools for assigning known and unknown plant taxa and to answer evolutionary and taxonomic questions (Arif et al., 2010). Important properties of ideal molecular markers include; high degrees of polymorphism, codominant inheritance, they frequently occur in the genome and are reproducible (Idrees and Irshad, 2014). Different types of molecular markers are available and more continue to be developed. Molecular markers such as AFLP and SSR are suitable for diversity analysis and fingerprinting (Varshney et al., 2007). These two markers were used in the current study.

AFLPs are highly reproducible and can be applied on different species (Semagn et al., 2006). They have been used extensively for phylogenetic analysis and for determining genetic diversity for the conservation of endangered plant species (Teyer et al., 2009). This method

combines restriction enzyme digestion followed by ligation of adaptor sequences and PCR involving primers modified by adding 2 to 4 selective nucleotides. The basis of observed polymorphism may be mutations in restriction site sequences or in sequences complementary to adaptor and selector nucleotides. They are distributed throughout the genome and can be used as a marker of choice to assess variation in closely related plant species (Despres et al., 2003).

SSRs have proven to be versatile molecular markers particularly for population analysis. They have also been used to establish conservation strategies of endangered species (Mcglaughlin et al., 2009: Noda et al., 2009). Their distribution throughout the genomes of many fully sequenced organisms is quite dense, unequal and seemingly non-random (Morgante et al., 2002). SSRs developed for a particular species can in some cases be applied to closely related species. They often present high levels of inter- and intra- specific polymorphism (Miah et al., 2013). Tandemly repeated sequences are amplified and are the source of polymorphism. All SSR types except trinucleotides and hexanucleotides are significantly less frequent in the predicted protein-coding sequences compared with the noncoding fraction in plant species (Kalia et al., 2011). Heterozygotes for different fragments can be easily distinguished. Individual loci corresponding to specific primer pairs are therefore co-dominant and can be multi-allelic. Microsatellites are often found in regulatory regions, e.g. untranslated regions as well as in introns (Zhang et al., 2004). Their distribution in the genome influences the practical implications of their use as molecular markers. For example microsatellite markers from noncoding regions are sufficiently polymorphic to discriminate between closely related taxa (Varshney et al., 2005).

SSRs may be classified as;

1. Perfect or simple perfect or pure repeats are composed of only one motif without interruption (CA)n.

2. Simple imperfect repeats consist of interrupted repeat motif (CA)nN(CA)n+1.
3. Compound or simple compound repeats contain stretches of two or more different repeat motifs (CA)n(GA)n.
4. Interrupted or compound imperfect repeats contain interruption in a compound repeat motif (CCA)nNN(GCA)n+1.

Imperfect repeats are more stable due to being less prone to slippage mutations than perfect repeats.

1.2.1 Genetic diversity parameters

One of the most widely used parameters to measure diversity within a population is heterozygosity. Often observed and expected heterozygosity or gene diversity are compared. These measures are sensitive to differences in allele frequencies. A decrease in observed heterozygosity can induce a decrease average fitness in a population (Reed and Frankham, 2003; Szulkin et al., 2010). Higher expected heterozygosity shows higher allele diversity in a population. This parameter is important in conservation strategies because it accounts for allele evenness which is important for population response to bottleneck events (Caballero et al., 2010).

Another criterion for measuring diversity is allelic richness. It is a measure of genetic diversity of a population's long term potential for adaptability and persistence (Greenbaum et al., 2014; Wagner, 2008). It is insensitive to allele frequencies and therefore favours rare alleles. While heterozygosity quantifies effective number of alleles, allelic richness quantifies actual number of alleles (Petit et al., 1998). Analysis of allelic richness may be complicated by effects of sample size. This however may be mitigated through rarefaction (Foulley and Ollivier, 2006). A third locus-level diversity measure recently developed is Shannon's index (Sherwin et al., 2006). With this index both common and rare alleles are weighted proportionally.

1.2.2 Population structure tools

Determination of the genetic structure of germplasm collections is important for both conservation and utilization of genetic resources. Population structure infers relationships among individuals and genetic similarities and differences within and between groups of genotypes and also their evolutionary history. Methods of reconstructing population structure include distance based phylogenetic methods (Pickrell and Pritchard, 2012), model based clustering approaches (Alexander et al., 2009; Pritchard et al., 2000) and multivariate consensus representation of genetic relationship among populations (Price et al., 2006). Various coefficients such as Dice, Jaccards, Squared Euclidean distance are used for genetic similarity or dissimilarity (Kosman and Leonard, 2005). In addition, analysis of molecular variance may be used to partition variance to infer population structure (Excoffier, et al., 1992). The choice of appropriate coefficients and methods are determined by the marker system used as well as the objective of the experiment. Descriptive procedures such as cluster analysis are useful in highlighting interesting populations. The reliability however, has to be tested with appropriate statistical methods such as bootstrapping.

1.3 Objectives

African nightshades have a potential to contribute nutritional security and also provide a source of livelihood. This potential has however not been fully met. The major bottleneck is lack of quality and affordable seed varieties. Analysis of genetic structure and diversity of African nightshade as well as investigation of reproductive biology will build a knowledge base for developing improved varieties to supply farmers with quality seeds. This study aimed at contributing towards developing superior varieties of African nightshades by undertaking the following objectives;

1. To analyse the genetic diversity in African nightshade entries using molecular markers
    i. Development and establishment of SSR and AFLP markers for diversity analysis.
    ii. Analysis of population structure and diversity within and between of African nightshade entries.
2. To analyse cytological and reproductive biology of African nightshade.
    i. Determination of ploidy levels and DNA content of selected African nightshade entries.
    ii. Assessment of pollen viability by *in vitro* germination.
3. Evaluation of African nightshade entries using phenotypic traits in field experiments to analyse the variation in morphological traits relevant for agronomic performance.

2 Publications and manuscripts

2.1 African nightshades: genetic, biochemical and metabolite diversity of an underutilized indigenous leafy vegetable and its potential for plant breeding

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REVIEW

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African nightshades: genetic, biochemical and metabolite diversity of an underutilised indigenous leafy vegetable and its potential for plant breeding

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ABSTRACT

African nightshades are becoming more important as leafy vegetables in sub-Saharan Africa. Previously considered as food for the poor, their cultivation is now being promoted, and some cultivars are commercialised; however, most farmers use self-produced seeds, leading to low and varying yields. Improvement through conventional breeding depends on the available genetic diversity, the possible breeding systems, and the nutritional value of the accessions. Therefore, we review the information on these topics with the following main outcomes: the most commonly discussed species, *S. nigrum, S. scabrum, S. villosum*, and *S. americanum*, could be differentiated using molecular markers, but further sub-clustering was rarely possible, and statistical support often missing. *S. nigrum* and *S. scabrum* seem to be most closely related to each other. The mainly self-pollinating African nightshades form a polyploidy series with diploid (2n = 2x = 24) to hexaploid taxa. Interploidy hybridisations between diploids and tetraploids are possible, whereas the hexaploid *S. nigrum* and *S. scabrum* could not be crossed to genotypes of lower ploidies. Solanine, solamargine, solasonine, and chaconine are the major steroidal alkaloid glucosides in African nightshades. Amounts are age and environment dependant. Mineral and vitamin contents in leaves are at least as high as in *Brassica oleracea* or *Spinacia oleracea*, underlining their relevance as local vegetables.

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Solanum nigrum; *Solanum scabrum*; genetic diversity; cytology; glycoalkaloids; polyploidy

Introduction

African nightshades comprise several species of the genus *Solanum* in the section *Solanum*, also referred to as the *Solanum nigrum* complex. The genus *Solanum* is the largest and most diverse genus in the family Solanaceae, and species belonging to African nightshades have been assigned to the section *Solanum*. They are mostly used as leafy vegetables and herbs in most parts of West and East Africa (Ojiewo, Mwai, Abukutsa, Agong, & Nono-Wandim, 2013). The most commonly grown species are *S. scabrum, S. villosum*, and *S. americanum* were –preferences differ with different regions. Unlike other major vegetable crops in the genus *Solanum*, such as potato, tomato, and eggplant, African nightshades are not widely utilised outside West and East Africa, as they are considered poisonous, or weeds, in many parts of the world. With the realisation of their rich nutritional value, their low input requirements for growth and cultivation, and their potential in nutrition security, they are emerging as an important food crop in sub-Saharan Africa to generate secure income, primarily for resource poor, rural and peri-urban, mainly female populations (Poczai & Hyvönen, 2011; Weinberger, Pasquini, Kasambulu, & Abukutsa-Onyango, 2011). Their cultivation has therefore been promoted by governmental and non-governmental institutions in the last few years. Although some cultivars were developed by public and private seed companies, working in collaboration with research institutions, most farmers rely on low-quality seed lots from local markets, or self-harvested seeds, leading to low and varying yields. Therefore, vegetable nightshades have attracted the interest of the research community, and are among the top priority African indigenous vegetables identified for cultivar improvement and development to increase their market share.

Historically incorrect nomenclature, due to phenotypic plasticity, has created confusion in the taxonomy of African nightshades. For instance, *S. scabrum* and *S. nigrum* are not clearly distinguished in different parts of the world (Olet, Heun, & Lye, 2005), and *S. nodiflorum* is used interchangeably with *S. americanum* in African accessions (Manoko, van den Berg, Feron, van der Weerden, & Mariani, 2007) by both consumers and taxonomists. Identification and classification of new

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and previously described taxa is still being undertaken (Manoko, van der Weerden, van den Berg, & Mariani, 2012), and many more taxa remain unexplored. Key research tools for studying the genetic diversity of plants include morphological traits, biochemical analysis, and molecular markers (Xiang, 2000). In particular, molecular markers promise to be an effective tool in resolving the taxonomic relationships within the *S. nigrum* complex. This review highlights recent research on the genetic diversity of African nightshades, their cytological variability and biochemical composition, in line with important fundamentals in crop variety development.

Taxonomy and genetic diversity of African nightshades

African nightshades are angiosperms belonging to the Solanaceae family. They are herbaceous annual plants, growing up to a height of 1.5 m, in upright to decumbent growth habits. They show phenotypic plasticity, and variations are observed in different populations of the same species (Manoko et al., 2007). There have been a number of studies to resolve taxonomic complexities regarding this section in Africa. Bukenya and Hall (1988) and Bukenya and Carasco (1995) made taxonomic studies of plant material from Ghana and Uganda, respectively, using herbarium material. They developed a key for the morphological description of the genus *Solanum*, following the classification of D'Arcy (1972). In their study, eight species belonging to the *Solanum nigrum* complex, namely *S. nigrum* L., *S. americanum, S. scabrum* Mill., *S. sarrachoides* Sendtn., *S. villosum* Mill., *S. grossedentatum* A. Rich., *S. florulentum* Bitter, and *S. tarderemotum* Bitter, were differentiated, based on descriptive morphological traits.

In order to evaluate the genetic diversity and biogeographic relationships, multilocus markers have been used in different studies. Jacoby, Labuschagne, and Viljoen (2003) used amplified fragment length polymorphism (AFLP) to study the genetic relationships of eight species of African nightshades sampled in South Africa (Table 1). The study involved 14 accessions, which were analysed using three EcoR I/Mse I primer combinations, generating 359 markers, of which 222 (62%) were polymorphic. Binary data was used to generate a dendrogram using NCSS 2000 (Number Cruncher Statistical Systems), and dissimilarity was expressed as Euclidean genetic distances. *S. villosum* formed a sub-cluster with *S. retroflexum*, with a dissimilarity coefficient of 0.51, while *S. scabrum* clustered separately with the diploid *S. americanum*, with a dissimilarity coefficient of 0.52. The phylogenetic grouping, however, did not provide information about the variability within species, since only one accession was used for every species, except for S. *retroflexum*, which showed low

Table 1. Summary of species used by different authors for genetic diversity analysis, and their sources.

Author	Polymorphic markers	Accessions used	Plant material used	Origin of plant material	Major results
Jacoby et al. (2003)	222 AFLP	14	*S. americanum, S. villosum, S. retroflexum, S. burbankii, S. chenopodioides, S. kwabense, S. scabrum, S. tomentosum, Solanum spp.*	Sourced from various regions in South Africa	Hexaploids more closely related to diploids than to tetraploids
Dehmer and Hammer (2004)	523 AFLP	44	*S. americanum, S. physalifolium, S. villosum, S. nigrum, S. scabrum*	Kenya, Cuba and Italy	Diploids (*S. americanum*) showed higher variation than other accessions. *S. scabrum* closely related to *S. nigrum.*
Manoko et al. (2007, 2008)	248 AFLP; 448 AFLP	48; 39	*S. americanum, S. chenopodioides, S. physalifolium, S. tarderemotum, S. florulentum, S. retroflexum, S. hirsutum, S. villosum, S. grossidentetum, S. scabrum, S. opacum, S. nigrum, S. chenopodioides, S. nodiflorum*	Africa (Kenya, Tanzania, South Africa, Cameroon, Uganda, Ghana and Zimbabwe), countries in Europe, America, Asia and Australia.	*S. scabrum* showed higher morphological variation but not reflected in molecular data. Plant material formally classified as *S. americanum* confirmed to be *S. nodiflorum.*
Van Biljon et al. (2010)	29 SSR	10	*S. americanum, S. chenopodioides, S. retroflexum, S. scabrum, S. burbankii.* Crosses of *S. scabrum* × *S.americanum, S. retroflexum* × *S. burbankii, S. retroflexum* × *S. chenopodioides, S. chenopodioides* × *S. burbankii, S. chenopodioides* × *S. scabrum*	South Africa	Microsatellite markers from other species were not very reliable for assessing population structure in section *Solanum* species.
Poczai et al. (2010)	252 RAPD		*S. retroflexum, S. villosum, S. scabrum, S. nigrum*	University of Pannonia botanical garden (Hungary)	There is a close relationship between *S. nigrum* and *S. retroflexum*
Olet et al. (2011)	510 AFLP	107	*S. americanum, S. florulentum, S. hirsutum, S. sarrachoides, S. tarderemotum, S. villosum, S. nigrum, S. scabrum*	Uganda, Brazil, Indonesia, China and Belgium	*S. americanum* and *S. villosum* showed highest levels of polymorphism. *S. nigrum* showed a closer relationship to tetraploids than to the other hexaploid *S. scabrum.*

variability. Additionally, the authors did not apply any statistical test estimating the support of their clustering, for example using bootstrapping.

Dehmer and Hammer (2004) also used AFLP to study taxonomic status and geographic provenance from a gene bank collection involving 44 accessions representing five species from different origins (Table 1). The two *EcoR* I/*Mse* I primer combinations used generated a total of 523 polymorphic bands. Genetic similarities, according to Dice, were used to construct a phenogram using the unweighted pair group with arithmetic mean method that generated four major clusters from the five species. The three accessions from *S. physalifolium* were nested within the *S. nigrum* and *S. villosum* clusters. In comparison to the other species examined, *S. americanum* was placed in a separate cluster, with only 43% genetic similarity to the other species, but also showed the lowest infraspecific similarity (49%) with two well supported subgroups from Cuba and Central America, respectively. *S. scabrum* and *S. nigrum* were the most closely related species, with 68% genetic similarity, and are related to the *S. villosum* cluster, with 62% genetic similarity. All three species were well separated, with high bootstrap support in independent clusters. Geographic provenances were only supported with bootstrap values above 60% for the *S. villosum* and *S. americanum* accessions. Those of *S. scabrum* and *S. nigrum* were inconclusive, due to a small number of accessions and unknown origins, respectively.

In their study on genetic diversity of the section *Solanum* using AFLP markers, Manoko et al. (2007) were able to separate *S. americanum* and *S. nodiflorum*, which had been treated as one species by earlier studies using morphological characterisation. The study also identified a new species from Tanzania, which was later described as *Solanum umalilaense* (Manoko et al., 2012).

The hexaploid *S. scabrum* and *S. nigrum* accessions did not cluster according to their geographical provenance, and showed low genetic differentiation in both neighbour-joining and maximum-parsimony phylogenetic trees, according to Manoko, van den Berg, Feron, van der Weerden, and Mariani (2008). The analysis did not reveal a clear distinction between wild and cultivated germplasm; however, using 448 AFLP markers, they were able to reliably differentiate the 80 *S. scabrum* accessions from the 41 *S. nigrum* and 15 *S. opacum* genotypes, with high Jacknife support. The study of Olet, Lye, and Heun (2011) also detected a high variability within *S. villosum*, showing 60% polymorphism, and within *S. americanum*, showing 64% polymorphism. For *S. villosum*, the previous differentiation into subspecies could not be confirmed by the AFLP data. The neighbour-joining dendrogram generated from 510 AFLP markers (481 polymorphic) generated six main clusters from the eight species used in the study (Table 1). Low variability was observed in *S. scabrum* (10% polymorphism), and genotypes from this species were placed in a separate main cluster. The *S. scabrum* accessions did not cluster according to their geographical origin, even when they originated from different continents. The 40 *S. villosum* and the 42 *S. americanum* accessions could also be grouped into two large clusters; however, the structure of the dendrogram was not supported by bootstrap or jack-knife analysis. The random amplified polymorphic DNA (RAPD) technique was used by Poczai, Mátyás, Taller, and Szabó (2010) to investigate the relationship between *S. scabrum, S. nigrum, S. retroflexum*, and *S. villosum* on 13 accessions provided by the Georgikon Botanical Garden (Hungary), for which the original sampling provenance was not indicated. All four species could be clearly separated from each other using the 252 RAPD markers with high bootstrap support. The authors stated that the morphological traits in *S. scabrum* exhibited high variations, which were not reflected by the molecular data, but a dendrogram constructed from the morphological data showed the same clustering as the dendrogram from the RAPD data; however, all morphological parameters were probably measured only from one individual per accession in two years.

Van Biljon, Labuschagne, and Koen (2010) used microsatellite markers in five species of the *S. nigrum* complex found in South Africa (*S. americanum, S. burbankii, S. chenopodioides, S. retroflexum*, and *S. scabrum*), and in crosses between them. Because only seven out of 29 SSR primer pairs detected polymorphisms between the accessions, the estimated genetic distances between the species might not be very reliable, and bootstrap analyses were again not presented. Two subspecies have been proposed for *S. scabrum*, on the basis of morphological data (Manoko et al., 2008; Olet et al., 2005; Poczai et al., 2010), with subsp. *scabrum* measuring up to 2 m in height, having a larger number of leaves and a smaller number of fruits, and subsp. *laevis* being less than 1 m in height, with fewer leaves and a larger number of fruits. But this division has not been supported by molecular data. The observed morphological differences could be due to selection by farmers, since in Kenya, *S. scabrum* is the most cultivated leafy vegetable in the section *Solanum*.

From the molecular data presented in the different publications, it could be concluded that *S. nigrum* and *S. scabrum* are more closely related to each other than to *S. villosum*, whereas *S. americanum* seems to be more distantly related. The four species could be separated from each other into different clusters in all mentioned publications. A further sub-clustering within a species could only be shown for *S. americanum* and *S. villosum*, by Dehmer and Hammer (2004). Overall, it is hard to draw general conclusions from the different studies, because the overlap in identical species and countries of origin

is low. Furthermore, many dendrograms are not statistically supported, either by bootstrap or jack-knife analyses, resulting in limited information content.

Cytological variation and hybridisation in African nightshades

The section *Solanum* forms a polyploidy series, with diploid (2n = 2x = 24), tetraploid (2n = 4x = 48), and hexaploid (2n = 6x = 72) taxa. Octoploids (2n = 8x = 96) have also been reported (Edmonds & Chweya, 1997). African nightshades are generally self-pollinating, but out-crossing is possible (Table 2). Interspecific hybridisations are considerably more successful when closely-related species of the same ploidy level are involved, and when they have the same floral sizes. Successful crosses between diploids produced hybrids with pollen fertilities lower than those of the parents (Heiser, Soria, & Burton, 1965). This, however, was dependent on the genetic distance between the species used for the crosses. For instance, crosses between the very closely related *S. americanum* and *S. nodiflorum*, which had often been treated as one species in studies using morphological characterisation (Manoko et al., 2007), led to progeny producing sterile seeds, while crossing the more distantly related *S. americanum* and *S. sarachoides* was not successful at all (Olet, Lye, & Stedje, 2015). Hybridisations between tetraploids were more successful than those between diploids. Viable progeny were obtained between *S. retroflexum* and *S. burbankii* (Jacoby & Labuschagne, 2006), and between *S. memphiticum* as the maternal parent crossed with *S. florulentum*, *S. tarderemotum* and *S. villosum* as paternal parents (Olet et al., 2015). Hybridisation between hexaploids has been attempted between *S. nigrum* and *S. scabrum*, which yielded fertile hybrids with over 90% viability. Self-pollination in these experiments was avoided by early emasculation and bagging the flowers.

Interploidy hybridisations were more successful between diploids and hexaploids. Crosses between the diploid *S. americanum* and *S. chenopodioides* crossed with *S. scabrum* produced viable progeny with ploidy levels as the maternal parent (Jacoby & Labuschagne, 2006; Olet et al., 2015). Progeny produced from crosses between diploids and tetraploids were triploids (2n = 36) and, therefore, not viable. Some were, however, successful when the paternal parent was of lower ploidy, for example in crosses between *S. americanum* × *S. memphiticum* and *S. americanum* × *S. villosum*. The progeny was of the same ploidy level as the maternal parent (tetraploid). Successful crosses between tetraploids and hexaploids produced pentaploids (2n = 60), as observed in the crossing between *S. nigrum* and *S. retroflexum*. These crosses were achieved in low frequency, and only if the hexaploid *S. nigrum* was used as the maternal parent. The germinated hybrids produced sterile pollen and, therefore, break down occurred in the second generation (Ganapathi & Rao, 1986). According to Olet et al. (2015) viable tetraploids were achieved when pollen from *S. scabrum* was used to pollinate the also tetraploid species *S. villosum*, *S. memphiticum*, and *S. florulentum*. Reciprocal crosses were, however, not done.

Steroidal glycoalkaloids in African nightshades

Steroidal alkaloid glucosides (SGAs) constitute the main secondary metabolites in African nightshades, and are found in all plant tissues, including leaves, fruits, stems and roots. Solanine, solamargine, solasonine, and chaconine are the major SGAs in African nightshades. Their diversity is determined by differences in sugar moieties attached to a glycone solanidine. Their nature and concentrations are genetically determined (Friedman, 2006). Biosynthesis normally starts at germination, and reaches a peak during fruiting. Environmental factors, such as high salinity, low temperature, and pest infestation, enhance production and accumulation (Bhat, Ahmad, Aslam, Mujib, & Mahmood, 2008; Potawale et al., 2008). They have no function in the primary growth of plants, but play an important role in the interaction of the plants with their

Table 2. Combinations of crosses from various studies summarising possible and unsuccessful hybridisations (Sources: Ganapathi & Rao, 1986; Heiser et al., 1965; Jacoby & Labuschagne, 2006; Olet et al., 2015).

♀ ♂=	1	2	3	4	5	6	7	8	9	10	11	12
Diploids												
1. S. americanum	++	–	–	+	+	+	+	+	+	+	++	
2. S. chenopodioides	+	++	–	+	+	+	+	+	+	+	++	
3. S. sarrachoides	–	–	++	–	–	–	–	–	–			
4. S. nodiflorum	+		+									
Tetraploids												
5. S. retroflexum	+	+	–		++	++				+	+	
6. S. burbankii	+	+	–		++			++				
7. S. florulentum	+	+	–				++	–	–	–	++	
8. S. villosum	++	+	–				++	++		++	++	
9. S. tarderemotum	+	+	–				++	++	++		++	
10. S. memphiticum	++		–				++	++	++	++		
Hexaploids												
11. S. scabrum											++	++
12. S. nigrum	+											

++ = fertile progeny; + = sterile progeny; – = unsuccessful crossing (seedless berries produced).

environment. They provide a chemical defence mechanism against a broad range of pathogens and predators. They also attract pollinators by producing scent and taste, and function as agents of plant-to-plant competition and plant-microbe symbiosis (Zhao, Wang, Norris, Chen, & Chen, 2013). However, consumption of glycoalkaloids in higher amounts (>5 mg kg^{-1}) results in toxic effects in mammals (Distl & Wink, 2009). Therefore, concentrations of glycoalkaloids in African nightshades have to be carefully estimated.

Carle (1981) investigated the content of SGAs in leaves, stems, fruits, and roots of different species in the section *Solanum*, through different stages of plant growth. Young leaves and unripe fruits had the highest concentrations, while ripe fruits and roots had the lowest. The study of Eltayeb, Al-Ansari, and Roddick (1997) also showed differences in concentrations during developmental stages of *S. nigrum*. Young leaves had the highest concentrations, compared to other organs. The concentration declined as the leaves matured, and contained only about 25% (~0.3 µg/mg dry wt.) of that of young leaves (1.2 µg/mg dry wt.). The absolute amount per leaf, however, increased with the age of the plants, from weeks eight to 16, because of the enlarging leaf mass and area, but decreased in older plants up to the age of 24 weeks, to about the same amount as in eight-week-old plants. Concentrations in the roots were three times those in the stems, increasing with time more markedly in the roots, and then reducing at maturity. Ripe fruits had approximately 3% of the concentration of the unripe ones. Phytochemical studies done by Kumar, Sagwal, and Rani (2012) and Djaafar and Ridha (2014) also indicated that leaves had the highest concentration of glycoside alkaloids. Figure 1 gives a generalised graphic representation of SGA accumulation in different organs during different developmental stages of African nightshades.

In contrast to these findings, Lehmann, Biela, Töpfl, Jansen, and Vögel (2007) reported higher SGA concentrations in ripe fruits of *S. scabrum* than in unripe fruits from African accessions. This could have been influenced by the growing conditions of the plants in the field. Lower autumn temperatures during fruiting were a stress factor, and consequently the unripe stage was prolonged. During harvest, only a small percentage of fruits had ripened, compared to *S. scabrum* of European origin. In West Africa, where the accessions originated, ripe fruits have been consumed without any known reports of health problems. Other *Solanum* species, such as potatoes, have also been found to accumulate glycoalkaloids at very low temperatures (Machado, Toledo, & Garcia, 2007).

Different species differ in quantity and type of steroidal alkaloids. According to the few studies that have been done, solanine, which is associated with bitterness in the section *Solanum*, is normally found in all parts of the plant. Solamargine, solasonine, and chaconine may be absent in some organs, such as flowers and roots. Leaf extracts of *S. villosum* contained the least amounts of α-solanine (1.5 mg/g dry wt.), compared to *S. nigrum* (4.7 mg/g dry wt.), *S. retrolexum* (5.7 mg/g dry wt.), *S. americanum* (3.29 mg/g dry wt.), and *S. chenopodioides* (3.08 mg/g dry wt.), but contained the highest amounts of solamargine (Mohyuddin, Khan, Ahmad, & Kashmir, 2010). *S. nigrum* contained the highest total SGA amounts; however, in Jared, Murungi, Wesonga, and Torto (2016), crude extracts obtained from *S. villosum* did not contain solamargine. A mixture of SGAs, including solasonine and solamargine, were, however, found in crude extracts of *S. sarrachoides*, but not detected in extracts from *S. scabrum*. The absence, or low amounts, of SGA detected could be attributed to the optimum conditions in the greenhouse under which the plants were grown. This is in contrast to plants grown in the field, where they are exposed to environmental

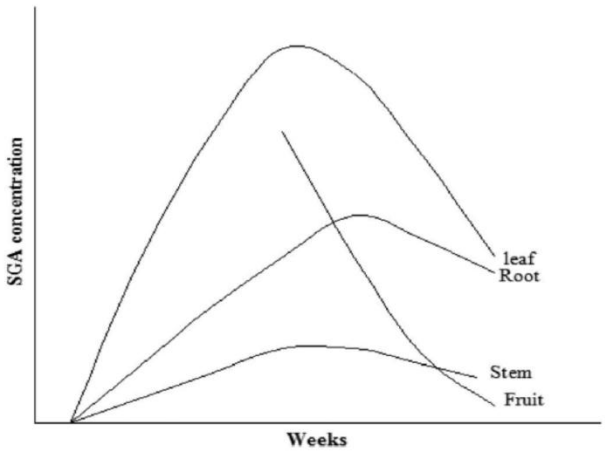

Figure 1. Changes in glycoalkaloid content in different plant organs during developmental stages; adapted from the studies of Carle (1981) and Eltayeb et al. (1997).

stresses. In comparison, α-solanine concentrations, from 1.14 to 3.37 mg/g dry wt., depending on the age of the leaf, are reported for leaf material of the commercial *S. tuberosum* cultivar Russet Burbank (Brown, McDonald, & Friedman, 1999). Also, in commercial *S. tuberosum*, younger and older leaves had significantly lower α-solanine concentrations, compared to leaves sampled in between. Analysis of fruit extracts has shown higher concentrations of solanine in *S. villosum* than in *S. nigrum* (26.6 and 9.7 w/w, respectively) (Sammani, Shammaa, & Chehna, 2013). It would be expected that *S. nigrum* contained higher concentrations, though, since *S. villosum* fruits are edible, while those of *S. nigrum* are not.

SGAs are therefore the main secondary metabolites in African nightshades, with functions in plant defence and attraction of pollinators, and are found in all plant tissues, including leaves, fruits, stems, and roots. The concentrations of α-solanine, measured in the different species belonging to the African nightshades, are in the range of the values estimated for the commercial *S. tuberosum* cultivar Russet Burbank. Young leaves and fruits have higher concentrations than ripe fruits and roots. Environmental stresses seem to influence the concentrations.

African nightshade seed production and breeding

The area under African nightshade cultivation is still low because it is traditionally produced at subsistence level, where it is grown in home gardens, around homesteads, or collected from the wild (Abukutsa-Onyango, Kavagi, Amoke, & Habwe, 2010). The majority of farmers use seeds either saved from a previous crop, which are processed using traditional methods, or obtained from local seed traders; however, these sources tend to have insufficient purity, especially due to mixing of different cultivars, and rarely display mean germination rates above 50% (Onyango, Kunyanga, Ontita, Narla, & Kimenju, 2013). Effective seed supply systems, and an assured market for seed, are critical in successfully unleashing the potential of nightshade to improve food security. Organisations and researchers are gaining interest in improving the quality and quantity of the vegetable. For instance, AVRDC has trained farmers in informal seed enterprises, with financial support from the Association for Strengthening Agricultural Research in East and Central Africa (ASARECA), and in collaboration with various partners in Kenya and Tanzania.

In an attempt to improve yield limited by early and prolific flowering and berry production, Ojiewo, Murakami, Masuda, and Agong (2009) reported that this could be reversed by inducing male sterility, which in turn reduces competition resulting from the reproductive functions that normally follow soon after anthesis. Temperature-sensitive male sterile mutants of *S. villosum* genotypes have been developed (Ojiewo, Murakami, & Masuda, 2007), and characterised (Ojiewo, Kubo, Murakami, & Masuda, 2010) with greater yield potential under sterility conditions, and seed production for propagation under fertility conditions (Ojiewo et al., 2009). In a study to improve seed yields of *S. scabrum*, Ojiewo, Agong, Murakami, and Masuda (2006) induced octoploids from the tetraploid *S. villosum* through seed treatments with colchicine. According to Masinde, Wesonga, Ojiewo, Agong, and Masuda (2009), an increase of up to 35% higher leaf dry weight at 40 days after transplanting was observed in octoploid plants. compared with the tetraploids. The octoploid plants tended to have fewer but larger leaves, yielding 1.3–1.6 times higher leaf fresh weight than their tetraploid progenitors.

Beside these mutagenesis experiments, selection within the existing diversity in germplasm collections could lead to significantly improved cultivars with various desirable horticultural traits, including late flowering and berry setting. This is the quickest and cheapest way of coming up with improved cultivars for commercialisation, especially at this infant stage of the crop improvement program for African nightshades. Selection within existing collections, and evaluation, has resulted in releases of at least two improved cultivars in Tanzania and three in Kenya (Dinssa et al., 2016; Ojiewo, Mbwambo, et al., 2013).

Importance of African nightshade as a vegetable

Although several publications on African nightshades have appeared in the last three decades, only few have focused on its importance as a vegetable. Five aspects of importance of African nightshade as a vegetable have been reported in the published studies, which include nutritional aspects, income generation, food security, its medicinal value, and its diversity (Abukutsa-Onyango et al., 2010; Grivetti & Ogle, 2000; Humphry, Clegg, Keen, & Grivetti, 1993; Ojiewo, Mwai, et al., 2013; Smith, Dueker, Clifford, & Grivetti, 1996).

Nutritional contents vary between species and within species from different localities (Weinberger & Msuya, 2004). For instance *S. villosum* is described to contain 241 mg calcium, 0.9 mg zinc and 5.09 mg iron per 100 g fresh weight, and *S. scabrum* contains 230 mg, 0.7 mg, and 3.32 mg, respectively, in the study from Luoh, Begg, Symonds, Ledesma, and Yang (2014), whereas the values reported by Yang and Keding (2009) are lower for both species (Table 3); however, compared to exotic vegetables, such as *Brassica oleracea* or *Spinacia oleracea*, higher contents for several nutrients are reported for *S. nigrum*, *S. scabrum*, *and S. villosum*, as shown in Table 3.

Table 3. Mineral and vitamin contents in leaves of *S. nigrum, S. scabrum* and *S. villosum* in comparison to other leafy vegetables grown in sub-Saharan Africa.

	Brassica oleracea[a]	*Vigna unguiculata*[a]	*Spinacia oleracea*[a]	*Amaranthus spec.*[a]	*Solanum nigrum*[b]	*Solanum scabrum*[c]	*Solanum villosum*[c]	*Brassica carinata*[c]	*Cleome gynandra*[c]
Nutrient	Cabbage	Cowpea	Spinach	Amaranth	Black nightshade	African nightshade	Red-fruited nightshade	Ethiopian kale	Spider plant
Ca (mg)	41	265	133	380	173	194	175	157	186
Fe (mg)	0.6	5.1	3.1	6.2	1.3	3	3.3	1.3	2.2
Mg (mg)	12	60	53	93	25	–	–	–	–
P (mg)	37	61	45	58	75	–	–	–	–
K (mg)	317	475	502	602	430	–	–	–	–
Na (mg)	12	6	87	13	3	–	–	–	–
Zn (mg)	0.2	0.5	0.8	0.72	0.1	0.5	0.8	0.9	0.8
Vit A (µg)	8	150	409	241	5	5.8	2.9	0.9	2.7
Vit E (mg)	0.15	2.36	2.31	0.24	9.7	2.3	2.1	1.3	0.7
Folate (µg)	48	129	176	79	12	70	61	86	198
Vit C (mg)	54	57	36	45	35	75	79	157	113

Values per 100 g fresh leaves from: [a]Stadlmayr et al. (2012), [b]Akubugwo, Obasi, and Ginika (2007), and [c]Yang and Keding (2009). The symbol "-" indicates that there were no data available.

Conclusions

African nightshades are important African leafy vegetables that are widely consumed in most parts of sub-Saharan Africa. The demand for the vegetable is steadily increasing, due to its nutritional value, income generation ability, and also people believe in its medicinal value. The production of the vegetable is constrained due to the lack of quality seeds of improved cultivars. There is, therefore, a need to develop high leaf- and seed-yielding cultivars with improved nutritional content, and resistance to biotic and abiotic stresses. For sustainable seed production, studies on optimal methods of seed processing are necessary to ensure the production and distribution of quality seed to farmers.

There are several taxa in the *Solanum nigrum* complex that are still poorly known. Although some molecular diversity studies have been published, an optimal utilisation of African nightshade germplasm, for use in breeding programs, needs a statistically supported taxonomic revision of germplasm from a wider geographic coverage. The genetic diversity data, in addition to available cytological data and results from yield trials, drought tolerance and resistance tests, could be used to develop improved breeding strategies for African nightshades. Furthermore, much of the molecular aspects of glycoalkaloid biosynthesis and regulation remain unknown, although such information is important in developing cultivars with high nutritional quality and improved horticultural traits.

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References

Abukutsa-Onyango, M.O., Kavagi, P., Amoke, P., & Habwe, F.O. (2010). Iron and protein content of seven priority indigenous vegetables in the Lake Victoria Basin. *Journal of Agricultural Science and Technology*, *4*, 67–69.

Akubugwo, I.E., Obasi, A.N., & Ginika, S.C. (2007). Nutritional potential of the leaves and seeds of black nightshade *Solanum nigrum* L. var *virginicum* from Afikpo-Nigeria. *Pakistan Journal of Nutrition*, *6*, 323–326. doi:10.3923/pjn.2007.323.326

Bhat, M.A., Ahmad, S., Aslam, J., Mujib, A., & Mahmood, U. (2008). Salinity stress enhances production of solasodine in *Solanum nigrum* L. *Chemical & Pharmaceutical Bulletin*, *56*, 17–21. doi:10.1248/cpb.56.17

Brown, M.S., McDonald, G.M., & Friedman, M. (1999). Sampling leaves of young potato (*Solanum tuberosum*) plants for glycoalkaloid analysis. *Journal of Agricultural Food Chemistry*, *47*, 2331–2334. doi:10.1021/jf981124m

Bukenya, Z.R., & Carasco, J.F. (1995). *Solanum* (Solanaceae) in Uganda. *Bothalia*, *25*, 43–59. doi:10.4102/abc.v25i1.711

Bukenya, Z.R., & Hall, J.B. (1988). *Solanum* (Solanaceae) in Ghana. *Bothalia*, *18*, 79–88. doi:10.4102/abc.v18i1.983

Carle, R. (1981). Investigation on the content of steroidal alkaloid and sapoganins within *Solanum* section *Solanum* (*Morella*) (Solanaceae). *Plant Systematics and Evolution*, *138*, 61–71. doi:10.1007/BF00984609

D'Arcy, W.G. (1972). Solanaceae studies 2. Typification of subdivisions of *Solanum*. *Annals of the Missouri Botanical Garden*, *59*, 262–278. doi:10.2307/2394758

Dehmer, K.J., & Hammer, K. (2004). Taxonomic status and geographic provenance of germplasm accessions in the *Solanum nigrum* L. complex: AFLP data. *Genetic Resources and Crop Evolution*, *51*, 551–558. doi:10.1023/B:GRES.0000024163.86762.fc

Dinssa, F.F., Hanson, P., Dubois, T., Tenkouano, A., Stoilova, T., Hughes, J.D., & Keatinge, J.D.H. (2016). AVRDC – The World Vegetable Centre's women-oriented improvement and development strategy for traditional African vegetables in sub-Saharan Africa. *European Journal of Horticultural Sciences*, *81*, 91–105. doi:10.17660/eJHS.2016/81.2.3

Distl, M., & Wink, M. (2009). Identification and quantification of steroidal alkaloids from wild tuber-bearing *Solanum* species by HPLC and LC-ESI-MS. *Potato Research*, *52*, 79–104. doi:10.1007/s11540-008-9123-0

Djaafar, Z., & Ridha, O.M. (2014). Phytochemical study on selected medicinal plants, *Solanum nigrum*. *International Letters of Chemistry, Physics and Astronomy*, *20*, 25–30. doi:10.18052/www.scipress.com/ILCPA.20.25

Edmonds, J.M., & Chweya, J.A. (1997). Black nightshades: *Solanum nigrum* L. and related species. *Bioversity International*, *15*, 1–115.

Eltayeb, E.A., Al-Ansari, A., & Roddick, J.G. (1997). Changes in the steroidal alkaloid solasodine during development of *Solanum nigrum* and *Solanum incanum*. *Phytochemistry*, *46*, 489–494. doi:10.1016/S0031-9422(97)00323-3

Friedman, M. (2006). Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. *Journal of Agricultural and Food Chemistry*, *54*, 8655–8681. doi:10.1021/jf061471t

Ganapathi, A., & Rao, G.R. (1986). The crossability and genetic relationship between *Solanum retroflexum* Dun. and *Solanum nigrum* L. *Cytologia*, *51*, 757–762. doi:10.1508/cytologia.51.757

Grivetti, L.E., & Ogle, B.M. (2000). Value of traditional foods in meeting macro- and micronutrient needs: The wild plant connection. *Nutrition Research Reviews*, *13*, 31–46. doi:10.1079/095442200108728990

Heiser, C.B., Soria, J., & Burton, D.L. (1965). A numerical taxonomic study of *Solanum* species and hybrids. *The American Naturalist*, *99*, 471–488. doi:10.1086/282392

Humphry, C., Clegg, M.S., Keen, C., & Grivetti, L.E. (1993). Food diversity and drought survival. The Hausa example. *International Journal of Food Sciences and Nutrition*, *44*, 1–16. doi:10.3109/09637489309017417

Jacoby, A., & Labuschagne, M.T. (2006). Hybridization studies of five species of the *Solanum nigrum* complex found in South Africa and two cocktail tomato cultivars. *Euphytica*, *149*, 303–307. doi:10.1007/s10681-005-9078-z

Jacoby, A., Labuschagne, M.T., & Viljoen, C.D. (2003). Genetic relationships between southern African *Solanum retroflexum* Dunal and other related species measured by morphological and DNA markers. *Euphytica*, *132*, 109–113. doi:10.1023/A:1024657827796

Jared, J.J., Murungi, L.K., Wesonga, J., & Torto, B. (2016). Steroidal glycoalkaloids: Chemical defence of edible African nightshades against the tomato red spider mite, *Tetranychus evansi* (Acari: Tetranychidae). *Pest Management Science*, *72*, 828–836. doi:10.1002/ps.2016.72.issue-4

Kumar, A., Sagwal, S., & Rani, S. (2012). An updated review on molecular pharmacology and physiology of black nightshade (*Solanum nigrum*). *International Journal of Pharmaceutical Science and Research*, *3*, 2956–2977.

Lehmann, C., Biela, C., Töpfl, S., Jansen, G., & Vögel, R. (2007). *Solanum scabrum* — A potential source of a coloring plant extract. *Euphytica*, *158*, 189–199. doi:10.1007/s10681-007-9442-2

Luoh, J., Begg, C., Symonds, R., Ledesma, D., & Yang, R. (2014). Nutritional yield of African indigenous vegetables in water-deficient and water-sufficient conditions. *Food and Nutrition Sciences*, *5*, 812–822. doi:10.4236/fns.2014.59091

Machado, R.M., Toledo, M.C., & Garcia, L.C. (2007). Effect of light and temperature on the formation of glycoalkaloids in potato tubers. *Food Control*, *18*, 503–508. doi:10.1016/j.foodcont.2005.12.008

Manoko, M.L.K., van den Berg, R.G., Feron, R.M.C., van der Weerden, G.M., & Mariani, C. (2007). AFLP markers support separation of *Solanum nodiflorum* from *Solanum americanum sensu stricto* (Solanaceae). *Plant Systematics and Evolution*, *267*, 1–11. doi:10.1007/s00606-007-0531-4

Manoko, M.L.K., van den Berg, R.G., Feron, R.M.C., van der Weerden, G.M., & Mariani, C. (2008). Genetic diversity of the African hexaploid species of *Solanum scabrum* Mill. and *Solanum nigrum* L. (Solanaceae). *Genetic Resources and Crop Evolution*, *55*, 409–418. doi:10.1007/s10722-007-9248-z

Manoko, M.L.K., van der Weerden, G.M., van den Berg, R.G., & Mariani, C. (2012). A new tetraploid species of *Solanum* L. section *Solanum* (Solanaceae) from Tanzania. *PhytoKeys*, *16*, 65–74. doi:10.3897/phytokeys.16.2884

Masinde, P.W., Wesonga, J.M., Ojiewo, C.O., Agong, S.G., & Masuda, M. (2009). Plant growth and leaf N content of *Solanum villosum* genotypes in response to nitrogen supply. *Dynamic Soil Dynamic Plant*, *3*, 36–47.

Mohyuddin, A., Khan, Z., Ahmad, M., & Kashmir, M.A. (2010). Chemotaxonomic value of alkaloid in *Solanum nigrum* complex. *Pakistan Journal of Botany*, *42*, 653–660.

Ojiewo, C.O., Agong, S.G., Murakami, K., & Masuda, M. (2006). Chromosome duplication and ploidy level determination in African nightshade (*Solanum nigrum* L. ssp. *villosum* (L.) Ehrh). *The Journal of Horticultural Science and Biotechnology*, *81*, 183–188. doi:10.1080/14620316.2006.11512048

Ojiewo, C.O., Kubo, Y., Murakami, K., & Masuda, M. (2010). Comparative analysis of differential gene expression in wild-type and 12C5+ ion beam-induced abnormal flower mutant of *Solanum villosum* by tomato cDNA macro array. *International Journal of Plant Developmental Biology*, *4*, 1–7.

Ojiewo, C.O., Mbwambo, O., Swai, I., Samali, S., Tilya, M. S., Mnzava, R.N., … Oluoch, M. (2013). Selection, evaluation and release of varieties from genetically diverse African nightshade germplasm. *International Journal of Plant Breeding*, *2*, 76–89.

Ojiewo, C.O., Murakami, K., & Masuda, M. (2007). A novel homeotic mutant with season-dependent floral organ and fertility restoration induced by 12C5+ ion beam irradiation of *Solanum villosum* seed. *Acta Horticulturae*, *760*, 311–317. doi:10.17660/ActaHortic.2007.760.42

Ojiewo, C.O., Murakami, K., Masuda, M., & Agong, S.G. (2009). Induced male-sterility alters dry matter distribution in African nightshade. *Acta Horticulturae*, *806*, 571–578. doi:10.17660/ActaHortic.2009.806.71

Ojiewo, C.O., Mwai, G.N., Abukutsa, M.O., Agong, S.C., & Nono-Wandim, R. (2013). Exploiting the genetic diversity of vegetable African nightshades. *Bioremediation, Biodiversity and Bioavailability*, *7*, 6–13.

Olet, E.A., Heun, M., & Lye, K.A. (2005). African crop or poisonous nightshade; the enigma of poisonous or edible black nightshade solved. *African Journal of Ecology*, *43*, 158–161. doi:10.1111/aje.2005.43.issue-2

Olet, E.A., Lye, K.A., & Heun, M. (2011). Amplified fragment length polymorphisms (AFLPs) analysis of species of *Solanum* section *Solanum* (Solanaceae) from Uganda. *African Journal of Biotechnology*, *10*, 6387–6395.

Olet, E.A., Lye, K.A., & Stedje, B. (2015). Crossing relationships and chromosome numbers of *Solanum* section *Solanum* in Uganda. *Nordic Journal of Botany*, *33*, 472–483. doi:10.1111/njb.2015.v33.i4

Onyango, C.M., Kunyanga, C.N., Ontita, E.G., Narla, R.D., & Kimenju, J.W. (2013). Current status on production and utilization of spider plant (*Cleome gynandra* L.) an underutilized leafy vegetable in Kenya. *Genetic Resources and Crop Evolution*, *60*, 2183–2189. doi:10.1007/s10722-013-0036-7

Poczai, P., & Hyvönen, J. (2011). On the origin of *Solanum nigrum*: Can networks help? *Molecular Biology Reports*, *38*, 1171–1185. doi:10.1007/s11033-010-0215-y

Poczai, P., Mátyás, K., Taller, J., & Szabó, I. (2010). Study of the origin of the rarely cultivated edible *Solanum* species: Morphological and molecular data. *Biologia Plantarum*, *54*, 543–546. doi:10.1007/s10535-010-0096-x

Potawale, S.E., Sinha, S.D., Shroff, K.K., Dhalawat, H.J., Boraste, S.S., Gandhi, S.P., & Tondare, A.D. (2008). *Solanum nigrum* L.: A phytopharmacological review. *Pharmacologyonline*, *3*, 140–163.

Sammani, A., Shammaa, E., & Chehna, F. (2013). Qualitative and quantitative steroidal alkaloids of *Solanum* species distributed widely in Syria by TLC and HPLC. *International Journal of Pharmaceutical Sciences Review and Research*, *23*, 23–27.

Smith, G.C., Dueker, S.R., Clifford, A.J., & Grivetti, L.E. (1996). Carotenoid values of selected plant foods common to southern Burkina Faso, West Africa. *Ecology of Food and Nutrition*, *35*, 43–58. doi:10.1080/03670244.1996.9991474

Stadlmayr, B., Charrondiere, U.R., Enujiugha, V.N., Bayili, R.G., Fagbohoun, E.G., Samb, B., … Burlingame, B. (2012). *West African food composition table*. Rome: FAO. Retrieved March 21, 2017, from http://www.fao.org/docrep/015/i2698b/i2698b00.pdf

Van Biljon, A., Labuschagne, M., & Koen, E. (2010). Microsatellite-based assessment of five *Solanum nigrum* complex species and their progeny. *Soil and Plant Science*, *1*, 1–6.

Weinberger, K., & Msuya, J. (2004). *Indigenous vegetables in Tanzania—Significance and prospects* (Technical Bulletin No. 31, AVRDC Publication 04-600, 70 pp). Shanhua: AVRDC—The World Vegetable Center.

Weinberger, K., Pasquini, M., Kasambulu, P., & Abukutsa-Onyango, M. (2011). Supply chains for indigenous vegetables in urban and peri-urban areas of Uganda and Kenya. In D. Mithöfer & H. Waibel (Eds.), *A gendered perspective in vegetable production and marketing in Africa* (p. 288). Nairobi: CABI.

Xiang, N. (2000). The AFLP technique and its application for plant study. *Plant Physiology Communication*, *36*, 236–240.

Yang, R.-Y., & Keding, G.B. (2009). Nutritional contributions of important African indigenous vegetables. Ch. 4. In C.M. Shackleton, M.W. Pasquini, & A.W. Drescher (Eds.), *African indigenous vegetables in urban agriculture*. London: Earthscan.

Zhao, N., Wang, G., Norris, A., Chen, X., & Chen, F. (2013). Studying plant secondary metabolism in the age of genomics. *Critical Reviews in Plant Sciences*, *32*, 369–382. doi:10.1080/07352689.2013.789648

2.2 Development of Next Generation Sequencing (NGS) based SSRs in African nightshades; Tools for Analysis of Genetic Diversity in Conservation and Breeding

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Development of next-generation sequencing (NGS)-based SSRs in African nightshades: Tools for analyzing genetic diversity for conservation and breeding

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ABSTRACT

African nightshade is an indigenous leafy vegetable in Eastern and Western Africa that is valued for its high nutrient content and potential to generate income. Efforts to improve this crop have been hampered by limited information available on intra- and interspecific genetic diversity. Here, we present data on the development and application of polymorphic simple sequence repeat (SSR) markers in a broad collection of entries (gene bank accessions, landraces from farmers, breeding lines and commercial cultivars) from two species of African nightshades, *Solanum scabrum* (Mill.) and *S. villosum* (Mill.). Next generation sequencing information was used to develop 16 polymorphic SSR markers that revealed genetic diversity within and between the 54 entries collected from various geographic locations in Sub-Saharan Africa. High levels of polymorphism were detected within entries, indicating gene flow between entries and/or a low intensity of selection during development of breeding lines and improved cultivars. As a consequence, they have retained sufficient variation for further selection within the improved germplasm. The two species were clearly distinct, and *S. scabrum* was found to be less diverse compared to *S. villosum*. Furthermore, some entries were identified as probably misclassified based on morphological markers as they can clearly be placed within the gene pool of either *S. scabrum* or *S. villosum* using the SSR data. In addition to the diversity studies, we determined the genome size and ploidy of a selection of 29 entries helpful for the design of future NGS-based marker projects. All entries of *S. scabrum* were found to be hexaploid, whereas entries of *S. villosum* were tetraploid with a haploid genome size of all samples slightly varying by approximately 1 pg. Finally, we also determined pollen viability parameters, which differed significantly between the two species but did not correlate to any of the other parameters measured.

1. Introduction

African nightshade is an important indigenous leafy vegetable in East and West Africa that is valued for its high nutritional value and its potential for generating income, especially for small scale farmers (Weinberger et al., 2011). In addition, the berries are used as source of dye, and some communities use various organs such as the roots as herbal medicine (Aguilar-Jiménez and Grusak, 2015; Neugart et al., 2017). Currently, there are approximately 30 species described for the so-called *Solanum nigrum* (L.) complex belonging to the section *Solanum*, but the most popularly used as vegetables are *S. scabrum* (Mill.), *S. villosum* (Mill.), *S. americanum* (Mill.) and *S. retroflexum* (Dunn.) (Ojiewo et al., 2013a). Until recently, little genomic research has been done on the section *Solanum* because it had been considered of little economic importance compared to other vegetables such as *Brassica* spp., which have been studied and marketed intensively by national agricultural research institutions and extension services (Fufa et al., 2011).

The section *Solanum* is known for its taxonomic complexity, and although a number of studies using molecular markers have provided data on the relationships among *Solanum* species (Olet et al., 2005; Poczai et al., 2008; Poczai et al., 2011; Poczai and Hyvönen, 2011; Poczai et al., 2014), very little information about the genetic diversity within species or within accessions is available. Robust locus-specific

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markers, SSR markers for example, for diversity studies or even marker-assisted selection in African nightshade are lacking. However, crop improvement can be achieved faster and more efficiently through knowledge of the genetic diversity that exists in the crop for selection of elite genotypes by combining genetically diverse germplasm in breeding programs and for systematic conservation of germplasm to avoid genetic erosion in genepools (Fu, 2015). SSRs have been used as the markers of choice in many other crops, as well as the *Solanaceae* family member, potato (Salmi et al., 2016), to construct genetic linkage maps (Das et al., 2012), diversity studies (Xiaoyan and Sanwen, 2016), QTLs and association analysis (Daware et al., 2016; Zhang et al., 2012). Currently, no published primer sequences are available for SSR markers for African nightshades. Only one study by van Biljon et al. (2010) used SSR markers developed for pepper, potato, sorghum and tomato, but the data were not sufficient to analyze the population structure of African nightshades. Other diversity studies on African nightshade have utilized dominant markers such as AFLPs (Dehmer and Hammer, 2004; Jacoby et al., 2003; Manoko et al., 2007; Manoko et al., 2008; Olet et al., 2011) and RAPDs (Poczai et al., 2010; Volis et al., 2009). However, transferring information about these dominant markers among different labs and inferring their allelic composition, which is an important factor in marker-assisted breeding, is not possible (Shan et al., 1999).

SSR markers are one of the most informative genome-based markers, but their development was labor- and cost-intensive before the advent of NGS technologies (Vieira et al., 2016). At present, next generation sequencing platforms such as Illumina sequencing technology are efficient tools for identifying many SSRs at a lower cost (Zapata et al., 2012) compared to first generation Sanger methods (Ekblom and Galindo, 2011; Shendure and Ji, 2008). Mining NGS data for SSR development has been used in a variety of crops such as pigeon peas, millet and safflower (Ambreen et al., 2015; Gimonde et al., 2016; Yang et al., 2015). SSRs have the advantages of being highly polymorphic and reproducible DNA markers with co-dominant inheritance and abundance in the genome, thus allowing for the detection of allelic diversity (Vieira et al., 2016).

In addition to developing new SSR markers for African nightshade, this study also assessed the germination percentage of pollen and the nuclear DNA content of a subset of 29 entries from WorldVeg. Because the DNA content is correlated with the ploidy level, it is important for determining phylogenetic relationships and the verification of taxonomic classifications (Dirihan et al., 2013). African nightshades are known to exhibit a ploidy series wherein diploids (2n = 2× = 24), tetraploids (2n = 4× = 48) and hexaploids (2n = 6× = 72) are found (Edmonds and Chweya, 1997). Pollen viability is one of the major measures of pollen quality, is important for incompatibility and fertility studies (Dafni and Firmage, 2000; Saha and Datta, 2014) and is a good indicator of successful reproduction and adaptation abilities of plants that may be used in conservation programs (Moza and Bhatnayar, 2007; Khan and Perveen, 2008; Pogorzelec et al., 2014). The newly established SSR markers could serve as an important future resource for population genetics, linkage-map construction, cultivar fingerprinting and selection for crop improvement. To achieve these, knowledge of germplasm diversity is a prerequisite. The aim of this study was therefore to assess the level of polymorphism within and between 54 entries of African nightshades using the newly developed SSR markers and to examine the variation in pollen viability and DNA content.

2. Materials and methods

2.1. Plant materials

The germplasm used in the SSR markers analysis comprise developed lines, cultivars and landraces of *S. scabrum* and *S. villosum*. A total of 54 entries (gene bank accessions, landraces from farmers, breeding lines and commercial cultivars) were used for the study (Table A1 Supplementary Material). From the total entries, nine landraces (farmers' cultivars) were obtained from farmers, and 16 cultivars from commercial outlets in Kenya. In addition, three cultivars were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Nairobi, Kenya. The rest of the entries, including 24 breeding lines, 3 advanced lines and 2 cultivars, were obtained from the World Vegetable Center (WorldVeg) in Arusha, Tanzania. The 29 entries obtained from WorldVeg (Table A1 Supplementary Material) were additionally characterized for their DNA content and pollen viability.

2.2. DNA isolation

Six genotypes from each of the 54 entries were used for SSR marker analysis. For DNA isolation, young leaves were harvested and dried over silica gel at 37 °C overnight. DNA was isolated from 20 mg of dried leaf tissue. Samples were placed in 2 ml reaction tubes with two steel beads and homogenized using a Retsch™ MM 400 Mixer Mill (Retsch, Haan, Germany). DNA isolation was done using the Macherey-Nagel NucleoSpin® plant II kit (Macherey-Nagel GmbH Düren Germany) following the manufacturer's protocol using lysis buffer PL1. The DNA was eluted from the columns two times using 40 µl PE buffer. The concentration of genomic DNA was assessed spectrophotometrically using Nano-drop spectrophotometer 2000 c (PeqLab Biotechnology Co. ltd Erlangen, Germany) at 260 nm and was evaluated for purity by determining the OD 260 nm/280 nm and the OD 260 nm/230 nm ratios. The DNA quality was further assessed by agarose gel electrophoresis.

2.3. Development of SSR primers

Primers were developed from sequences obtained from genomic DNA of *S. scabrum* cultivar 'Olevolosi' sequenced by the GATC Biotech AG (Konstanz, Germany) using an Illumina HiSeq2500. The raw reads have been uploaded on the NCBI sequence read archive at http://www.ncbi.nlm.nih.gov under SRP132031and registered at BioProject database with ID PRJNA432637 and at the BioSample database as accession SAMN08456262. The generated sequence reads were uploaded onto CLC-bio "Genomics Workbench" 7.5.1 and analyzed using the *de novo* assembly algorithm with the default settings. The assembled contigs in FASTA format were analyzed with the SSRLocator software (da Maia et al., 2008) for motif detection. The sequences were searched for di-, tri-, tetra-, penta-, and hexanucleotide SSR motifs. The minimum number of repeats for identifying SSRs for di- nucleotide was 10 repeats, 7 repeats for trinucleotides, 5 repeats for tetra-, and 4 repeats for penta- and hexanucleotides. The primers were designed using the Primer3 software (Untergasser et al., 2012) implemented in SSRLocator. The default parameters were used for the design of all primers: an amplicon size 100–280 bp, optimum annealing temperature of 60 °C, primer optimal length of 20 bp and 50% GC content. For detection on an automated sequencer, the forward primer of each primer pair was connected to an M13- tail at the 5′ end with the sequence 5′-GTAAAA CGACGACGGCCAGT-3′ (Schuelke, 2000).

2.4. PCR conditions

Initially, primers were selected at random, and those showing clear bands were selected for further analyses. Amplification was done following the method adopted from Schuelke (2000). PCR was carried out in a total volume of 10 µl, consisting of 20 ng genomic DNA, 0.025 µM forward primer and reverse primer, 0.125 µM M13 primer end-labeled with the IRD 700 dye (Eurofins MWG, Ebersberg, Germany), 0.5 U DCS Taq polymerase, 0.15 mM dNTPs and Williams buffer (200 mM Tris-HCL (pH 8.4), 15 mM $MgCl_2$, 500 mM KCL). The following PCR profile was used: 5 min at 94 °C; 45 s at 94 °C, 58–60 °C depending on the primer pair for 1 min, 20 cycles; 72 °C for 1 min. A further 7 cycles were performed with the following conditions; 94 °C for 30 s, 52 °C for 45 s and 72 °C for 1 min, followed by a final incubation at 72 °C for 10 min.

The PCR products were separated on 6% acrylamide gels running at 30 W on an LI-COR 4300 sequencer (Li-Cor Biotechnology Co. Ltd, Hamburg Germany), and detection was performed at 700 nm. All alleles were manually scored for each SSR marker to obtain a data matrix.

2.5. SSR data analysis

The obtained binary data from six individuals per entry were used to generate tab-delimited text files as input data. Allele frequencies were used to calculate the number of alleles and PIC with Molkin v3.0 (Gutiérrez et al., 2005), and the observed and expected heterozygosity were calculated using the GenAlex software (Peakall and Smouse, 2012) to validate the discriminatory power of SSR markers. Multivariate analysis using principal coordinates analysis was performed to visualize genetic patterns among the 54 entries using genetic distance as input data. The analysis was carried out in GenAlex software (Peakall and Smouse, 2012). The population structure of African nightshade entries used in the study was determined using an admixture model with allele frequency correlation based on a Monte Carlo Markov Chain (MCMC) algorithm implemented in STRUCTURE software version 2.3.4 (Pritchard et al., 2000). Prior information on sampling locations was included for all entries. The estimated proportion of each individual was calculated for K values ranging from 1 to 10, with 10 iterations for each K value. Burn-in periods of 50,000 and MCMC replications of 100,000 were used for every run. The optimum K value was calculated using structure harvester (Earl and VonHoldt, 2012), which compares the log likelihood of data [LnP(D)] and an ad hoc statistic ΔK based on the rate of change in [LnP(D)] between successive K (Evanno et al., 2005). The results of each replicate were combined using the CLUMPP software (Jakobsson and Rosenberg, 2007).

2.6. Analysis of nuclear DNA content

For the isolation of nuclei, three genotypes from each of the 29 entries obtained from WorldVeg were used. Fresh young leaf tissue of approximately 0.5 cm^2 was placed in a petri dish and chopped after adding 1 ml of ice-cold nuclei isolation buffer CysStain PI Absolute P (Partec Co. Ltd. Germany). *Solanum lycopersicum* (pg/2C DNA content 1.96) accession number LYC418 obtained from the Gatersleben genbank was used as a reference standard. The homogenate was then filtered through a 30-µm CellTrics filter (Partec) into sample tubes and 1 ml propidium iodide containing 50 µg ml^{-1} RNase was added. The preparation was then incubated on ice for 1 h in the dark with occasional shaking. The fluorescence intensity of the homogenate was measured using CyFlow ploidy-analyzer (Partec, Münster, Germany), measuring at least 2000 nuclei for each sample. Sample nuclear DNA content was calculated as follows: sample 2 C value = (sample peak mean/reference peak mean) × reference 2 C value (Dolezel et al., 2007).

2.7. Pollen viability analysis

Pollen viability was determined via in vitro germination using a solid medium adapted from Brewbaker and Kwack (1963). Only entries obtained from WorldVeg were used for this analysis. Plants were grown in the greenhouse under semi-controlled conditions. Incubation was performed in petri-dishes overnight at room temperature. The medium consisted of 100 g L^{-1} sucrose, 0.2 g L^{-1} magnesium sulfate, 0.1 g L^{-1} potassium nitrate, 0.4 g calcium nitrate, 0.2 g L^{-1} boric acid and 7.5 g L^{-1} Plant agar (Duchefa, Haarlem, The Netherlands) at pH 6. Approximately 300 pollen grains per sample were counted at ×400 magnification using a polarized light microscope (Axio vision scope, A1, Zeiss), and viability was scored as a percentage. Samples were derived from three genotypes from each entry. Pollen with pollen tubes with a length of at least the diameter of the pollen grains was counted as viable (Dafni and Firmage, 2000).

Table 1
Descriptive statistics for the 16 SSR markers used in the study. 54 entries with six genotypes each were analysed with the markers.

Locus	Expected fragment size	Repeat motif	Na	Ho	PIC	He
Tet_Ole27092	161	(AAAT)6	3	0.92	0.48	0.02
Tet_Ole36530	150	(TTTC)8	8	0.98	0.71	0.54
Tet_Ole50895	158	(TAAA)6	5	0.30	0.56	0.23
Tet_Ole61583	232	(AAAT)6	5	0.89	0.58	0.47
Tet_Ole77901	169	(TTTC)6	3	0.04	0.37	0.08
Tet_Ole101141	174	(GTAT)6	3	0.97	0.54	0.50
Tet_Ole106079	168	(CAAA)6	3	0.98	0.47	0.51
Tet_Ole20163	140	(TTTA)6	2	0.00	0.26	0.01
Pen_Ole14507	165	(TTCTC)5	4	0.98	0.68	0.50
Pen_Ole56306	251	(TTTTA)5	6	0.74	0.62	0.40
Pen_Ole1865	258	(TAAAA)5	5	0.76	0.66	0.44
Pen_Ole69791	219	(TCAAA)5	3	0.63	0.37	0.32
Hex_Ole56218	260	(TTTCTT)4	2	0.01	0.08	0.03
Hex_Ole111446	121	(CTGAAA)4	4	0.98	0.56	0.50
Hex_Ole120086	258	(TTTTCG)4	3	0.67	0.45	0.35
Hex_Ole51860	162	(GAGTTG)4	3	0.34	0.46	0.20
Total			62			
Mean			3.9	0.62	0.54	0.35

Na- number of alleles; Ho- observed heterozygosity; PIC- polymorphic information content; He- expected heterozygosity.

3. Results

3.1. SSR marker development

A total of 6,824,250 paired sequence reads were analyzed using de novo assembly, generating 122,663 contigs with an average length of 394 bp and an N50 of 391 bp. Of these, 38,901 (33.1%) contained SSR motifs (Table A2 Supplementary Material). Mononucleotide repeats were excluded from the SSR search. The total number of bases was 1,706,237,500, and the GC content was 35%. SSR motifs with more repeats were selected for primer design. A total of 68 primer pairs were designed and tested for amplification using two *S. scabrum* and two *S. villosum* genotypes. Table A3 (Supplementary Material) shows the sequences, annealing temperature and repeat motifs of the primer pairs. Of the 68 primer pairs, 44 produced amplicons for all four genotypes. The primers for di- and trinucleotide motifs did not amplify a product. The 44 primer pairs were tested on a panel of 10 genotypes, and a total of 16 primers that showed polymorphisms were selected for further analysis (Table 1).

3.2. Descriptive statistics of SSR markers

Six individuals each from a total of 54 entries, 20 of *S. villosum*, 33 of *S. scabrum* and the diploid entry IP02, were analyzed using the 16 primer pairs described above. A total of 62 alleles were generated from the 16 SSR loci (Table 1). The average number of alleles per locus ranged from 2 to 8 alleles with a mean of 3.9 alleles. The mean PIC value for all the markers was 0.54. Eight of the 16 markers were highly informative, with PIC values ranging from 0.54 to 0.71. The remaining markers were reasonably informative (0.50 > PIC > 0.25) except for marker Hex_Ole56218, which was only slightly informative, with a PIC value of 0.08 according to Botstein et al. (1980). The mean observed heterozygosity was 0.62, with approximately 70% of the markers showing values above 0.7. The expected heterozygosity was lower than observed heterozygosity for all loci.

3.3. Population structure

A principal coordinates analysis (PCoA) divided the accessions into two main clusters corresponding to *S. villosum* and *S. scabrum* accessions (Fig. 1a and b). The first three axes explained approximately 92.1% of

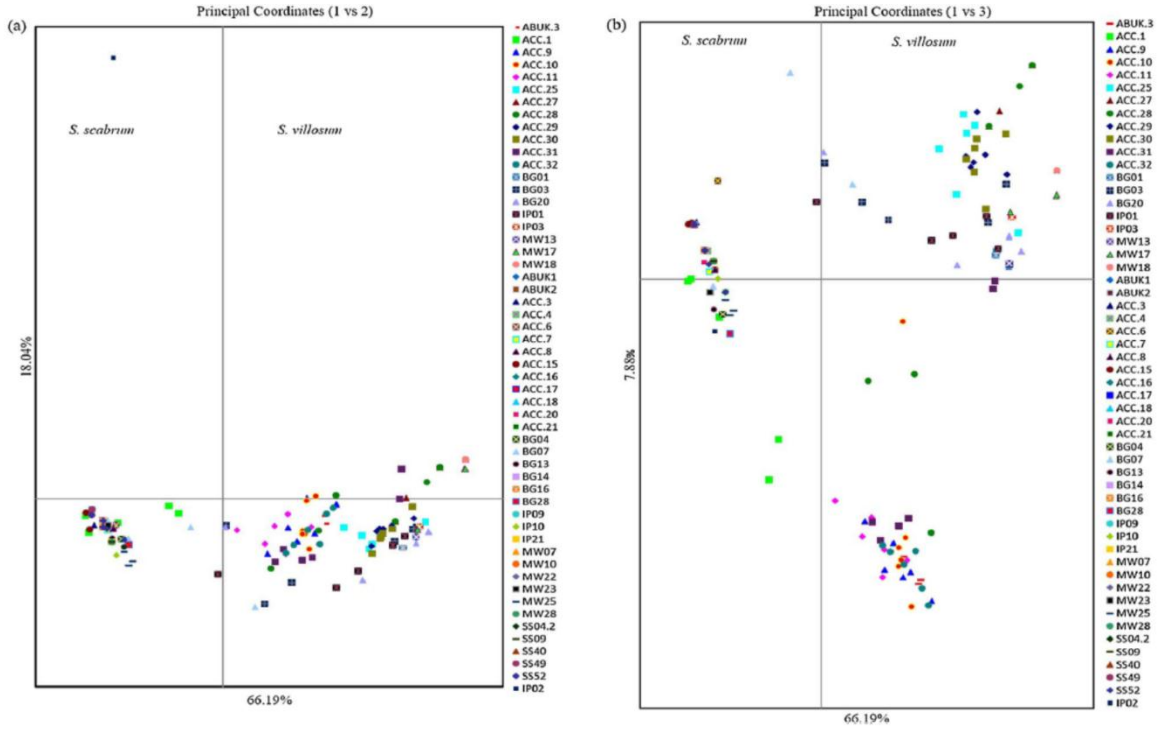

Fig. 1. The first three axes of principal co-ordinate analysis generated from the distance matrix of 324 genotypes using 16 SSR markers.

the variation. Axes 1, 2 and 3 accounted for 66.2, 18.0 and 7.9% of the total variation, respectively. Entries in *S. villosum* were characterized by higher average variability among entries compared to *S. scabrum* and were separated into two sub-clusters. In contrast, entries of *S. scabrum* were characterized by less diversity and clustered more closely together. Entry IP02 was treated as an outlier from *S. villosum* and *S. scabrum* entries. When analyzing the dataset, including all entries using Bayesian modeling in STRUCTURE, two clusters were again observed representing *S. scabrum* and *S. villosum* (Fig. 2a and b). When looking at the entries belonging to the two species separately, *S. villosum* entries were partitioned into two sub-clusters (Fig. A1, Supplementary Material). The first cluster consisted of landraces, and the second sub-cluster consisted of cultivars and breeding lines. The best partition for *S. scabrum* was also K = 2, corresponding to advanced lines and cultivars in the first cluster and breeding lines in the second cluster, as shown in Fig. A2 (Supplementary Material). Indications of admixture were more often detected in *S. villosum* clusters than in *S. scabrum*. There was, however, no partitioning according to the geographic origin of the entries.

3.4. Nuclear DNA content

Among the 29 analyzed entries, the nuclear DNA content ranged from 2.26 pg/2 C to 7.04 pg/2 C with coefficients of variance (CVs) from 2.2% to 6.5% (Table 2). Average nuclear DNA contents for *S. villosum* entries varied between 4.01 and 4.6 pg/2 C, whereas for *S. scabrum* entries, the range was between 5.28 and 7.04 pg/2 C. This result shows more pronounced differences in nuclear DNA content among the *S. scabrum* entries than among the *S. villosum* entries. Entry IP02, which was separated from the other accessions by PCoA and Bayesian analysis, had a comparably low nuclear DNA content of 2.26 pg/2 C, and this can be assumed to be a diploid. Fig. A3 (Supplementary Material) shows the histograms of the reference standard, diploid sample entry IP02, tetraploid sample BG01 and the hexaploid sample entry BG28.

3.5. Pollen viability

Pollen viability expressed as the average percentage of germinated pollen grains is shown in Table 3. Mean viability ranged between 12.25% (BG20) and 96.05% (MW22), with a mean of 66.7% for all entries The *S. villosum* entries showed a generally lower mean viability compared to *S. scabrum* entries (Fig. A4, Supplementary Material). The mean viability for *S. villosum* ranged between 12.25 and 53.4%, with a mean of 33.66%. For *S. scabrum*, viability ranged between 31.95 and 97.3% with a mean of 79.7%.

4. Discussion

Information about genetic variation and population structure is important not only for selection in advanced breeding programs but also for the development of conservation strategies (Fu, 2015). Here, we present data on 16 SSR markers representing the best performing subset of primers that were developed for African nightshades. To date, this is the most extensive study on genetic variability within and between entries of two African nightshade species, *S. scabrum* and *S. villosum*, utilized as leafy vegetables. The markers were able to discriminate entries between and within species, which is a useful aspect in variety identification and genetic assessment. The results from SSR mining showed hexanucleotide repeats to be the most abundant type in the sequenced genomic DNA from *S. scabrum* cultivar 'Olevolosi,' accounting for 74%, followed by tetranucleotides, which accounted for 10% of the repeats under our search conditions. In other crops of the *Solanaceae* family, mononucleotide repeats are the most abundant, followed by trinucleotides (Cheng et al., 2016). In pepper, for example, mononucleotides account for 43% and trinucleotides for 29% (Xiao-Min et al., 2016). This finding is consistent with studies on potato and tomato, where mononucleotide repeats accounted for 50% and 37% while trinucleotides accounted for 27% and 30%, respectively (Cheng et al., 2016). The reason for the discrepancy remains elusive as we only sequenced a comparatively small portion of the genome, and bias

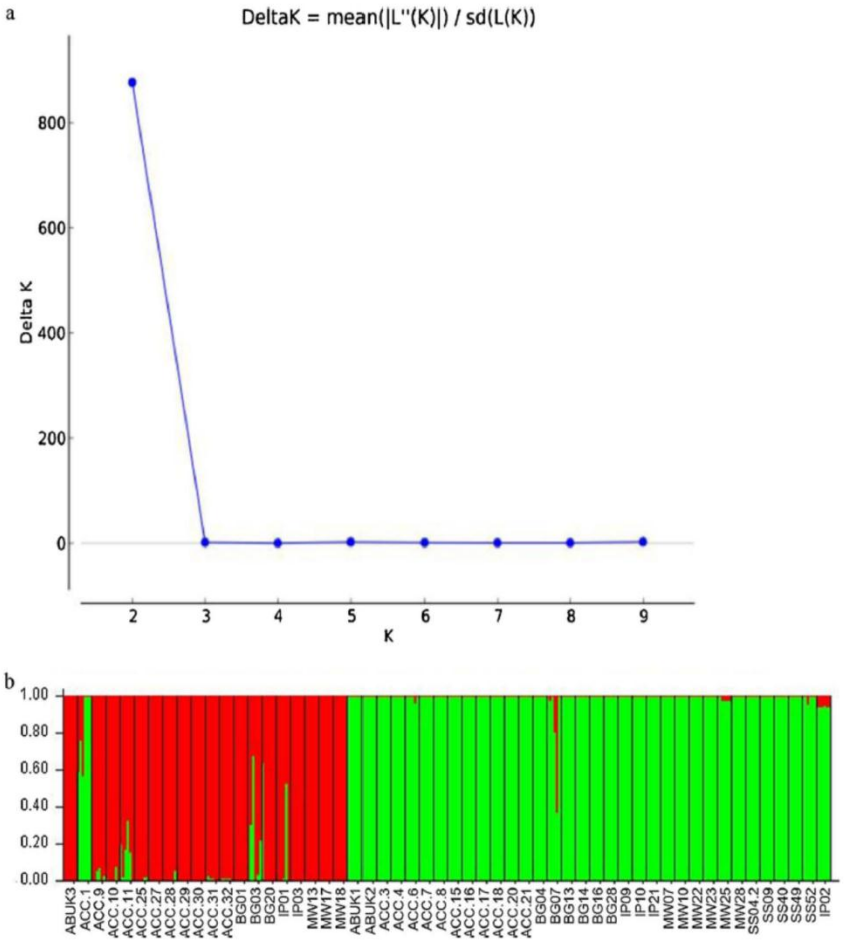

Fig. 2. (a) A plot of delta K to show optimal K for all entries. (b) Bayesian admixture analysis grouped the entries to the optimal K = 2. For the analysis 324 genotypes from 54 entries were used. Each vertical bar represents one genotype. The order of the entries is according to Fig. 1.

introduced by the sequencing procedure (library preparation) as well as through the assembly process cannot be excluded.

To describe the genetic variation in the populations, we used gene diversity according to Nei (1973), which is equal to the expected Hardy-Weinberg heterozygosity in non-random mating populations. This index can be applied to species with different reproduction systems, independent of the ploidy level and the number of alleles per locus (Hedrick, 2010; Nei, 1973). The estimated gene diversities were relatively high for most loci, ranging from 0.01 to 0.54 with a mean of 0.35. These values were higher than those reported by Poczai et al. (2014), who used nuclear intron-targeting markers, and found values ranging from 0.06 to 0.1 with a mean of 0.24. This difference can be explained by the fact that intron-targeting markers are genic markers whereas markers from Poczai et al. (2014) could only differentiate at the species level but not between accessions within one species. Furthermore, we used a much broader set of genotypes, six genotypes for each of the 54 entries, compared to the smaller number of six accessions of *S. scabrum* and nine accessions of *S. villosum* (with only one sample used for each) in the study by Poczai et al. (2014). Our SSR markers resolved genetic differences even between closely related entries within the species used in this study. Limited levels of polymorphism have also been found in improved cultivars of other crops in the *Solanaceae* family such as eggplant (*Solanum melongena* L.) (Augustinos et al., 2016), potato (Salmi et al., 2016), pepper (Cheng et al., 2016; Xiao-Min et al., 2016) and tomato (San-Yi et al., 2008). This might be because the composition of the original gene pool is affected in improved cultivars by methods applied for breeding, most especially through phenotypic selection and the small number of individuals used as parental genotypes (Munoz-Falcon et al., 2009).

The allele number as a measure of genetic diversity showed a mean of 3.9 alleles per locus over all entries. This result is most likely influenced by the SSR loci used and the composition of the plant material being analyzed. In other underutilized crops, like *Amaranthus* spp., a mean of 11.1 alleles per locus was reported using 11 loci and 33 species (Suresh et al., 2015), whereas a mean of 3 alleles per locus was reported for cowpeas in 5 loci (Menssen et al., 2017), and a mean of 5 alleles were detected using 9 SSRs in *Cleome gynandra* by Omondi et al. (2017). Allelic diversity is important for a plant population to respond to bottleneck situations such as selection pressure acting over longer time periods or a temporally drastic reduction in population size (Greenbaum et al., 2014). Heterozygosity was high, with 62% of loci being heterozygous in any given individual, showing a capacity to respond to narrowed gene diversity attributed to fixed heterozygosity found in allopolyploids such as African nightshades. All the loci under study showed higher observed heterozygosity than expected heterozygosity, suggesting some degree of gene flow (Greenbaum et al., 2014; Veron et al., 2005) between accessions during the breeding and/or maintenance process. Although African nightshades are generally self-pollinating, spontaneous hybridization may also occur, which is one of the sources of diversity in the section *Solanum* (Edmonds and Chweya. 1997). As seed multiplication, even for advanced lines, is usually done without strict separation of different accessions by distance or other methods,

Table 2
Nuclear DNA contents of 29 African nightshade entries determined by flow cytometry using *S. lycopersicum* accession LYC418 as a diploid reference standard.

Entry	Nuclear DNA content (pg/2C)	CV (%)	Proposed ploidy
S. scabrum			
BG04	7.04	2.8	6×
BG07	6.66	3.4	6×
BG13	5.96	2.2	6×
BG14	6.05	4.9	6×
BG16	6.53	5.2	6×
BG28	5.75	3.6	6×
IP09	6.15	3.8	6×
IP10	6.31	4.2	6×
IP21	5.98	4.6	6×
MW07	6.50	4.7	6×
MW10	5.29	6.2	?
MW22	5.72	2.9	6×
MW23	5.97	4.6	6×
MW25	6.58	6.2	6×
MW28	5.28	3.4	?
SS09	6.03	6.0	6×
SS40	5.73	3.8	6×
SS04,2	6.69	5.0	6×
SS52	6.14	3.1	6×
SS49	6.50	5.0	6×
Average ± SD	6.14 ± 0.46	4.3 ± 1.2	
S. villosum			
BG01	4.24	4.2	4×
BG03	4.01	2.4	4×
BG20	4.25	5.0	4×
IP01	4.51	3.3	4×
IP03	4.60	6.4	4×
MW13	4.81	6.5	4×
MW17	4.17	2.6	4×
MW18	4.38	5.1	4×
Average ± SD	4.37 ± 0.26	4.4 ± 1.6	
IP02	2.26	3.6	2×

Table 3
Mean percentage pollen viability of African nightshade entries germinated in vitro.

Entry	Mean ± SD	Entry	Mean ± SD
S. scabrum		*S. villosum*	
BG04	36.65 ± 8.95	BG01	23.45 ± 0.95
BG07	85.75 ± 0.95	BG03	16.55 ± 0.35
BG13	75.20 ± 1.90	BG20	12.25 ± 0.05
BG14	90.25 ± 1.35	IP01	51.40 ± 6.50
BG16	77.80 ± 1.40	IP03	46.15 ± 1.35
BG28	75.65 ± 1.15	MW13	53.40 ± 8.80
IP09	94.40 ± 2.00	MW17	34.45 ± 0.35
IP10	92.10 ± 2.00	MW18	31.65 ± 0.95
IP21	95.70 ± 1.50		
MW07	69.75 ± 2.55	Mean ± SE	33.66 ± 5.57
MW10	31.95 ± 6.65		
MW22	96.05 ± 1.25	IP02	73.30 ± 6.50
MW23	65.75 ± 3.35		
MW25	95.15 ± 1.25		
MW28	95.35 ± 3.05		
SS04,2	95.25 ± 1.05		
SS09	82.60 ± 0.90		
SS40	91.10 ± 1.30		
SS49	88.00 ± 1.30		
SS52	58.85 ± 3.45		
Mean ± SE	79.66 ± 4.20		

cross pollination between lines may occur from time to time. Furthermore, mechanical mixture is possible, especially on farmers' fields, during seed processing. Additionally, the selection methods used during cultivar development could play a role, as breeding lines and improved cultivars used in the current study were developed through mass selection.

Bayesian modeling with STRUCTURE was used to assess the population structure present in the collection without any a priory classification of taxonomy but with prior grouping according to sampling location based on correlated allele frequencies. The analysis showed optimal clusters at K = 2, indicating each entry nicely fits into either the cluster representing *S. scabrum* or *S. villosum*. This led us to propose a different classification of nine entries in this manuscript which had previously been treated as different species. The entries IP01 and IP03 had been classified as *S. nigrum*, MW13 as *S. sararrachoides* and MW18 as only *S.* spp. According to our results, they all should be treated as *S. villosum*. The entry MW07 had been classified as *S. eldoretianum*, MW10 as *S. retroflexum*, IP09 and IP10 as *S. villosum*. We propose to group them under *S. scabrum* as these entries formed a homogenous group in the STRUCTURE analysis. The entry *S. eldoretianum* was also proposed to be classified as *S. scabrum* by Manoko et al. (2008). This was corroborated by the PCoA analysis and further supported by DNA content measurements, which are consistent with the ploidy level expected for the clade to which the genotypes are now regrouped. However, for a final conclusion on potential misclassification it might be useful to re-analyze some of the entries together with clearly taxonomically described genotypes of *S. nigrum*, *S. saccharoides*, *S. eldoretianum* and *S. retroflexum* to rule out other possibilities, for example hybridity, as the cause of the close association to *S. scabrum*. Among the *S. scabrum* entries, two distinct sub-populations were identified showing very limited admixture. Admixture was more apparent among the *S. villosum* entries, which were also partitioned into two sub-populations. The variability was generally higher in *S. villosum* compared to *S. scabrum*, but, apart from a possibly lower intensity of selection in *S. villosum*, reasons for this finding remain obscure.

Nuclear DNA content is a cytological tool that is very useful for systematic and evolutionary considerations. Even though DNA content is an important aspect in genomic research in plants, and most especially in cultivated crops, there is no extensive DNA content assessment for African nightshades available. Our genome size data are the first for the two species under study. DNA content estimates are correlated to the ploidy of a cell, and data from this study support the existence of ploidy series in African nightshades (Edmonds and Chweya, 1997) and that *S. villosum* is tetraploid whereas *S. scabrum* is hexaploid. However, the question of whether they are allopolyploids or autopolyploids remains unclear, although some studies have argued that they are probably allopolyploids since they show polysomy in metaphase cells (Ojiewo et al., 2013b). Here, SSR markers might be useful in future studies in that recombination between alleles might be used to analyze the type of recombination in sexual progeny from crosses between divergent genotypes.

The mean genome sizes in Table 2 show intraspecific variations of approximately 1.3 pg between the highest and lowest 2 C values for the 20 *S. scabrum* accessions with an overall mean of 6.1 pg and a 0.6 pg difference in the eight *S. villosum* accessions with a mean of 4.4 pg. Because there are no other data published for African nightshades, the DNA contents obtained in this study could not be compared to previous data except for *S. nigrum*, which is reported to have a 1 C value of 3.1 pg (as listed in the "Plant DNA C-values database," www.kew.org/cvalues/), corresponding to a 2 C value of 6.2 pg and therefore matching well our estimates of the genome size of *S. scabrum*, to which it is closely related. The 2 C value of the diploid reference accession of 2.3 corresponds to a haploid C value of 1 and fits well with tetraploidy in *S. villosum*, with an average 2 C value of 3.96 and hexaploidy of *S. scabrum*, with an average 2 C value of 5.9. Our data point to a haploid genome size approximately 1 pg, which corresponds roughly to 980 Mb of genomic DNA, a number seen for many species of the genus *Solanum* (www.kew.org/cvalues/). This is an important finding for the future development of markers based on next generation sequencing, for example, by GBS or RAD, as the coverage obtained with these technologies is highly dependent on the genome size of the genotypes under study. All histogram profiles produced single 2C peaks, suggesting

absence of endopolyploidy in African nightshade genomes.

The amount and quality of pollen is an important component of the reproductive fitness of a plant (Saha and Data, 2014). The quality of pollen is measured by the amount of viable pollen. Pollen viability tests are useful in identifying favorable genotypes that can be used as source of pollen in breeding initiatives (Sutyemez, 2011). Analysis of in vitro germination of pollen is a popular method for assessing viability in breeding programs to ensure higher success rates of crosses (Tuinstra and Wedel, 2000). This method may also be used to determine pollen vigor by assessing the rate of germination and/or the length of pollen tubes (Sulusoglu and Cavasoglu, 2014). Information about pollen quality and DNA content of African nightshade is important in utilization of its genetic resources in conservation and crop improvement (Olet et al., 2015).

There were no significant differences between the entries in pollen viability, but we detected a significant difference at $P < 0.05$ between the species *S. scabrum* and *S. villosum*. Most of the *S. scabrum* entries (70%) had higher than 80% pollen viability, whereas the highest viability for *S. villosum* was 66%. This variation, however, may not be conclusive, because only one type of germination medium was used. Pollen viability may be influenced by external factors such as temperature, moisture, genotypic differences, plant vigor and physiological stage (Lyra et al., 2011). In our study however, the observed differences are most likely attributed to genotypic differences, because the pollen were sampled at the same physiological stage and germinated under similar conditions. Pollen samples that showed low viability showed poor tube growth, which in the end may affect fertilization, although all values measured here are relatively high and effects may only be seen under particular environmental conditions. It has been argued that there is a linear correlation between pollen viability and fruit set (Sutyemez, 2011). However, vitality stains as well as germination tests do not exactly predict the germination of pollen on floral stigmas and are therefore only rough estimates of pollen fertility (Sulusoglu and Cavusoglu, 2014).

In conclusion, we provide information on SSR markers that is useful for the study of genetic diversity in *Solanum scabrum* and *S. villosum* and show that although selection has reduced genetic diversity, significant variability is present, mainly within entries, that might support the further selection of advanced lines. Furthermore, our genome size data support tetraploidy in *S. villosum* and hexaploidy in *S. scabrum*, which may help to develop NGS-based marker systems in the future. Finally, we show that there is higher average pollen viability in *S. scabrum* compared to *S. villosum*, but there were no significant differences among entries. These data may serve as a valuable resource for germplasm conservation in African nightshades as well as tools in more sophisticated breeding strategies for developing superior germplasm in the future.

Competing interests statement

No potential conflicts of interest were reported by the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2018.03.003.

References

Aguilar-Jiménez, G.M., Grusak, D.M., 2015. Evaluation of minerals, phytochemical compounds and antioxidant activity of Mexican, Central American and African green leafy vegetables. Plant Foods Hum. Nutr. 70, 357. http://dx.doi.org/10.1007/s11130-015-0512-7.

Ambreen, H., Kumar, S., Variath, M.T., Joshi, G., Bali, S., Agarwal, M., Kumar, A., Jaganaath, A., Goel, S., 2015. Development of genomic microsatellite markers in *Carthmanus tinctorius* L. (Safflower) using next generation sequencing and assessment of their cross-species transferability and utility for diversity analysis. PLoS One 10, 1–22.

Augustinos, A.A., Petropoulos, C., Karasoulou, V., Bletsos, F., Papasotiropoulos, V., 2016. Assessing diversity among traditional Greek and foreign eggplant cultivars using molecular markers and morphometrical descriptors. Span. J. Agric. Res. 14, e0710 13 pages.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32, 314–331.

Brewbaker, J.L., Kwack, B.H., 1963. The essential role of calcium ion in pollen germination and tube growth. Am. J. Bot. 50, 859–865.

Cheng, J., Zhao, Z., Li, B., Qin, C., Wu, Z., Trejo-Saavedra, D.L., Luo, X., Cui, J., Rivera-Bustamante, R.F., Hu, K., 2016. A comprehensive characterization of simple sequence repeats in pepper genomes provides valuable resources for marker development in *Capsicum*. Sci. Rep. 6, 18919. http://dx.doi.org/10.1038/srep18919.

Dafni, A., Firmage, D., 2000. Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Syst. Evol. 222, 113–132.

da Maia, L.C., Palmieri, D.A., de Souza, V.Q., Mauricio Marini Kopp, M.M., de Carvalho, F.I.F., de Oliveira, A.C., 2008. SSR locator: tool for simple sequence repeat discovery integrated with primer design and PCR simulation. Int. J. Plant Genom. 2008, 9. http://dx.doi.org/10.1155/2008/412696. Article ID 412696.

Das, M., Banerjee, S., Dhariwal, R., Topdav, N., Kundu, A., Khurana, J.P., Tyagi, A.K., Sarkar, D., Sinha, M.K., Balya, H.S., Gupta, P.K., 2012. Development of SSR markers and construction of a linkage map in Jute. J. Genet. 91, 21–31.

Daware, A., Das, S., Srivastava, R., Badoni, S., Singh, Ak., Agarwal, P., Parida, S.K., Tyagi, A.K., 2016. An efficient strategy combining SSR markers- and advanced QTL-seq-driven QTL mapping unravels candidate genes regulating grain weight in rice. Front. Plant Sci. 7, 1535. http://dx.doi.org/10.3389/fpls.2016.01535.

Dehmer, K.J., Hammer, K., 2004. Taxonomic status and geographic provenance of germplasm accessions in the *Solanum nigrum* L. complex: AFLP data. Genet. Resour. Crop Evol. 51, 551–558.

Dirihan, S., Terho, P., Helander, M., Saikkonen, K., 2013. Efficient analysis of ploidy levels in plant evolutionary ecology. Caryologia 66, 251–256.

Dolezel, J., Greilhuber, J., Suda, J., 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nat. Protoc. 2, 2233–2244.

Earl, D.A., VonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing Evanno method. Conserv. Genet. Resour. 4, 359–361.

Edmonds, J.M., Chweya, J.A., 1997. Black nightshades. *Solanum nigrum* L. and related species. Promoting the conservation and use of underutilized and neglected crops. Rome: Int. Plant Genet. Resour. Inst. (IPIGRI) 1–115.

Ekblom, R., Galindo, J., 2011. Application of next generation sequencing in molecular ecology of non-model organisms. Heredity 107, 1–15.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Fu, Y., 2015. Understanding crop genetic diversity under modern plant breeding. Theor. Appl. Genet. 128, 2131–2142.

Fufa, F., Henson, P., Dagnoko, S., Dhaliwal, M., 2011. AVRDC. The world vegetable center tomato breeding in Sub-Saharan Africa: lessons from the past, present work and future prospects. Acta Hortic. 911, 87–98.

Gimonde, D., Odeny, D., Villiers, E., Wanyonyi, S., Dida, M., Mneney, E.E., Muchugi, A., Machuka, J., de Villiers, S., 2016. Identification of SNP and SSR markers in finger millet using next generation sequencing technologies. PLoS One 11, 1–23.

Greenbaum, G., Templeton, A.R., Zarmi, Y., Bar-David, S., 2014. Allelic richness following population founding events-a stochastic modeling framework incorporating gene flow and genetic drift. PLoS One 912, e115203. http://dx.doi.org/10.1371/journal.pone.0115203.

Gutiérrez, J.P., Royo, L.J., Álvarez, I., Goyache, F., 2005. MolKin v2.0: a computer program for genetic analysis of populations using molecular co-ancestry information. J. Hered. 96, 718–721.

Hedrick, P.W., 2010. Genetics of Populations, 4th ed. Jones and Bartlett Publishers International, Sudbury, USA, pp. 99.

Jacoby, A., Labuschagne, M.T., Viljoen, C.D., 2003. Genetic relationships between southern African *Solanum retroflexum* Dunal and other related species measured by morphological and DNA markers. Euphytica 132, 109–113.

Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP. A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806.

Khan, S.A., Perveen, A., 2008. Germination capacity of stored pollen of *Morus alba* (*Moraceae*) and their maintenance. Pak. J. Bot. 40, 1823–1826.

Lyra, D.H., Sampaio, L.S., Pereira, D.A., Silva, A.P., Amaral, C.F., 2011. Pollen viability and germination in *Jathropha ribifolia* and *Jathropha mollissima* (*Euphorbiaceae*): species with potential for biofuel production. Afr. J. Biotechnol. 10, 368–374.

Manoko, M.L.K., van den Berg, R.G., Feron, R.M.C., van der Weerden, G.M., Mariani, C., 2007. AFLP markers support separation of *Solanum nodiflorum* from *Solanum americanum* sensu stricto (*Solanaceae*). Plant Syst. Evol. 267, 1–11.

Manoko, M.L.K., van den Berg, R.G., Feron, R.M.C., van der Weerden, G.M., Mariani, C., 2008. Genetic diversity of the African hexaploid species *Solanum scabrum* Mill. and Solanum *nigrum* L. (*Solanaceae*.). Genet. Resour. Crop Evol. 55, 409–418.

Menssen, M., Linde, M., Omondi, E., Onyango, M.A., Dinssa, F.F., Winkelmann, T., 2017. Genetic and morphological diversity of Cowpea (*Vigna unguiculata* (L.) Walp.) entries from East Africa. Sci. Hortic 226, 268–276.

Moza, M.K., Bhatnayar, A.K., 2007. Plant reproductive biology studies crucial for conservation. Curr. Sci. 92, 243–244.

Munoz-Falcon, J.E., Prohens, J., Vilanova, S., Nuez, F., 2009. Diversity in commercial varieties and land races of black eggplants and implications for broadening the breeders genepool. Anal. Appl. Biol. 154, 453–465.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. 70, 3321–3323.

Neugart, S., Baldermann, S., Ngwene, B., Wesonga, J., Schreiner, M., 2017. Indigenous leafy vegetables of Eastern Africa- a source of extraordinary secondary plant metabolites. Food Res. Int. http://dx.doi.org/10.1016/j.foodres.2017.02.014.

Ojiewo, C.O., Mbwambo, O., Swai, J., Samali, S., Tilya, M.S., Mnzara, R.N., Mrosso, L., Minja, R., Oluoch, M., 2013a. Selection, evaluation and release of varieties from genetically diverse African nightshade germplasm. Int. J. Plant Breed. 7, 76–89.

Ojiewo, C.O., Mwai, G.N., Abukutsa, M.O., Agong, S.C., Nono-Wandim, R., 2013b. Exploiting the genetic diversity of vegetable African nightshades. Biorem. Biodevers. Bioavailab. 7, 6–13.

Olet, E.A., Heun, M., Lye, K.A., 2005. African crop or poisonous nightshade; the enigma of poisonous or edible black nightshade solved. Afr. J. Ecol. 43, 158–161.

Olet, E.A., Lye, K.A., Heun, M., 2011. Amplified fragment length polymorphisms (AFLPs) analysis of species of *Solanum* section *Solanum* (*Solanaceae*) from Uganda. Afr. J. Biotechnol. 10, 6387–6395.

Olet, E.A., Lye, K.A., Stedge, B., 2015. Crossing relationships and chromosome numbers of *Solanum* section *Solanum* in Uganda. Nord. J. Bot. 33, 472–483.

Omondi, E.O., Debener, T., Linde, M., Abukutsa-Onyango, M., Dinssa, F.F., Winkelmann, T., 2017. Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breed. 136, 578–589.

Peakall, R., Smouse, P.E., 2012. GenAlex 6.5: genetic analysis in excel. Population genetics software for teaching and research- an update. Bioinformatics 28, 2537–2539.

Poczai, P., Taller, J., Szabo, I., 2008. Analysis of phylogenetic relationships in the genus *Solanum* (*Solanaceae*) as revealed by RAPD markers. Plant Syst. Evol. 275, 59–67.

Poczai, P., Matyas, K., Taller, J., Szabo, I., 2010. Study of the origin of the rarely cultivated edible *Solanum* species: morphological and molecular data. Biol Plant. 54, 543–546.

Poczai, P., Hyvönen, J., 2011. On the origin of *Solanum Nigrum*. Can network help? Mol. Biol. Reprod. 38, 1171–1185.

Poczai, P., Varga, I., Bell, N.E., Hyvonen, J., 2011. Genetic diversity assessment of bittersweet (*Solanum dulcamara, Solanaceae*) germplasm using conserved DNA-derived polymorphism and intron-targeting markers. Ann. Appl. Biol. 159, 141–153.

Poczai, P., Cernak, I., Varga, I., Hyvonen, J., 2014. Nuclear intro-targeting markers in genetic analysis of black nightshade (*Solanum* sect. *Solanum, Solanaceae*) accessions. Genet. Resour. Crop Evol. 61, 247–266.

Pogorzelec, M., Glebocka, K., Nowak, B., Parymies, M., 2014. Reproduction and diversity of endangered *Salix lapponum* L. populations in Eastern Poland. Turk. J. Bot. 38, 1239–1247.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Interface of population structure using multilocus genotype data. Genetics 155, 945–959.

Saha, M., Datta, B.K., 2014. Reproductive biology of *S. viarum* Dunal (Solanaceae) in Northeast India. Pleion 8, 258–266.

Salmi, H., Bahar, M., Mirlohi, A., Talebi, M., 2016. Assessment of the genetic diversity among potato cultivars from different geographical areas using genomic and EST microsatellites. Iran. J. Biotechnol. 14. http://dx.doi.org/10.15171/isb.1280.

San-Yi, S.J., Atoi, S.A., Fujimura, T., Yamanaka, S., Watanabe, J., Watanbe, K.N., 2008. Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-center of origin. Plant Breed. 127, 189–196.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18, 233–234. http://dx.doi.org/10.1038/72708.

Shan, X., Blake, T.K., Talbert, L.E., 1999. Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor. Appl. Genet. 98, 1072–1078.

Shendure, J., Ji, H., 2008. Next generation DNA sequencing. Nat. Biotechnol. 26, 1135–1145.

Sulusoglu, M., Cavusoglu, A., 2014. In vitro pollen viability and pollen germination in cherry laurel (*Prunus laurocerasus* L.). Sci. World J. 7. http://dx.doi.org/10.1155/2014/657123.

Suresh, S., Chung, J.W., Cho, G.T., Sung, J.S., Park, J.H., Gwang, J.C., Baek, H.J., 2015. Analysis of molecular genetic diversity and population structure in *Amaranthus* germplasm using SSR markers. Plant Biosyst. 148, 635–644.

Sutyemez, M., 2011. Pollen quality, quantity and fruit set of some self-compatible cherry cultivars with artificial pollination. Afr. J. Biotechnol. 10, 3380–3386.

Tuinstra, M.R., Wedel, J., 2000. Estimation of pollen viability in grain sorghum. Crop Sci. 40, 968–970.

Untergasser, A., Cutcutache, I., Koressar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S.G., 2012. Primer3-new capabilities and interfaces. Nucleic Acid Res. 40, e115.

van Biljon, A., Labuschagne, M., Koen, E., 2010. Microsatellite-based assessment of five *Solanum nigrum* complex species and their progeny. Soil Plant Sci. 1, 1–6.

Veron, V., Caron, H., Degen, B., 2005. Gene flow and mating systems of the tropical tree *Sextonia* rubra. Silvae Genet. 54, 275–280.

Vieira, M.L.C., Santini, L., Diniz, A.L., Munhoz, C.F., 2016. Microsatellite markers: what they mean and why they are useful. Genet. Mol. Biol. 39, 312–328.

Volis, S., Shulgina, I., Mendlinger, S., Oluoch, M., 2009. Genetic structure in two African vegetable nightshade species. Int. J. Plant Breed. 3, 81–85.

Weinberger, K., Pasquini, M., Kasambulu, P., Abukutsa-Onyango, M., 2011. Supply chains for indigenous vegetables in urban and peri-urban areas of Uganda and Kenya: a gendered perspective. In: Waibel, H., Mithöfer, D. (Eds.), Vegetable Production and Marketing in Africa. CABINairobi, pp. 288.

Xiao-Min, Z., Zheng-hai, Z., Xiao-Zhen, G., Sheng-Li, M., Xi-Xiang, Li, Chadoeuf, J., Palloix, A., Li-hao, W., Bao-Xi, Z., 2016. Genetic diversity of pepper (*Capsicum spp.*) germplasm resources in China reflects selection for cultivar types and spatial distribution. J. Integr. Agric. 15, 1991–2001.

Xiaoyan, S., Sanwen, H., 2016. SSR analysis of genetic diversity among 192 diploid potato cultivars. Hortic. Plant J. 2, 163–171.

Yang, T., Fang, L., Zhang, X., Hu, J., Bao, S., Hao, J., Li, L., He, Y., Jiang, J., Wang, F., Tian, S., Zong, X., 2015. High-throughput development of SSR markers from pea (*Pisum sativum* L.) based on next generation sequencing of a purified Chinese commercial variety. PLoS One 10, 1–14.

Zapata, J.E., Cueras, H., Zhu, H., Steffen, S., Senalik, D., Zeldin, D., McCown, B., Harbut, R., Simon, P., 2012. Using next generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. Am. J. Bot. 99, 193–208.

Zhang, Q.Q., Wu, C.L., Ren, F.L., Li, Y., Zhang, C.Q., 2012. Association analysis of important agronomical traits of maize inbred lines with SSRs. Aust. J. Crop Sci. 6, 1131–1138.

Supplementary figures and tables

Table A.1: African nightshade entries used in the study, their affiliation and original accession sources

Entry	Received as	Proposed as	Germplasm type*	Source	Original accession source
ABUK3	*S. villosum*	*S. villosum*	Cultivar	JKUAT	Kenya
ACC.1	*S. villosum*	*S. villosum*	Landrace	Kericho	Kenya
ACC.9	*S. villosum*	*S. villosum*	Landrace	Kisii	Kenya
ACC.10	*S. villosum*	*S. villosum*	Landrace	Kisii	Kenya
ACC.11	*S. villosum*	*S. villosum*	Landrace	Kisii	Kenya
ACC.25	*S. villosum*	*S. villosum*	Cultivar	Nakuru	Kenya
ACC.27	*S. villosum*	*S. villosum*	Landrace	Nakuru	Kenya
ACC.28	*S. villosum*	*S. villosum*	Landrace	Nakuru	Kenya
ACC.29	*S. villosum*	*S. villosum*	Cultivar	Nakuru	Kenya
ACC.30	*S. villosum*	*S. villosum*	Landrace	Nakuru	Kenya
ACC.31	*S. villosum*	*S. villosum*	Landrace	Kisii	Kenya
ACC.32	*S. villosum*	*S. villosum*	Landrace	Kisii	Kenya
BG01	*S. villosum*	*S. villosum*	Line	WorldVeg	Tanzania
BG03	*S. villosum*	*S. villosum*	Line	WorldVeg	Tanzania
BG20	*S. villosum*	*S. villosum*	Line	WorldVeg	Tanzania
IP01	*S. nigrum*	*S. villosum*	Line	WorldVeg	Kenya
IP03	*S. nigrum*	*S. villosum*	Line	WorldVeg	Kenya
MW13	*S. sarrachoides*	*S. villosum*	Line	WorldVeg	Kenya
MW17	*S. villosum*	*S. villosum*	Line	WorldVeg	Kenya
MW18	*S. spp*	*S. villosum*	Line	WorldVeg	Kenya
ABUK1	*S. scabrum*	*S. scabrum*	Cultivar	JKUAT	Kenya
ABUK2	*S. scabrum*	*S. scabrum*	Cultivar	JKUAT	Kenya
ACC.3	*S. scabrum*	*S. scabrum*	Cultivar	Kisumu	Kenya
ACC.4	*S. scabrum*	*S. scabrum*	Cultivar	Kisumu	Kenya
ACC.6	*S. scabrum*	*S. scabrum*	Cultivar	Kakamega	Kenya
ACC.7	*S. scabrum*	*S. scabrum*	Cultivar	Kakamega	Kenya
ACC.8	*S. scabrum*	*S. scabrum*	Cultivar	Kisumu	Kenya
ACC.15	*S. scabrum*	*S. scabrum*	Cultivar	Kakamega	Kenya
ACC.16A	*S. scabrum*	*S. scabrum*	Cultivar	Kakamega	Kenya
ACC.16B	*S. scabrum*	*S. scabrum*	Cultivar	Kakamega	Kenya
ACC.18	*S. scabrum*	*S. scabrum*	Cultivar	Kisumu	Kenya
ACC.20	*S. scabrum*	*S. scabrum*	Cultivar	Kisumu	Kenya
ACC.21	*S. scabrum*	*S. scabrum*	Cultivar	Kakamega	Kenya
BG04	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Tanzania
BG16	*S. scabrum*	*S. scabrum*	Cultivar	WorldVeg	Tanzania
BG07	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Tanzania
BG13	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Tanzania
BG14	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Tanzania

BG28	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Tanzania
IP09	*S. villosum*	*S. scabrum*	Line	WorldVeg	Kenya
IP21	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Cameroon
IP10	*S. villosum*	*S. scabrum*	Line	WorldVeg	Kenya
MW07	*S. eldoretianum*	*S. scabrum*	Line	WorldVeg	Tanzania
MW10	*S. retroflexum*	*S. scabrum*	Line	WorldVeg	Kenya
MW22	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Kenya
MW23	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Kenya
MW25	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Kenya
MW28	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Kenya
SS09	*S. scabrum*	*S. scabrum*	Line	WorldVeg	Cameroon
SS40	*S. scabrum*	*S. scabrum*	Advanced line	WorldVeg	Cameroon
SS042	*S. scabrum*	*S. scabrum*	Advanced line	WorldVeg	Cameroon
SS49	*S. scabrum*	*S. scabrum*	Cultivar	WorldVeg	Tanzania
SS52	*S. scabrum*	*S. scabrum*	Advanced line	WorldVeg	Cameroon
IP02	*S. nigrum*	*Solanum* spp.	Line	WorldVeg	Kenya

*Landraces obtained from famers; Cultivars obtained from commercial outlets

Table A.2: Types of SSR motifs and number of DNA contigs containing these types, number of SSRs selected for primer design, and number of primer pairs obtained by Primer3

Motif type	No. of contigs with SSR motifs	Selected contigs	No. of primer pairs
Dinucleotide	1452	31	17
Trinucleotide	3671	20	7
Tetranucleotide	4141	35	16
Pentanucleotide	959	21	8
Hexanucleotide	28678	43	20
Total	38901	150	68

Table A.3: Name, motive type, primer sequences, annealing temperature and estimated fragment size in bp of the 68 microsatellite loci designed for African nightshades

Name	Motif	Forward primer	Reverse primer	AT	bp
Di_Ole2160	(TC)19	GTAAAACGACGGCCAGTATCTGAAGCGCGGTGTATCT	GCATGGGCTCTCTCTCTCTC	59	230
Di_Ole7313	(TG)16	GTAAAACGACGGCCAGTAAGTTCACGTGGAGATATTGAGC	CAAGTGATACAAGAGCGAATGA	58	161
Di_Ole14831	(TA)15	GTAAAACGACGGCCAGTTTTTGGAGTTTGGGATCGAG	AATAGGGATAGGGGCGAGTG	60	231
Di_Ole16454	(AG)20	GTAAAACGACGGCCAGTATTTGTGCCTGACCAAGACC	TCCAACGTATGTGGGATTGA	59	217
Di_Ole16650	(TC)19	GTAAAACGACGGCCAGTATCTGAAGCGCGGTGTATCT	GGTTCCCTGTCAATGCAAAC	60	244
Di_Ole21469	(TG)23	GTAAAACGACGGCCAGTGAGGATTGTGTTTTTAGGGGG	TGGGGAGAGAGGGATTTCTT	60	265
Di_Ole26543	(TA)15	GTAAAACGACGGCCAGTTTTGAGTTCAGTACAACTCGTGG	GCGTTTCCAATCAACCCTAA	59	103
Di_Ole30104	(TC)18	GTAAAACGACGGCCAGTAACCTTTTGCTTCATTTGCG	GGGGAAAACACAGGAACAGA	59	261
Di_Ole38244	(GA)19	GTAAAACGACGGCCAGTTCTTCCATCTTGACGACCCT	ATCTGAAGCGCGGTGTATCT	59	163
Di_Ole48966	(AT)17	GTAAAACGACGGCCAGTCCACAAATGGAATTGTCTTCG	TTGAAGGTTTGATGCTTGACC	60	191
Di_Ole58556	(AG)19	GTAAAACGACGGCCAGTAATCGAACCAATCCAATCCA	CGTTTCGCATCCTCATTTTT	60	118
Di_Ole59630	(TA)19	GTAAAACGACGGCCAGTGTCATGCCTGGTTTTCGATT	CTCCTCTCAGACTCTCTCTTTGC	59	277
Di_Ole60059	(TA)16	GTAAAACGACGGAGTCCCTTTTCTTTCCACAACCA	TCCCATGGATACCAAGTGTG	59	157
Di_Ole84285	(TA)15	GTAAAACGACGGCCAGTATTTTCCGGTACTCCGTGGT	GAACTGAGCAAACGGAGGAG	59	191

DI_Ole87021	(GA)16	GTAAAACGACGGCCAGTGAAAAAGGGGGATCTTGTGA	TATGCTTGCACACTAAGGCG	60	165
Di_Ole111281	(AT)15	GTAAAACGACGGCCAGTGACTTGAGTGGTGGAAAACG	CCCGTGTTTCTTTTGATTAGC	58	130
Di_Ole114286	(AT)15	GTAAAACGACGGCCAGTCCAAACTCGAAAAGGGATTG	TTTACCTCATTGGCCGAAAC	59	257
Tri_Ole7458	(TAT)16	GTAAAACGACGGCCAGTGGGAAAAGGGTAAAAATGTCC	TGAATGGAAAGGAACGGAGT	59	181
Tri_Ole17608	(ATA)15	GTAAAACGACGGCCAGTAGGGATCGTTTGGTAGAGCA	TACTGGATTTTGGACCCCTG	59	160
Tri_Ole26856	(AAG)20	GTAAAACGACGGCCAGTAGAGCACATTTGCCACTCCT	TGACCCTATTCTTCCTCTTCCTC	60	131
Tri_Ole48678	(TTA)15	GTAAAACGACGGCCAGTCATGTGACAATCAGTTCTGGG	CGATGGTTAATCTTCATAGGTTCAG	60	231
Tri_Ole49229	(CTT)16	GTAAAACGACGGCCAGTTGCTGCTTGCCGCTATTATT	TAGAAGCAAAACGAAACGGC	60	176
Tri_Ole55169	(AAT)15	GTAAAACGACGGCCAGTAGATGCTCAGGACCCAAATG	ACCTAATTTGACGCGTGCTT	59	229
Tri_Ole102542	(TTA)15	GTAAAACGACGGCCAGTGTCTTGCTTAAAGTCAATTGGTAGC	GATTGGCAATTTGTGGATGA	59	206
Tet_Ole1488	(TTGT)6	GTAAAACGACGGCCAGTTAATGCTCTGTGATGCCTGC	CCTGGTTATAGTGGGCAAGG	59	260
Tet_Ole7092	(AATA)6	GTAAAACGACGGCCAGTGGGAGTTGTTCGGATGATAAA	CAGCAAGAAAGTGGCATTGA	59	256
Tet_Ole17646	(TTAT)6	GTAAAACGACGGCCAGTGGTCTTTGATCTGCTGGGAA	AACAACTCCCTCGGCTTGTA	59	235
Tet_Ole20163	(TTTA)6	GTAAAACGACGGCCAGTGCCACTGCTTTCTTATGATTTTCC	GGAAAGTTCAATTTGACACGG	59	140
Tet_Ole27092	(AAAT)6	GTAAAACGACGGCCAGTCTTGAGGAGCATGCAGAACA	ATCTGTTACCTTCGAGGCGA	59	161
Tet_Ole36530	(TTTC)8	GTAAAACGACGGCCAGTCGCGCTCAGAATTGGTTATT	GGAAGAGATACATACGCATACCC	59	150
Tet_Ole38559	(ATAG)10	GTAAAACGACGGCCAGTGTCAACCTACATTTATATCCGTGC	CTGCAACGACAAACACAAGAA	59	270

Tet_Ole45471	(TATG)10	GTAAAACGACGGCCAGTTGGTCGTTACCTGCGTACAA	TTGAGTTCTTGTGTGTGTGTGTG	59	222
Tet_Ole50723	(AATA)6	GTAAAACGACGGCCAGTAGAAAAAGCAGTGAATGCCC	CAAACTATTGTGGGGTTCCA	58	192
Tet_Ole50895	(TAAA)6	GTAAAACGACGGCCAGTCGTGCCTAGTTTTACCCCAA	TTAGTCCAACCCAAAACCCA	60	158
Tet_Ole61583	(AAAT)6	GTAAAACGACGGCCAGTTTAAACATAGCCAACCCCCA	TTCTTTGGCATGCTAGGATAGA	58	232
Tet_Ole68971	(ATAA)7	GTAAAACGACGGCCAGTCGGTTTTGATGTTAGGGTATGA	CCAAATGTGGTATTTGCCCT	59	209
Tet_Ole77901	(TTTC)6	GTAAAACGACGGCCAGTAGTCCGTTTCCTTTTGAACG	GCAAATGCTGAATCATCACA	58	169
Tet_Ole81848	(TTAT)6	GTAAAACGACGGCCAGTGACTGTTACGTTTGCAGCCA	AGCAGAACTGACCAAATGAACA	59	216
Tet_Ole101141	(GTAT)6	GTAAAACGACGGCCAGTTTGATACAATGGGGGTGGTT	TCCATCCATTAAAACGGAGC	59	174
Tet_Ole106079	(CAAA)6	GTAAAACGACGGCCAGTCCTCTTGAGAAGCTCCAACG	CGATGACCCAGATCCTGAAT	59	168
Pen_Ole1865	(TAAAA)5	GTAAAACGACGGCCAGTTGCTTAAGAGGTGCTTACGTG	GGAACAAATGCTTGTGAAGGA	60	258
Pen_Ole2020	(GTAAA)6	GTAAAACGACGGCCAGTCCCGATTTATATGTACACCACTGA	TGCTGAAAGTCCCTGTCCTC	60	253
Pen_Ole14507	(TTCTC)5	GTAAAACGACGGCCAGTGAACCGTCCATCACCTGACT	TTGAGACGCACCTTTTGTTG	59	165
Pen_Ole20708	(AAACA)5	GTAAAACGACGGCCAGTTTGATTCTTGTGCCCATGTC	GGCAGCCCTGAACTTAGGTA	59	225
Pen_Ole56306	(TTTTA)5	GTAAAACGACGGCCAGTAGCAGATTACGCGGCTAGAA	TTTCCTTTAGAAGAATGGGACG	59	251
Pen_Ole69791	(TCAAA)5	GTAAAACGACGGCCAGTTTGCATGCATTTCAACCAAC	TCATAGGTCCCCCACCACTA	59	219
Pen_Ole72993	(TATAT)8	GTAAAACGACGGCCAGTTGCTTAGCCCAAGAGACGTT	ATGCATGCAATTCTAATGCG	59	245
Pen_Ole118759	(TTATA)8	GTAAAACGACGGCCAGTTTCTTAGGGTCGGAATTACTGAG	ACAGACCTTTTCCAACTCCG	59	178

Hex_Ole24089	(CTCATC)4	GTAAAACGACGACGGCCAGTATCATGTGAACCCCTTGCTC	GGGAAGGAATAGGGTGAGGA	60	242
Hex_Ole25912	(GAAGAG)10	GTAAAACGACGGCCAGTGGAAGGATGGACGAGAATCA	CTCTCTCAATTCTCTCCAAATCTGA	60	242
Hex_Ole31638	(GGGATT)4	GTAAAACGACGGCCAGTGAGCAAAGGTCTTGACGGAG	TTTGATGCTGTCTGGCACTC	60	190
Hex_Ole31972	(TTCAAT)4	GTAAAACGACGGCCAGTGCGCTCTACTTTTGTTTCGC	GTTCACACCCACTTCGAACA	59	114
Hex_Ole34210	(AAAAAC)4	GTAAAACGACGGCCAGTTCAGGAGCCATCCAATAAGG	CAGATGTGGTAGCCAAGCAA	59	232
Hex_Ole34975	(TAATTT)4	GTAAAACGACGGCCAGTGACGGATCAAGAGTCAACCC	GTGGTGAACACATGTCATATTGTCT	60	160
Hex_Ole47850	(TTTTTG)4	GTAAAACGACGGCCAGTTCGATGTTTCCCTCATCTCC	TGAAATACACTGCGGTTGGA	60	264
Hex_Ole48689	(AGAAAA)6	GTAAAACGACGGCCAGTCAGTCACCAAAGTAATAGTAGCCC	TCCTTCACATGAAATGGTGG	59	107
Hex_Ole52252	(GAGTTG)4	GTAAAACGACGGCCAGTCTGGTTCAACGGACAACCTT	AGCTTCACTAACGGCCACAC	60	204
Hex_Ole55182	(TAAATA)5	GTAAAACGACGGCCAGTTGGTCAATGCCTTTTCTTCC	TAATGTTGCCTTCCCCATTC	59	155
Hex_Ole56218	(AAAAAC)4	GTAAAACGACGGCCAGTCACCAAATAAATGGCTGCAT	CCTGGATTGAACCTCTCCAA	60	260
Hex_Ole70403	(TTTCTT)4	GTAAAACGACGGCCAGTCACACTAAAGGCTGTTTAATTCCC	TCAATTCCGGGATTAATGGA	60	110
Hex_Ole78838	(TAAAAA)4	GTAAAACGACGGCCAGTGTCGGATTGATTTGTTCTCG	CGACCCCTGAATGTCTTTGT	60	211
Hex_Ole94855	(ATAAAA)4	GTAAAACGACGGCCAGTGTTGACAAAGGGAAAGGACC	CACGCGGAATTGTCTAATCA	59	115
Hex_Ole95897	(AAAAAT)4	GTAAAACGACGGCCAGTGGACCGGAGGGAGTAAGAAA	TGTGAAAGCAAAGGAGGTTG	58	275
Hex_Ole99194	(AAGTGA)4	GTAAAACGACGGCCAGTAAGGTTGGGGGTTTTCGTAG	TAATGAAGATTCGGGGCAAA	60	145
Hex_Ole108690	(GGTGAA)4	GTAAAACGACGGCCAGTGCCGGTGAAGAAACAAAAGA	ATCCCCGACTGTATCACCAG	59	238

Hex_Ole111446	(TATTTT)4	GTAAAACGACGGCCAGTGCTGCGAGGCTTTTAAACAA	ACCAAACTACGCCAACGAAA	60	121
Hex_Ole120086	(CTGAAA)4	GTAAAACGACGGCCAGTAATGGGCAAACGGTAGAGTG	TGGAGAAGGAACAACATCTGG	60	258
Hex_Ole51860	(TTTTCG)4	GTAAAACGACGGCCAGTCATCAATGGCTTCCTCATCA	CCAGAAATTGAATGTCAGATGC	59	162

7.2 Supplementary figures

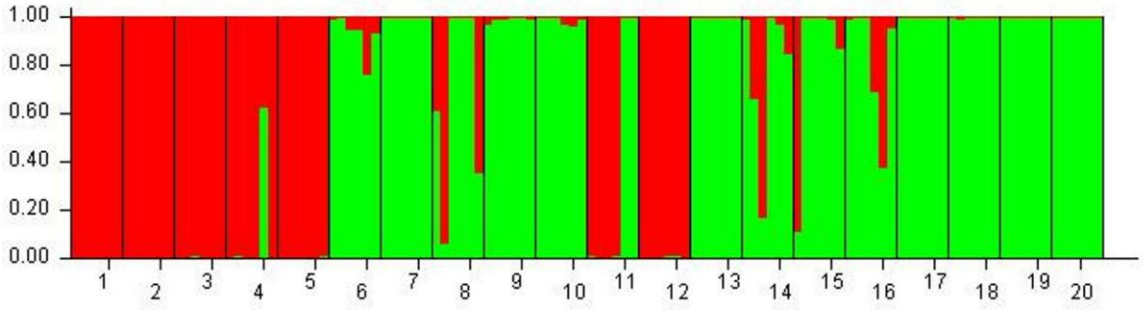

Figure A.1: Bayesian model analysis of the 20 *S. villosum* entries grouped to the optimal K=2. Each vertical bar represents one entry. The order is as presented in Table A.1. The entry IP02 was not included in these analyses

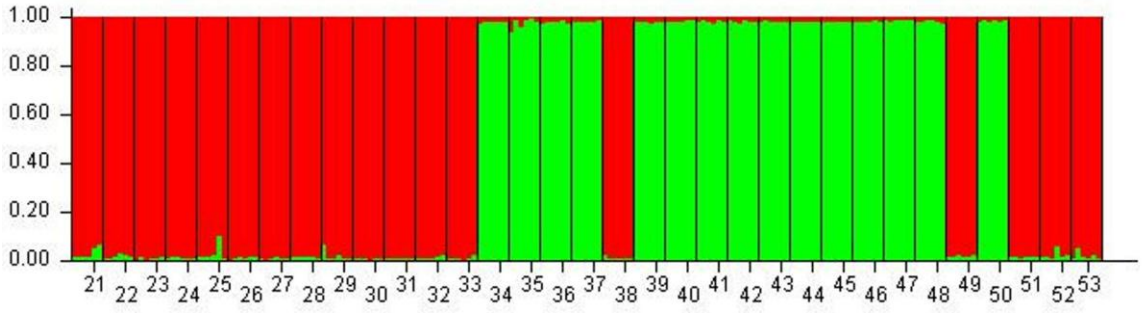

Figure A.2: Figure A.2: Bayesian model analysis of the 33 *S. scabrum* entries grouped to the optimal K=2. Each vertical bar represents one entry. The order is as presented in Table A.1. The entry IP02 was not included in these analyses

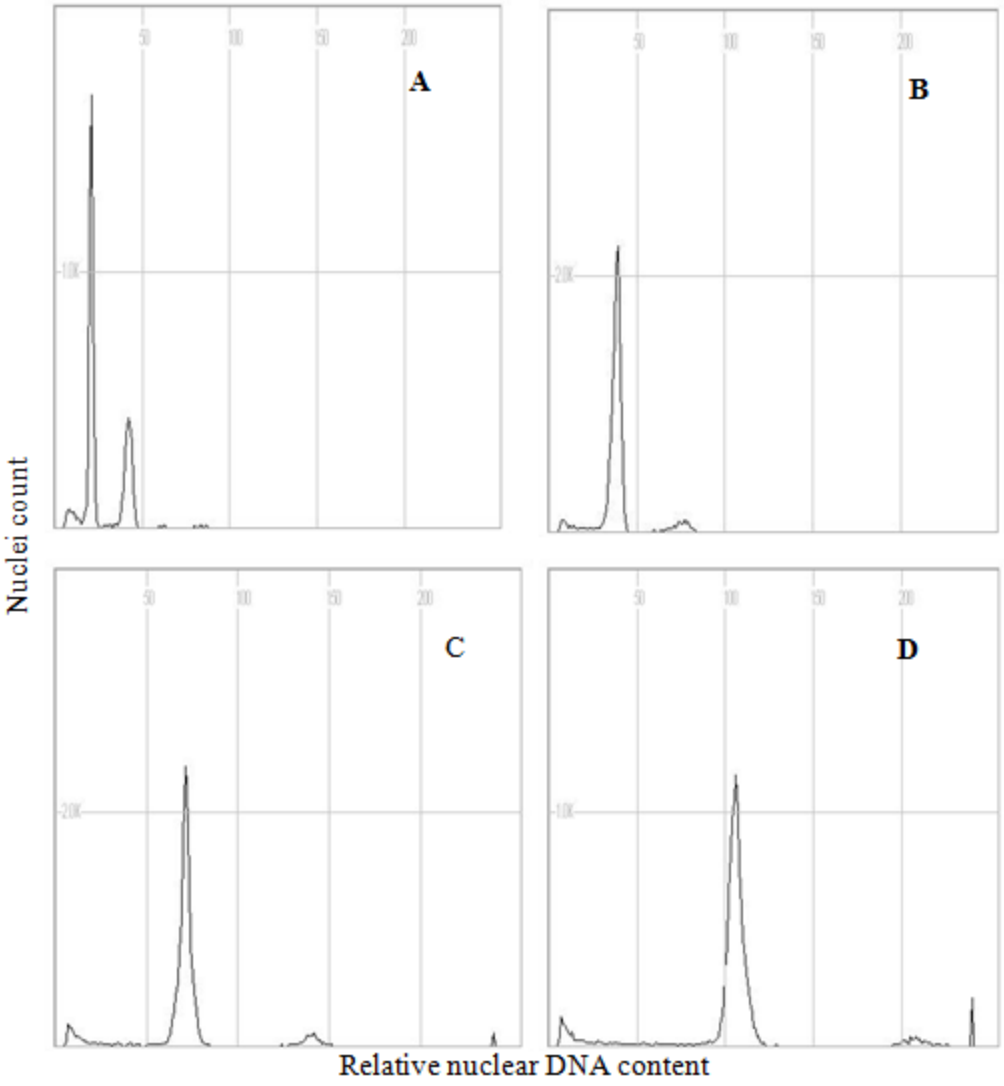

Figure A.3: Relative nuclear DNA content from leaf tissue of the reference standard- *S. lycopersicum* accession LYC418 (A), the diploid entry IP02 (B), tetraploid entry BG01 (C) and hexaploid entry BG28 (D).

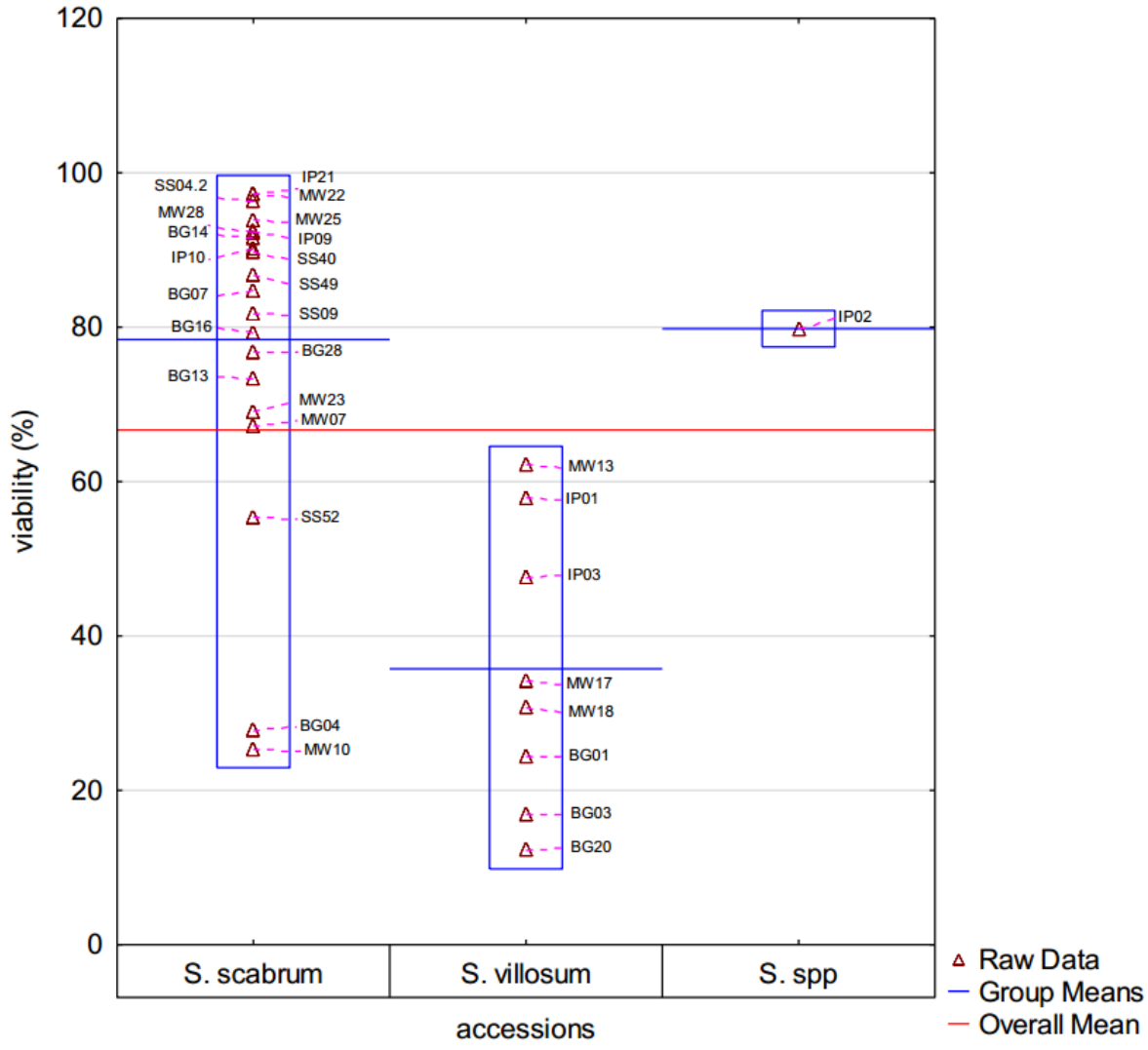

Figure A.4: Pollen viability variation expressed as the average percentage of germinated pollen grains among 20 *S. scabrum*, 8 *S. villosum* entries and ACC. IP02.

2.3 Morphological characterization, genetic diversity and population structure of African nightshade (*Solanum* section *Solanum* L.)

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RESEARCH ARTICLE

Morphological characterization, genetic diversity and population structure of African nightshades (section *Solanum* L.)

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Abstract The production of African nightshade is below its potential due to a lack of superior varieties. The development of varieties through breeding programmes has been initiated in order to supply farmers with quality seeds. However, systematic studies on the agronomic performance of African nightshade germplasm have been started only recently, and publications in this area are very scarce. In this study, we analysed morphological traits relevant for the agronomic performance of 54 entries comprising two species: *Solanum villosum* and *S. scabrum*. In addition, AFLP markers and newly developed SSR markers were used to assess genetic diversity and differentiation in the African nightshade entries. An analysis of molecular variance showed higher variation within than among entries. A cluster analysis grouped the entries into two clusters representing the two species. The analysis of agronomic traits revealed significant differences among entries. The genetic diversity of the released cultivars and accessions was comparable to but lower than that of the local cultivars. Additionally, the local cultivars had higher allelic richness and a larger number of unique alleles than did the developed cultivars and could serve as a useful gene pool for future breeding of superior germplasm.

Keywords AFLP · Agronomic traits · Molecular markers · *S. scabrum* · SSR · *S. villosum*

Introduction

African nightshades (cultivated species from the genus *Solanum*, section *Solanum*) are underutilized crops with the potential to improve food and nutrition security in sub-Saharan Africa. It is mainly used as a leafy vegetable, and some communities utilize it as an herbal medicine (Schippers 2002). Despite the potential of this indigenous vegetable, most species remain at the level of landraces, with only a few developed cultivars (Afari-Sefa et al. 2012). Advanced lines of African nightshades have been developed by mass and single-plant selection within germplasm collections from different countries in Africa, and the superior ones have been released for commercial production (Dinssa et al. 2015). However, to advance the breeding programme in the crop, knowledge of its genetic and phenotypic variability as well as the inheritance of major characteristics, such as yield, pest and disease resistance, and nutritional properties, is a prerequisite. Despite preliminary analyses of genetic diversity

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between species of African nightshades (Dehmer and Hammer 2004; Jacoby et al. 2003; Manoko et al. 2007, 2008; Olet et al. 2005, 2011; Poczai and Hyvönen 2011; Poczai et al. 2008, 2010, 2014), no study describing genetic variation has been conducted with more than five entries of one species for the two main species *S. scabrum* and *S. villosum* Mill.

In addition, there is a lack of molecular markers for use in breeding programmes and genetic studies for African nightshades. The use of molecular markers for characterization would substantially facilitate appropriate parental selection in the breeding programmes and speed up the selection for improved lines as it has been the case for other Solanaceae crops such as eggplant (Kaushik et al. 2018), potato (Salmi et al. 2016), pepper (Cheng et al. 2016) and tomato (San-Yi et al. 2008). Molecular markers are a fast and more reliable way of assessing variation compared with conventional selection approaches that use phenotypic descriptors, which are influenced by environmental factors (Aggarawal et al. 2008).

A wide panel of entries, including advanced breeding lines, accessions and local cultivars, was used in the current study for diversity comparison, which is an important component in crop improvement. Assessment of available diversity in plant material with varying degrees of breeding has been reported as an important component of crop improvement in other crops such as wheat (Henkrar et al. 2016) and rice (Choudhary et al. 2013). Amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers were used in the current study. AFLPs are more reliable than other dominant markers such as RAPD (Random amplified polymorphic DNA) and RFLPs (Restriction fragment length polymorphisms) and can be easily used without prior sequence information which is still of major interest in non-model species. While AFLPs detect a large number of polymorphic fragments in a single reaction, SSRs identify high levels of polymorphism or have high discriminating power at each locus (Fu 2015; Garcia et al. 2004). SSRs are also useful in germplasm management and breeding and can be used in studying closely related genotypes and determine levels of heterozygosity (Vilanova et al. 2014). Species-specific SSRs were developed and used for the current study. The AFLP and SSR markers were able to distinguish local cultivars from improved cultivars and will form a basis for informed breeding programmes and conservation strategies. The objective of this study was therefore to determine the genetic diversity and population structure of a wide panel of African nightshade entries and to characterize them for important phenotypic and agronomic traits.

Materials and methods

Plant material

The plant material included both improved and local cultivars together called entries. A total of 54 entries were used for this study (Table 1). Of these, 29 entries consisting of advanced lines and accessions were provided by the World Vegetable Center (WorldVeg) in Arusha, Tanzania; 25 entries from Kenya consisting of advanced lines were from Jomo Kenyatta University of Agriculture and Technology (JKUAT); released cultivars were obtained from retail seed outlets; and local cultivars were obtained from farmers' own seed stores.

Characterization of morphological traits

Experimental layout

The measurement of morphological traits was performed under field conditions in Kenya at the Jomo Kenyatta University Horticulture Farm (1°11′0″ South, 37°7′0″ East, 1416 m a.s.l.) from November 2016 to April 2017. A randomized complete block design was adopted with three blocks, with each entry represented once in each block. Soil was mixed with decomposed manure in each row during sowing. Seeds were sown at double density and then thinned to 10 plants per plot when seedlings were approximately 10 cm tall, with a spacing of 30 cm between plants and 40 cm between rows. The experiment was performed under rain-fed conditions, and when required, the rain was supplemented with overhead irrigation applied three times every week. Weeding was performed manually throughout the experiment. No artificial fertilizer or any other input was applied.

Data collection

Data for morphological traits were recorded from six randomly selected plants per entry in each block. Plant

Table 1 African nightshade entries used in the study with their ploidy, germplasm type and sources

Entry	Species	Ploidy[a]	Germplasm type[b]	Source	Original source
ABUK1	*S. scabrum*	NA	Advanced line	JKUAT	Kenya
ABUK2	*S. scabrum*	NA	Advanced line	JKUAT	Kenya
ABUK3	*S. villosum*	NA	Advanced line	JKUAT	Kenya
ACC.1	*S. villosum*	NA	Local cultivar	Kericho	Kenya
ACC.10	*S. villosum*	NA	Local cultivar	Kisii	Kenya
ACC.11	*S. villosum*	NA	Local cultivar	Kisii	Kenya
ACC.15B	*S. scabrum*	NA	Released cultivar	Kakamega	Kenya
ACC.16A	*S. scabrum*	NA	Released cultivar	Kakamega	Kenya
ACC.16B	*S. scabrum*	NA	Released cultivar	Kakamega	Kenya
ACC.18	*S. scabrum*	NA	Released cultivar	Kisumu	Kenya
ACC.20	*S. scabrum*	NA	Released cultivar	Kisumu	Kenya
ACC.21	*S. scabrum*	NA	Released cultivar	Kakamega	Kenya
ACC.25	*S. villosum*	NA	Released cultivar	Nakuru	Kenya
ACC.27	*S. villosum*	NA	Local cultivar	Nakuru	Kenya
ACC.28	*S. villosum*	NA	Local cultivar	Nakuru	Kenya
ACC.29	*S. villosum*	NA	Released cultivar	Nakuru	Kenya
ACC.3	*S. scabrum*	NA	Released cultivar	Kisumu	Kenya
ACC.30	*S. villosum*	NA	Local cultivar	Nakuru	Kenya
ACC.31	*S. villosum*	NA	Local cultivar	Kisii	Kenya
ACC.32	*S. villosum*	NA	Local cultivar	Kisii	Kenya
ACC.4	*S. scabrum*	NA	Released cultivar	Kisumu	Kenya
ACC.6	*S. scabrum*	NA	Released cultivar	Kakamega	Kenya
ACC.7	*S. scabrum*	NA	Released cultivar	Kakamega	Kenya
ACC.8B	*S. scabrum*	NA	Released cultivar	Kisumu	Kenya
ACC.9	*S. villosum*	NA	Local cultivar	Kisii	Kenya
BG01	*S. villosum*	4 ×	Accession	WorldVeg	Tanzania
BG03	*S. villosum*	4 ×	Accession	WorldVeg	Tanzania
BG04	*S. scabrum*	6 ×	Accession	WorldVeg	Tanzania
BG07	*S. scabrum*	6 ×	Accession	WorldVeg	Tanzania
BG13	*S. scabrum*	6 ×	Accession	WorldVeg	Tanzania
BG14	*S. scabrum*	6 ×	Accession	WorldVeg	Tanzania
BG16 (Nduruma)	*S. scabrum*	NA	Released cultivar	WorldVeg	Tanzania
BG20	*S. villosum*	NA	Accession	WorldVeg	Tanzania
BG28	*S. scabrum*	NA	Accession	WorldVeg	Tanzania
IP01	*S. villosum*	4 ×	Accession	WorldVeg	Kenya
IP02	*Solanum* sp.	NA	Accession	WorldVeg	Kenya
IP03	*S. villosum*	4 ×	Accession	WorldVeg	Kenya
IP09	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
IP10	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
IP21	*S. scabrum*	6 ×	Accession	WorldVeg	Cameroon
MW07	*S. scabrum*	6 ×	Accession	WorldVeg	Tanzania
MW10	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
MW13	*S. villosum*	4 ×	Accession	WorldVeg	Kenya
MW17	*S. villosum*	4 ×	Accession	WorldVeg	Kenya
MW18	*S. villosum*	4 ×	Accession	WorldVeg	Kenya

Table 1 continued

Entry	Species	Ploidy[a]	Germplasm type[b]	Source	Original source
MW22	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
MW23	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
MW25	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
MW28	*S. scabrum*	6 ×	Accession	WorldVeg	Kenya
SS042	*S. scabrum*	6 ×	Advanced line	WorldVeg	Cameroon
SS09	*S. scabrum*	6 ×	Accession	WorldVeg	Cameroon
SS40	*S. scabrum*	6 ×	Advanced line	WorldVeg	Cameroon
SS49 (Olevolosi)	*S. scabrum*	6 ×	Released cultivar	WorldVeg	Tanzania
SS52	*S. scabrum*	6 ×	Advanced line	WorldVeg	Cameroon

[a]Ploidy levels of the entries as determined in Ronoh et al. (2018); entries where no measurements were available are marked with "NA"

[b]Local cultivar—cultivars propagated by farmers from landraces; advanced lines—entries developed by mass selection from germplasm collections or gene bank accessions and evaluated for some desirable horticultural traits; accessions- germplasm collection held in genebank; released cultivars—entries released for commercial production (from Simlaw Seeds Co. Ltd. Kenya). Entries BG16 (Nduruma) and SS49 (Olevolosi) were released by World Vegetable Centre

height was determined by measuring from the base of the plant to the tip of the longest stem. Leaf yield usually harvested as vegetables was considered fresh mass. Mature leaves of each of the selected plants were harvested every week to avoid loss of leaves due to senescence until the fruiting stage of each entry when fresh mass declines. The sum of the weekly yield was used to compute fresh mass for each entry. To obtain dry mass, fresh material was dried at 70 °C until a constant weight was attained, and the sum of the weekly measurements was calculated. Stem diameter was measured at the first internode for every plant using a micrometre screw gauge, and leaf area was taken for fully grown leaves using leaf-area meter LI-3100C (Li-COR Biosciences). The number of branches was recorded as the total number of primary and secondary branches just after the onset of flowering. The numbers of fruits and individual masses were measured at the onset of fruit ripening when fruits change colour. Peduncle length was measured for six fully grown leaves from each entry. Days to flowering was recorded as the number of days from sowing to when approximately half the plants in each plot had flowered. Five qualitative descriptors were examined at different growth phases. Inflorescent types and leaf shapes were recorded at flowering stage. Plant growth habit and stem ridges were evaluated at maturity when plants were already fruiting, while colour of ripe fruits was evaluated when fruits changed colour from green.

Statistical analysis

The statistical analyses (ANOVA, Pearson correlation, coefficient of variance (CV), and descriptive statistics) for quantitative traits were performed using Minitab® 18.1, which is available at http://minitab.com/product/minitab. Analysis of variance (ANOVA) was performed with significance set at $P < 0.05$. Comparisons of means were carried out using Tukey's test.

Molecular marker characterization

DNA isolation

Six plants from each entry were used for analysis. For DNA isolation, young leaves were harvested and dried over silica gel at 37 °C overnight. DNA was isolated from 20 mg of dried leaf tissue. The samples were placed in 2 mL reaction tubes with two steel beads and homogenized using a Retsch™ MM 400 Mixer Mill (Retsch, Haan, Germany). DNA isolation was performed using the Macherey-Nagel NucleoSpin® Plant II kit (Macherey-Nagel GmbH, Düren, Germany) following the manufacturer's protocol using PL1 lysis buffer. The DNA was eluted from the columns two times using 40 µL PE buffer. The concentration of genomic DNA was assessed spectrophotometrically using a Nanodrop Spectrophotometer 2000c (PeqLab Biotechnology Co. Ltd., Erlangen, Germany) at

260 nm and was evaluated for purity by determining the OD 260/280 nm ratio and OD 260/230 nm ratio. DNA integrity was assessed by agarose gel electrophoresis.

SSR analysis

The SSR primers used in this study were developed in a previous study (Ronoh et al. 2018). PCR and visualization were carried out as described therein under similar conditions. The primer sequences and repeat motifs are presented in supplementary Table S1.

AFLP analysis

For AFLP analysis, 100 ng of DNA was restricted at 37 °C overnight using Hind III and MseI followed by ligation at 37 °C for 3.5 h. Pre-amplification reactions were carried out using the non-selective primers Hind III and MseI in the following profile: 94 °C for 5 min followed by 20 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min with a final step of 72 °C for 10 min. The final amplification reaction mix consisted of the primer Hind III-IRD with AAC as selective bases in combination with different MseI selective bases. Amplification was performed in the following profile: 94 °C for 5 min; 1 cycle of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min; an additional 11 cycles of the same profile with a reduction in annealing temperature by 0.7 °C per cycle; 24 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min; and 72 °C for 10 min. The samples were then incubated at 94 °C for 5 min after adding 20 µL formamide loading dye and separated on 6% acrylamide gels on a LI-COR 4300 sequencer at the 700 nm channel. Banding patterns were scored manually and transformed into a presence (1) or absence (0) matrix.

Genetic diversity analysis

Genetic diversity was assessed for each entry (represented by six individual genotypes each) and for different groups (accessions, released cultivars and local cultivars) with GenAlEx 6.5 (Peakall and Smouse 2012). Mean numbers of alleles and effective alleles were calculated for each population. Shannon's diversity index (I) was used as the measure of diversity. Heterozygosity was analysed as observed heterozygosity (Ho) and gene diversity (He). Genetic diversity and rarity were derived from allelic richness (Ar) and private alleles (P) using ADZE v 1.0 (Szpiech et al. 2008) using a rarefaction approach for counting alleles private to combinations of populations. To determine how informative the AFLP primers were, the polymorphism information content (PIC) was calculated according to the formula $PIC_i = 2f_i(1 - f_i)$, where f_i is the frequency of present fragments and $1 - f_i$ is the frequency of absent fragments (Roldan-Ruiz et al. 2000).

Genetic structure

Genetic differences between and within species and groups were estimated with analysis of molecular variance (AMOVA) using ARLEQUIN 3.5 (Excoffier and Lischer 2010). Population subdivision was estimated from the F-statistics generated from variance components obtained with analysis of molecular variance. The variance components and F-indices were tested for significance at $P < 0.05$ with 999 permutations. The genetic differentiation (Fst) for groups (accessions, released cultivars and local cultivars) was also computed using GenAlEx 6.5 (Peakall and Smouse 2012). Pairwise distances between genotypes were calculated using Jaccard's similarity index. Dendrogram construction was carried out using the unweighted pair group with arithmetic mean (UPGMA) method. The reliability of the tree topology was assessed using bootstrap analysis with 1000 replicates. All the above mentioned analyses were carried out using FreeTree software (Pavlicek et al. 1999). The dendrogram was visualized using TreeView (Page 1996).

Results

Characterization of morphological traits

For morphological characteristics, we recorded nine quantitative and five qualitative phenotypic traits from six selected plants of each of the 54 entries. The qualitative traits inflorescence type and colour of ripe berries were found to be species-specific: all *S. scabrum* entries had purple berries, while those of *S. villosum* had yellow/orange berries. All inflorescences of *S. scabrum* were simple cymes differentiated by the umbel form, whereas *S. villosum* inflorescences were

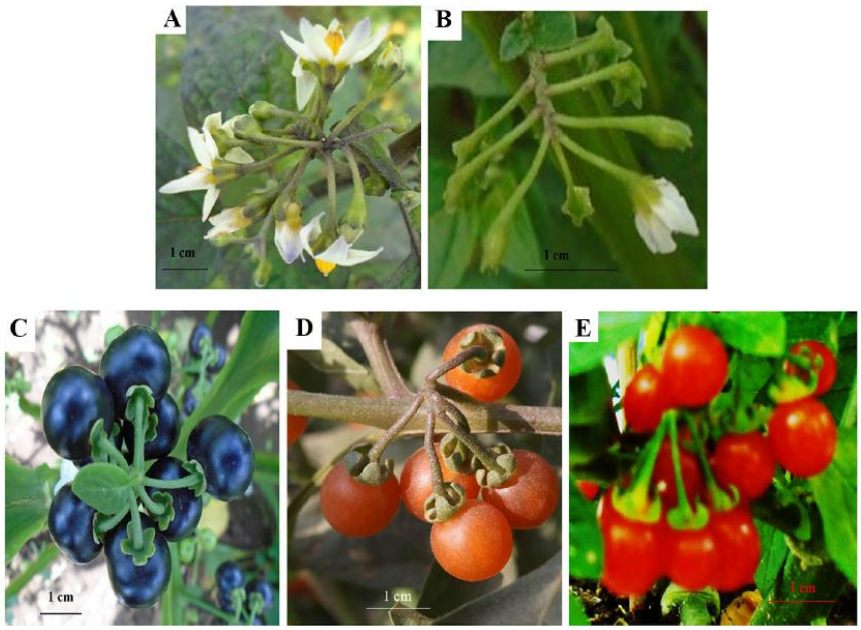

Fig. 1 Inflorescence types for entries SS49 (**A**) and ACC.25 (**B**) and ripe fruit colour for entries SS49 (**C**), ACC.25 (**D**), and IP03 (**E**). SS49 is an *S. scabrum* entry, and ACC.25 and IP03 are *S. villosum* entries

all of the raceme shape (Fig. 1, supplemental Table S2). Only seven of the 20 *S. villosum* entries (ACC.1, ACC.9, ACC.10, ACC.11, ACC.31, ACC.32 and ABUK3) had a prostrate growth habit, whereas all other entries comprised genotypes with an upright growth habit (supplemental Table S2). There was higher variation for the *S. scabrum* entries than those of *S. villosum* in fresh mass and leaf area (Fig. 2). The *S. scabrum* entry SS40 showed the highest variability in both fresh mass and leaf area. The traits of agronomic importance, such as leaf yield expressed as leaf fresh mass, leaf area, height and stem diameter, varied significantly between entries and between the two species. The number of branches and the number of fruits were significantly different among entries within species but not significantly different between the species (Table 2). The mean fresh mass for *S. scabrum* entries was 172.71 g (supplemental Table S3) and ranged from 31.02 g (ACC.7) to 350 g (ACC.4 and ACC. 21). The mean fresh mass for *S. villosum* entries was considerably lower, with an average of 74.39 g (supplemental Table S4) and a range from 29.6 g (ACC.9) to 122.5 g (MW17). Only seven *S. scabrum* entries had a lower mean fresh mass than the best *S. villosum* entry (MW17). The mean leaf

Fig. 2 Fresh mass (**A**) and leaf area (**B**) of 54 African nightshade entries. *S. scabrum* entries are on the left of the dashed line, while *S. villosum* entries are on the right. Crosses within the boxes mark the mean values

area for *S. scabrum* entries ranged from 20.98 cm^2 (ACC.16B) to 371.46 cm^2 (BG16) with a mean of 138.11 cm^2, while for *S. villosum*, the leaf area ranged from 4.68 to 59.09 cm^2 with a mean of 30.75 cm^2. The mean height for *S. scabrum* entries was 94.7 cm and ranged from 46.6 cm (ACC.6) to 125.3 cm (SS40). The height of *S. villosum* plants ranged from 21.5 cm (ACC.10) to 121.6 cm (MW13) with a mean of 61.6 cm. For both species, the advanced lines and accessions showed a larger plant height than did the released cultivars and local cultivars. Stem diameter ranged between 11.1 mm (ACC.6) and 18 mm (SS52) with a mean of 14.6 mm for *S. scabrum* and between 3 mm (ACC.10) and 12.5 mm (ACC.30) with a mean of 7.9 mm for *S. villosum*. The mean number of days to flowering ranged between 65.7 (MW23) and 116.5 (SS49) with a mean of 85.6 for the *S. scabrum* entries. On average, the *S. villosum* entries were flowering earlier than were the entries of *S. scabrum* at approximately 67 days after sowing, with the lowest

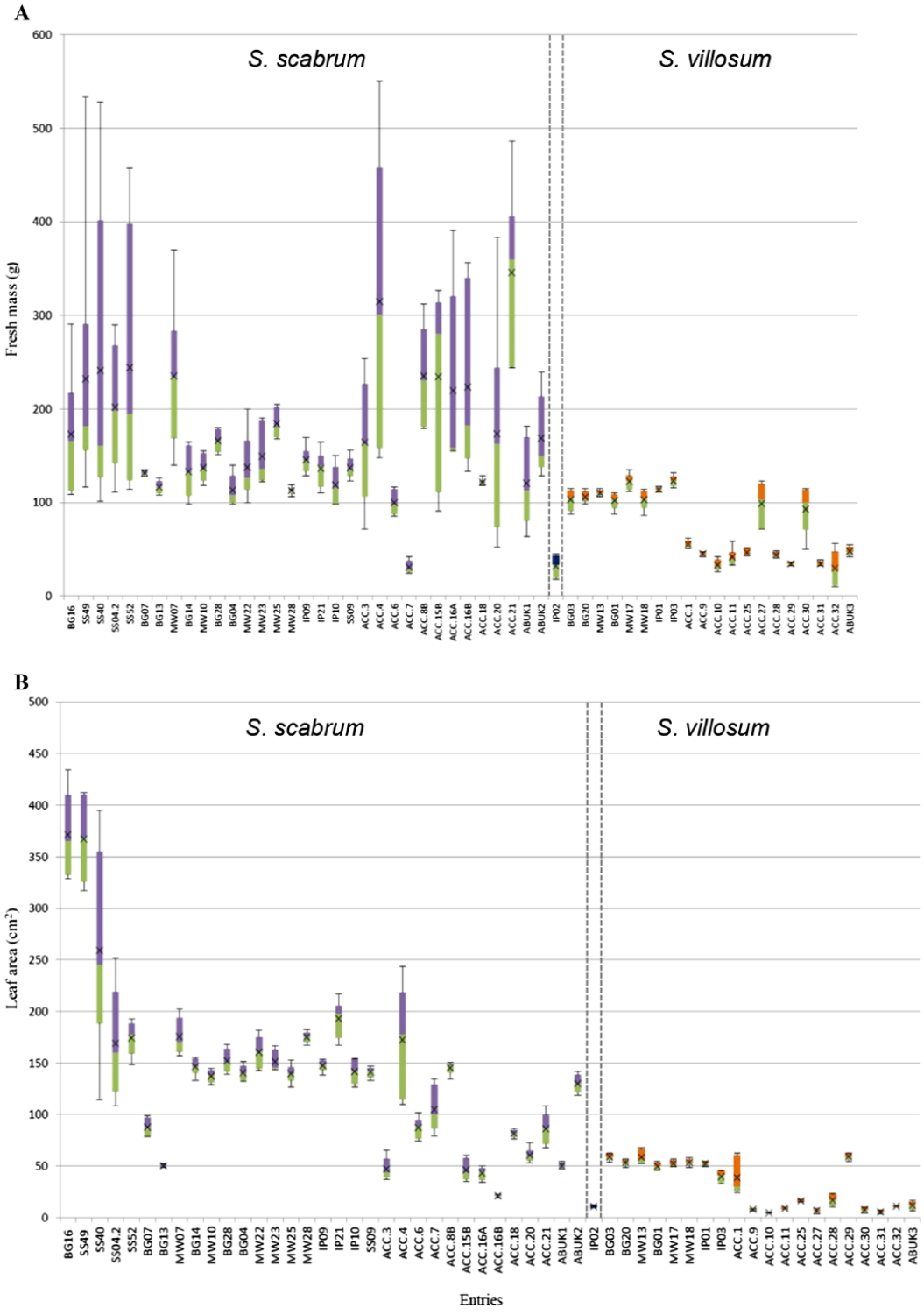
A
S. scabrum
S. villosum
Fresh mass (g)
0
100
200
300
400
500
600
BG16 SS49 SS40 SS04.2 SS52 BG07 BG13 MW07 BG14 MW10 BG28 BG04 MW22 MW23 MW25 MW28 IP09 IP21 IP10 SS09 ACC.3 ACC.4 ACC.6 ACC.7 ACC.8B ACC.15B ACC.16A ACC.16B ACC.18 ACC.20 ACC.21 ABUK1 ABUK2 IP02 BG03 BG20 MW13 BG01 MW17 MW18 IP01 IP03 ACC.1 ACC.9 ACC.10 ACC.11 ACC.25 ACC.27 ACC.28 ACC.29 ACC.30 ACC.31 ACC.32 ABUK3
B
S. scabrum
S. villosum
Leaf area (cm²)
0
50
100
150
200
250
300
350
400
450
500
BG16 SS49 SS40 SS04.2 SS52 BG07 BG13 MW07 BG14 MW10 BG28 BG04 MW22 MW23 MW25 MW28 IP09 IP21 IP10 SS09 ACC.3 ACC.4 ACC.6 ACC.7 ACC.8B ACC.15B ACC.16A ACC.16B ACC.18 ACC.20 ACC.21 ABUK1 ABUK2 IP02 BG03 BG20 MW13 BG01 MW17 MW18 IP01 IP03 ACC.1 ACC.9 ACC.10 ACC.11 ACC.25 ACC.27 ACC.28 ACC.29 ACC.30 ACC.31 ACC.32 ABUK3
Entries

Table 2 Analysis of variance within and between *S. scabrum* and *S. villosum* entries for quantitative morphological traits

Source	*df*	*SS*	*MS*	*F* value	*P* value
Height					
Between species	1	13,725.0	13,725.2	16.39	0.001
Within *S. scabrum*	32	106,043.2	3313.9	12.52	0.001
Within *S. villosum*	19	151,880.5	7993.7	73.33	0.001
Stem diameter					
Between species	1	566.0	565.9	111.56	0.001
Within *S. scabrum*	32	674.5	21.1	2.82	0.001
Within *S. villosum*	19	878.0	46.2	11.19	0.001
Leaf area					
Between species	1	143,551.0	143,551.0	34.15	0.001
Within *S. scabrum*	32	1,229,440.6	38,420.0	56.46	0.001
Within *S. villosum*	19	56,786.1	2988.7	117.54	0.001
Fruit mass					
Between species	1	15.9	15.9	226.05	0.001
Within *S. scabrum*	32	11.1	0.3	4.85	0.001
Within *S. villosum*	19	10.5	0.5	63.55	0.001
Fruit number					
Between species	1	1602.0	1602.0	0.13	0.722
Within *S. scabrum*	32	2,275,734.7	71,116.7	19.37	0.001
Within *S. villosum*	19	1,342,506.4	70,658.2	20.71	0.001
Fresh mass					
Between species	1	122,850.0	122,850.0	37.17	0.001
Within *S. scabrum*	32	807,768.8	25,242.8	4.45	0.001
Within *S. villosum*	19	140,360.0	7387.3	67.19	0.001
Dry mass					
Between species	1	1823.0	1823.3	36.53	0.001
Within *S. scabrum*	32	11,911.6	372.2	2.98	0.001
Within *S. villosum*	19	3362.9	176.9	48.23	0.001
Peduncle length					
Between species	1	224.0	223.9	34.90	0.001
Within *S. scabrum*	32	1143.5	35.7	31.41	0.001
Within *S. villosum*	19	820.4	43.2	229.19	0.001
Number of branches					
Between species	1	292.5	292.5	3.73	0.059
Within *S. scabrum*	32	14,124.4	441.4	12.50	0.001
Within *S. villosum*	19	10,208.2	537.3	39.45	0.001
Days to flowering					
Between species	1	4019.0	4018.5	62.27	0.001
Within *S. scabrum*	32	16,013.9	500.4	44.13	0.001
Within *S. villosum*	19	3725.7	196.1	27.67	0.001

df degrees of freedom, *SS* sum-of-squares, *MS* mean squares

mean number of days being 60 (ACC.32) and the highest being 78 (BG03). The entry IP02 had the lowest number of days to flowering from all entries with a mean of 57 days. The *S. scabrum* entries had a higher fresh mass, dry mass, leaf area, height, peduncle length and stem diameter on average than did the entries of *S. villosum* (supplemental Figures S2–S4).

The fruit mass also was distinct between the two species. The entries of *S. scabrum* generally had larger fruits than did those of *S. villosum* with the exception of entry BG01, which had an approximately sevenfold higher mean fruit mass than that of all other *S. villosum* entries (supplemental Figure S5). Together with the very high number of fruits (306) for *S. villosum* this leads to highest total fruit mass of all *S. villosum* entries (supplemental Table S4). In general, the released cultivars had a higher number of branches and a lower number of fruits (supplemental Figures S6 and S7).

The correlation analysis showed a significant positive correlation ($P < 0.05$) between fresh mass and stem diameter, fresh mass and the number of branches, height and leaf area, height and the number of fruits, leaf area and days to flowering, and leaf area and peduncle length for the *S. scabrum* entries (Table 3). There was a negative correlation between leaf area and the number of branches. For the *S. villosum* entries, nearly all traits were positively correlated with each other (Table 3). There was a significant and strong positive correlation between height and stem diameter and between height and leaf area. Additionally, a strong positive correlation was noted between fresh mass and all the other traits except fruit size. There was, however, a weak positive correlation between height and the number of branches and a moderate correlation between height and days to flowering. Of all the recorded traits, leaf area was the most variable (CV = 86.6%), and stem diameter was the least variable (CV = 37.4%) for all entries (supplemental Table S5). Amongst the *S. scabrum* entries, fruit number had the highest variability of 66.0% (CV), while stem diameter was the least variable (CV = 21.3%). For the *S. villosum* entries, fruit mass had a CV of 93.9% and stem diameter was the least variable (CV = 41.9%).

Genetic diversity

To compare the genetic diversity between and within the entries, DNA was extracted from six individuals for each of the 54 entries and analysed with 16 polymorphic SSR markers. The total mean number of alleles for the *S. scabrum* entries was 1.75 ± 0.03, and it was 2.00 ± 0.38 for the *S. villosum* entries (Table S6). The total number of private alleles for all entries was 30. Out of these, 12 private alleles were detected for entry IP02, which was separated from the other entries; fourteen were shared among the *S. villosum* entries; and only four occurred exclusively in *S. scabrum*. The local cultivars had the highest allelic richness of 1.71 ± 0.35 compared with the *S. scabrum* accessions and cultivars, which had values of 1.09 ± 0.21 and 1.11 ± 0.05, respectively. The *S. villosum* accessions had an allelic richness of 1.36 ± 0.43, which was slightly higher than that of the *S. scabrum* accessions. *S. villosum* had a higher diversity index of 0.56 ± 0.09 than that of *S. scabrum*, which was 0.48 ± 0.09. The observed heterozygosity of 0.66 ± 0.11 for *S. scabrum* was slightly higher than that for *S. villosum* (0.63 ± 0.1).

Population structure

According to the structure of the populations, the released cultivars showed the highest similarity ($Fst = 0.04$), while the local cultivars displayed the highest differentiation with a value of 0.27 (supplemental Table S7). Although we included a number of advanced entries developed by the World Vegetable Center, the analysis of molecular variance showed higher variation within than between entries for all populations (supplemental Table S8). The fixation indices were highly significant at $P < 0.001$ except for those of the *S. scabrum* entries. *S. villosum* had much higher variation between entries (20.1%) compared with *S. scabrum* (2.1%). The genetic differentiation of 0.2 in *S. villosum* was almost tenfold higher than that in *S. scabrum*.

To obtain better-supported phylogenetic data, in addition to the 16 SSR markers, we used information from a set of 5 AFLP primer combinations analysed in three genotypes per entry except for entries ACC.9, ACC.11, ACC.31 and SS52, in which two genotypes were used due to poor-quality fragments for scoring in one genotype.

Table 3 Pearson correlation analysis for quantitative morphological traits of African nightshade entries

	Height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Fruit mass (g)	Fruit number	Fresh mass (g)	Dry mass (g)	Peduncle length	Branch number
S. scabrum entries									
Stem diameter (mm)	$0.288_{(.105)}$								
Leaf area (cm^2)	$\mathit{0.454}_{(.008)}$	$0.314_{(.075)}$							
Fruit mass (g)	$-\mathit{0.162}_{(.366)}$	$-0.302_{(.088)}$	$-0.140_{(.437)}$						
Fruit number	$\mathit{0.642}_{(.001)}$	$-0.048_{(.791)}$	$0.164_{(.362)}$	$0.145_{(.420)}$					
Fresh mass (g)	$0.175_{(.330)}$	$\mathit{0.755}_{(.001)}$	$0.154_{(.393)}$	$-0.300_{(.090)}$	$-0.179_{(.319)}$				
Dry mass (g)	$0.142_{(.432)}$	$\mathit{0.567}_{(.001)}$	$-0.009_{(.960)}$	$-0.068_{(.706)}$	$-0.055_{(.763)}$	$\mathit{0.888}_{(.001)}$			
Peduncle length	$\mathit{0.580}_{(.001)}$	$0.342_{(.051)}$	$\mathit{0.733}_{(.001)}$	$-0.308_{(.082)}$	$0.290_{(.101)}$	$0.154_{(.391)}$	$0.018_{(.922)}$		
Branch number	$-0.131_{(.468)}$	$0.327_{(.063)}$	$-\mathit{0.418}_{(.015)}$	$-0.090_{(.618)}$	$-0.035_{(.845)}$	$\mathit{0.535}_{(.001)}$	$\mathit{0.536}_{(.001)}$	$-0.293_{(.098)}$	
Days to flowering	$0.152_{(.398)}$	$0.137_{(.448)}$	$\mathit{0.544}_{(.001)}$	$-0.239_{(.180)}$	$-0.220_{(.218)}$	$0.241_{(.178)}$	$0.081_{(.653)}$	$0.227_{(.205)}$	$-0.193_{(.282)}$
S. villosum entries									
Stem diameter (mm)	$\mathit{0.789}_{(.001)}$								
Leaf area (cm^2)	$\mathit{0.721}_{(.001)}$	$\mathit{0.467}_{(.038)}$							
Fruit mass (g)	$0.215_{(.363)}$	$0.206_{(.384)}$	$0.240_{(.308)}$						
Fruit number	$\mathit{0.878}_{(.001)}$	$\mathit{0.769}_{(.001)}$	$\mathit{0.679}_{(.001)}$	$0.275_{(.240)}$					
Fresh mass (g)	$\mathit{0.873}_{(.001)}$	$\mathit{0.859}_{(.001)}$	$\mathit{0.633}_{(.003)}$	$0.249_{(.290)}$	$\mathit{0.919}_{(.001)}$				
Dry mass (g)	$\mathit{0.617}_{(.004)}$	$\mathit{0.803}_{(.001)}$	$0.207_{(.382)}$	$0.039_{(.870)}$	$\mathit{0.657}_{(.002)}$	$\mathit{0.802}_{(.001)}$			
Peduncle length	$\mathit{0.830}_{(.001)}$	$\mathit{0.643}_{(.002)}$	$\mathit{0.805}_{(.001)}$	$\mathit{0.531}_{(.016)}$	$\mathit{0.825}_{(.001)}$	$\mathit{0.829}_{(.001)}$	$\mathit{0.475}_{(.034)}$		
Branch number	$0.300_{(.199)}$	$0.156_{(.512)}$	$0.117_{(.623)}$	$0.153_{(.521)}$	$0.285_{(.223)}$	$0.338_{(.145)}$	$0.263_{(.263)}$	$0.242_{(.304)}$	
Days to flowering	$\mathit{0.544}_{(.013)}$	$\mathit{0.478}_{(.033)}$	$\mathit{0.658}_{(.002)}$	$0.038_{(.873)}$	$\mathit{0.574}_{(.008)}$	$\mathit{0.577}_{(.008)}$	$0.340_{(.143)}$	$\mathit{0.471}_{(.036)}$	$0.051_{(.832)}$

Numbers in parentheses are *P* values. Italicise numbers are significant at alpha < .05

The five AFLP primer combinations generated a total of 447 bands of which 202 were polymorphic, representing 45% polymorphism (supplemental Table S9) and therefore increasing the genetic information on our entries significantly. The mean PIC for the five AFLP primer combinations was 0.4 and ranged from 0.33 (H-AAC/M-ACA, H-AAC/M-CCA) to 0.56 (H-AAC/M-ACT). The phylogenetic analysis using the 202 AFLP and 16 SSR markers separated the entries into two major clusters representing *S. villosum* and *S. scabrum*. Entry IP02 did not fall into either of the two clusters but showed some affiliation with *S. scabrum* (Fig. 3). Two sub-clusters could be detected within the *S. scabrum* cluster. Sub-cluster I consisted of advanced breeding lines and released cultivars, while sub-cluster II formed three clades consisting of accessions (IIc), cultivars (IIb) and entry BG07 (IIa). The *S. villosum* cluster formed two sub-clusters. Sub-cluster I consisted of local cultivars with a prostrate growth habit and was supported by a high bootstrap value of 100%. Sub-cluster II had two clades consisting of cultivars (IIb) and accessions (IIc). Entry BG03 was grouped into its own clade (IIa) separated from the rest of the accessions.

Discussion

In the present study, we demonstrate that morphological characteristics important for agronomic use vary significantly between entries of African nightshades. In addition, extensive genetic variability both within and between accessions indicates that a large potential for breeding advanced germplasm exists in this crop.

Among the morphological characteristics that we measured in our collection of entries, biomass (measured as both dry mass and fresh mass) of leaf yield, leaf area and the number of days to flowering contribute to the amount of harvested biomass that can be used for consumption. Here, we publish the first comparative study on morphological and genetic parameters of a large number of African nightshade entries. The number of days to flowering influences the harvest in that with the onset of flowering, the generation of new leaf biomass is drastically reduced (Ojiewo et al. 2013). For all of these traits, we found significant variation between entries, indicating a large potential for breeding superior populations/entries (Awas et al. 2015).

Correlation analysis is useful for determining traits influencing variation as well as interrelatedness when selecting desirable parental lines towards yield improvement (Mohammed et al. 2012). A positive correlation between fresh mass and descriptors related to yield such as leaf area, height and stem diameter indicates, that these traits should be prioritized when selecting for high yield. Positively correlated traits may have a common genetic or physiological basis as reported in eggplant (Kaushik et al. 2018). The negative correlation between fruit traits and other desirable yield traits indicates that high fresh yield may not be achieved simultaneously with high fruit yield. This implies that an increase in one trait would result in the decrease in another (Ganguly and Bhat 2012). In addition, quality parameters such as bitterness and taste and post-harvest traits (for example, shelf life) are also very important characteristics that determine the quality of an entry and should be addressed in future research.

In addition to information about the variability of agronomical traits, the genetic diversity present in a crop species is of prime importance for both the development of breeding strategies and conservation strategies (Caballero et al. 2010). Conservation of genetic resources in gene banks is costly due to the high costs of the preservation of seeds or living plants and the sophistication needed for evaluation and documentation (Arif et al. 2010). Information on the genetic diversity within and between entries of a crop therefore helps to focus on a broad diversity of the gene pool of a species and to not waste resources on redundant samples.

The development of breeding strategies benefits from information about the genetic diversity of a crop in several ways. Diversity within an entry of a species implies potential genetic variation useful for selection (Costa et al. 2016). If the diversity within entries is high, then this also means that selection conducted on these entries was not substantial in the past, that this entry cannot necessarily be treated as a genetic unit in further crosses and that selection within the entry might contribute to further development of superior germplasm.

African nightshades have been investigated with molecular markers in several studies (Dehmer and Hammer 2004; Jacoby et al. 2003; Manoko et al. 2007, 2008; Olet et al. 2011). Most of the studies focused on the analysis of the so-called "*Solanum*

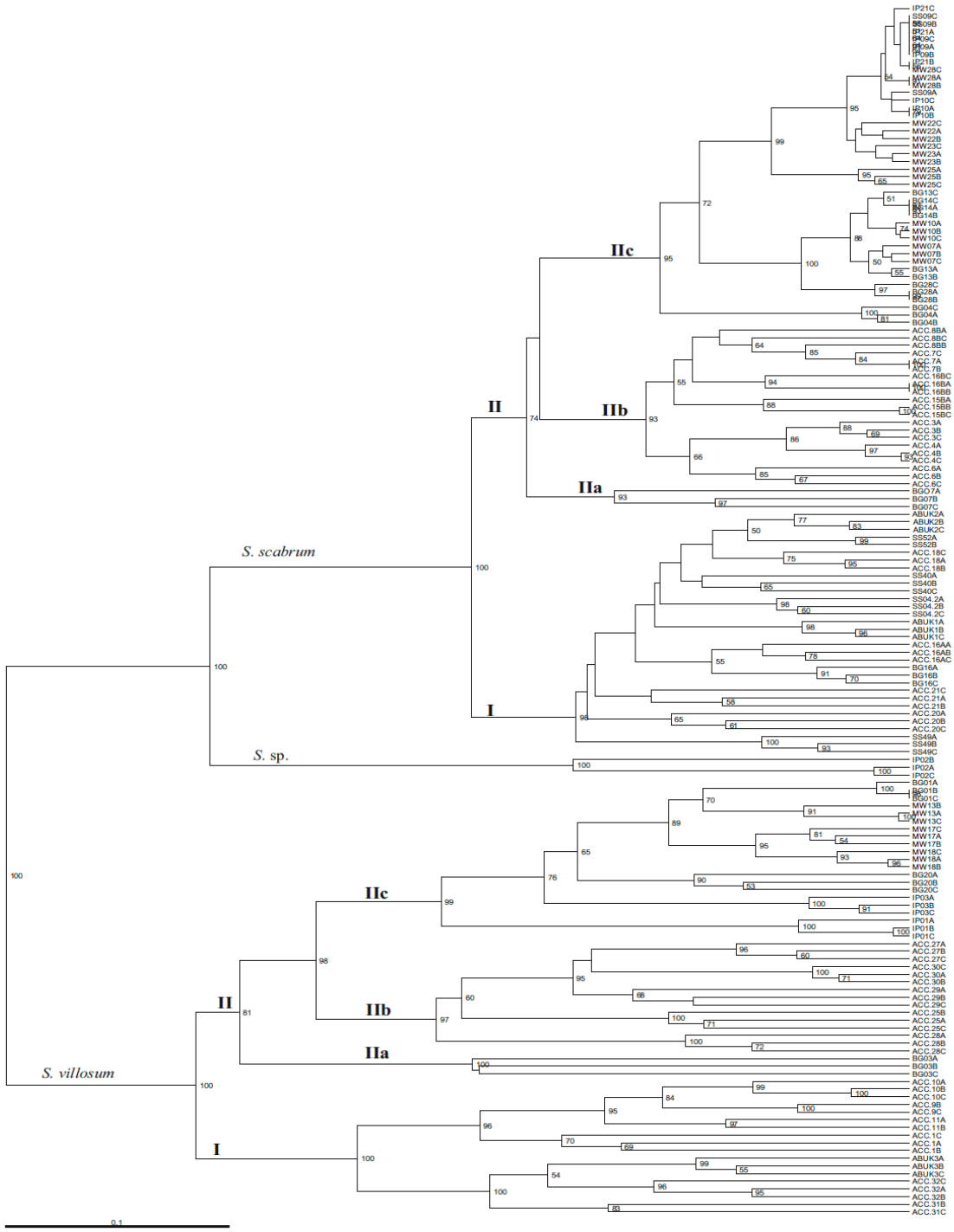

Fig. 3 UPGMA dendrogram generated from pooled 202 AFLP and 16 SSR markers. Numbers at the nodes are bootstrap values > 50. Letters A, B and C denote different genotypes of the same entry

nigrum complex" species closely related to *S. nigrum*. In these analyses, *S. villosum* and *S. scabrum* were found to be closely related to each other but clearly formed separate clades. However, to date, only two studies have investigated a considerable number of entries of both *S. villosum* and *S. scabrum* (Poczai et al. 2010; Poczai and Hyvönen 2011). Although these studies analysed six *S. scabrum* and nine *S. villosum* entries, only single genotypes/samples per entry were analysed, and thus no information about the variability within entries is available.

In the present study, we analysed 33 entries of *S. scabrum*, 20 entries of *S. villosum* and one entry with unclear taxonomic assignment with six individuals per entry for the SSR analyses and three individuals per entry for the AFLP analyses. The number of individuals in the AFLP analyses was reduced in order to avoid errors during scoring of fragments due to the large number of samples and fragments observed on the acrylamide gels. The diversity analysis was based on recently developed SSR markers (Ronoh et al. 2018), which were complemented with AFLP markers to increase the reliability of the diversity measures. High bootstrap values for the species clusters and for most of the sub-clusters indicate that the topology of the dendrogram is reliable.

Genetic diversity was analysed using SSR markers because of their high discriminating capacity and ability to detect high levels of polymorphism at each locus (Vieira et al. 2016). Furthermore, these markers can easily be adopted by laboratories in developing countries, as the technical requirements are considerably low (Zapata et al. 2012). AFLP markers were used for the phylogenetic analysis because they have a higher marker index due to the effective multiplex ratio (Indrees and Irshad 2014). The plant material used in this study consisted of entries with varying degrees of breeding history in order to understand the available diversity within the germplasm collection.

The 54 entries yielded a mean number of 1.834 alleles across 16 loci, an equivalent of 99 alleles and 30 private alleles, of which 12 were found in entry IP02. When local cultivars and developed lines were analysed together, 33 entries of *S. scabrum* generated 4 private alleles, and 20 *S. villosum* entries generated 14 private alleles. The diversity index for *S. villosum* was 0.56, while the diversity index for *S. scabrum* was 0.48. This indicates that the *S. scabrum* entries were more similar to each other than were the *S. villosum* entries, even when only the breeding lines were compared. This may be attributed to the selection *S. scabrum* entries have undergone for a longer period of time, since this species is more popular to farmers due to its higher leaf yield and later flowering (Manoko et al. 2008). The slightly higher observed heterozygosity in *S. scabrum* could be explained by its higher ploidy level (Ojiewo et al. 2013; Ronoh et al. 2018).

Observed heterozygosity of local cultivars was slightly lower than that of developed cultivars. This has also been reported for eggplant complexes where local landraces had lower observed heterozygosity than commercial varieties (Muñoz-Falcón et al. 2009; Vilanova et al. 2014). Low observed heterozygosity indicates high degree of homozygosity due to autogamy and in non-hybrids such as landraces (Gramazio et al. 2017). *S. villosum* had higher expected heterozygosity compared to *S. scabrum* due higher allele diversity. Except for observed heterozygosity, *S. villosum* showed higher variability in all other aspects of diversity analysed.

Other studies using AFLPs also found that *S. villosum* showed more variability than did *S. scabrum*, although only a few genotypes were analysed compared to the present study (Dehmer and Hammer 2004; Olet et al. 2011).

The genetic diversity within entries showed a marked difference between the local cultivars and developed genotypes. As expected, higher allelic richness (1.71) was found among the local cultivars than among the accessions and released cultivars. Unique alleles also indicated considerable variation between the local cultivars and developed entries. The local cultivars also had higher genetic differentiation ($Fst = 0.27$), while the released cultivars showed the highest similarity (Table S7). These entries have already been released to the farmers and were more uniform than the breeding lines. The uniformity in germplasm may be attributed to continuous selection and crossing of related cultivars, which has led to narrowing of the genetic base. It has been proven that breeding efforts over time lead to a reduction in genetic diversity (van de Wouw et al. 2010). This makes the crop prone to attacks from pests and diseases and is a drawback to gains made in crop improvement (Keneni et al. 2012). It is therefore important to strike a balance between achieving high yield and the risk of crop failure due to changes in environmental conditions (Shukla and Mattoo 2013).

The impact of breeding on genetic diversity may be assessed in improved genotypes through different generations of breeding cycles (Henkrar et al. 2016; Mardi et al. 2011). Information from the assessment will enable determination of whether there is a need to incorporate more diverse gene pools such as landraces or even wild relatives (Duvick et al. 2004). This may be achieved by identifying allelic combinations of released cultivars by using molecular markers. Keeping track of these changes will therefore provide a genetic blueprint for future selection. Furthermore, it would be desirable to obtain molecular markers linked to key traits, such as biomass, stem diameter or leaf area, and to use these markers for selection of superior cultivars while keeping genetic diversity as high as possible (Fu 2015).

In summary, the molecular variance analysis showed greater variation within groups and species than between them. The corresponding fixation indices were highly significant ($P < 0.001$). The F-index between species is the highest, but there is a reduction in this index when the groups are compared (Table S8). This corroborates the result of the genetic and differentiation analysis that there is a reduction in genetic diversity in the released cultivars and accessions (Kaushik et al. 2018). The observed clustering suggests considerable differentiation according to the level of breeding. The cluster consisting of the *S. villosum* entries formed two sub-clusters. Sub-cluster I consists of individuals that are morphologically distinct in growth habit. While they are similar to the rest of the *S. villosum* entries in other aspects such as the colour of ripe berries and the type of inflorescence, this group exhibits a prostrate growth habit. This sub-cluster is supported by a high bootstrap value of 100%, which indicates that the entries of this cluster are derived from a separate gene pool carrying the allele combinations of genes responsible for the particular growth phenotype (Augustinos et al. 2016). Some accessions were also placed in a separate sub-cluster. The entry IP02, which was separated from the other entries with 12 private alleles in between the *S. scabrum* and the *S. villosum* clusters, had interestingly the lowest mean number of days to flowering from all 54 entries. In all other quantitative morphological traits IP02 showed values in the range of the *S. villosum* entries but had a purple fruit colour as all *S. scabrum* entries (supplemental Figures S2-S7, Table S2). Entry BG03 was placed in a separate clade from the other accessions. The genetic diversity analysis showed this entry to have the highest number of private alleles and higher allelic richness compared to the other *S. villosum* accessions. It is also late-flowering (approximately 78 days) and was one of the entries with the largest height, stem diameter and leaf area compared with the other *S. villosum* entries (supplemental Table S4). For *S. scabrum*, genetic variation was higher within entries than between entries. There were two sub-clusters of *S. scabrum*, with the advanced lines and released cultivars in one sub-cluster and the other accessions in another sub-cluster. Entry BG07 was separated from the rest of the accessions and formed a separate clade. This entry had the highest number of alleles, diversity index and allelic richness among the developed entries of both *S. scabrum* and *S. villosum*, but showed no noticeable phenotype beside the relatively high fruit number (supplemental Table S4). The clustering patterns were not correlated with the other morphological traits or with the geographic origin (data not shown).

In summary, we have shown that African nightshades display significant variability at both the morphological and genetic level, demonstrating the potential for selection of superior cultivars in the future. However, stronger selection within *S. scabrum* has already led to a decrease in genetic diversity compared to that in *S. villosum*.

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Compliance with ethical standards

Conflict of interest No potential conflicts of interest were reported by the authors.

References

Afari-Sefa V, Tenkouano A, Ojiewo CO, Keatinge JDH, Hughes J (2012) Vegetable breeding in Africa: constraints, complexity and contributions towards achieving food and nutritional security. Food Secur 4:115–127

Aggarawal RK, Hendre PS, Varshney RK, Bhat PR, Krishna-Kumar V, Singh L (2008) Identification, characterization and utilization of EST-derived genetic microsatellite marker for genome analyses of coffee and related species. Theor Appl Genet 114:359–372

Arif IA, Bakir MA, Khan HA, Farhan AH, Homaidan AA, Bahkali A, Sadoon M, Shobrak M (2010) A brief review of molecular techniques to asses plant diversity. Int J Mol Sci 11:2079–2096

Augustinos AA, Petropoulos C, Karasoulou V, Bletsos F, Papasotiropoulos V (2016) Assessing diversity among traditional Greek and foreign eggplant cultivars using molecular markers and morphometrical descriptors. Span J Agric Res 14(4):e0710. https://doi.org/10.5424/sjar/2016144-9020

Awas G, Mekbib F, Ayana A (2015) Variability, heritability and genetic advance for some yield related traits and oil content in Ethiopian coriander (*Coriandrum sanvum* L.) genotypes. Int J Plant Breed 9:116–125

Caballero A, Rodrıguez-Ramilo ST, Avila V, Fernandez J (2010) Management of genetic diversity of subdivided populations in conservation programmes. Conserv Genet 11:409–419

Cheng J, Zhao Z, Li B, Qin C, Wu Z, Trejo-Saavedra DL, Luo X, Cui J, Rivera-Bustamante RF, Hu K (2016) A comprehensive characterization of simple sequence repeats in pepper genomes provides valuable resources for marker development in *Capsicum*. Sci Rep 6:18919. https://doi.org/10.1038/srep18919

Choudhary G, Ranjit-Kumar N, Surapaneni M, Deborah AD, Anuradha G, Siddiq EA, Vernireddy LR (2013) Molecular genetic diversity of major Indian rice cultivars over decades. PLoS ONE. https://doi.org/10.1371/journal.pone.0066197

Costa R, Pereira G, Garrido I, Tavares-de-Sousa M, Espinosa F (2016) Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchard grass (*Dactylis glomerata* L.) germplasm variations. PLoS ONE 11(4):e0152972. https://doi.org/10.1371/journal.pone.0152972

Dehmer KJ, Hammer K (2004) Taxonomic status and geographic provenance of germplasm entries in the *Solanum nigrum* L. complex: AFLP data. Genet Resour Crop Evol 51:551–558

Dinssa FF, Stoilova T, Nenguwo N, Aloyce A, Tenkouano A, Henson P, Hughes J, Keatinge JDH (2015) Traditional vegetables improvement and development in sub-Saharan Africa at AVRDC—The World Vegetable Center. Acta Hortic 1102:21–28

Duvick DN, Smith JSC, Coope M (2004) Changes in performance parentage, and genetic diversity of successful corn hybrids 1930 to 2000. In: Smith WC, Betrán J, Runge ECA (eds) Corn: origin, history technology and production. Wiley, Hoboken, pp 65–97

Excoffier L, Lischer H (2010) Arlequin version 3.5: an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Fu Y (2015) Understanding crop genetic diversity under modern plant breeding. Theor Appl Genet 128:2131–2142

Ganguly PR, Bhat KV (2012) Analysis of agronomically important morphological traits diversity in *Vigna radiata*. Int J Biomed Life Sci 3:84–92

Garcia A, Benchimol LL, Barbosa AM, Geraldi I, Sauza CL, Sauza AP (2004) Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genet Mol Biol 27:579–588

Gramazio P, Prohens J, Borrás D, Plaza M, Harraiz FJ, Villaniva S (2017) Comparison of transcriptome-derived simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers for genetic fingerprinting, diversity evaluation and establishment of relationships in eggplants. Euphytica 213:264. https://doi.org/10.1007/s10681-017-2057-3

Henkrar F, El-haddoury J, Ouabbou H, Nsarellah N, Iraqi D, Bandaou N, Udupa SM (2016) Genetic diversity reduction in improved durum wheat cultivars of Morocco as revealed by microsatellite markers. Sci Agric 2:134–141

Indrees M, Irshad M (2014) Molecular markers in plants for analysis of genetic diversity; a review. Eur Acad Res 2:1513–1540

Jacoby A, Labuschagne MT, Viljoen CD (2003) Genetic relationships between southern African *Solanum retroflexum* Dunal and other related species measured by morphological and DNA markers. Euphytica 132:109–113

Kaushik P, Plaza M, Prohens J, Vilanova S, Gramazio P (2018) Diallelic genetic analysis for multiple traits in eggplant and assessment of genetic distances for predicting hybrids performances. PLoS ONE 13(6):0199943

Keneni G, Bekele E, Imtiaz M, Dagne K (2012) Genetic vulnerability of modern crop cultivars: causes, mechanism and remedies. Int J Plant Res 2:69–79

Manoko MLK, van den Berg RG, Feron RMC, van der Weerden GM, Mariani C (2007) AFLP markers support separation of *Solanum nodiflorum* from *Solanum americanum* sensu stricto (*Solanaceae*). Plant Syst Evol 267:1–11

Manoko MLK, van den Berg RG, Feron RMC, van der Weerden GM, Mariani C (2008) Genetic diversity of the African hexaploid species *Solanum scabrum* Mill. and *Solanum nigrum* L. (Solanaceae). Genet Resour Crop Evol 55:409–418

Mardi M, Naghavi MR, Pirseyedi SM, Alamooti MK, Monfared SR, Ahkami AH, Omidbakhsh MA, Alavi NS, Shanjani PS, Katsiotis A (2011) Comparative assessment of SSAP, AFLP and SSR markers for evaluation of genetic diversity of Durum wheat (*Triticum turgidum* L.var. *durum*). J Agric Sci Technol 13:905–920

Mohammed A, Geremey B, Amsalu A (2012) Variation and association of quality parameters in Ethiopian Durum wheat (*Triticum turgidum* L. var. *durum*) genotypes. Int J Plant Breed Genet 6:17–31

Muńoz-Falcón JE, Prohens J, Vilanova S, Ribas F, Castro A, Nuez F (2009) Distinguishing a protected geographical indication vegetable (*Almagro* eggplant) from closely related varieties using morphological traits and molecular markers. J Sci Food Agric 89:320–328

Ojiewo C, Mwai GN, Abukutsa-Onyango MO, Agong S, Nono-Wamdim R (2013) Exploiting the genetic diversity of vegetable African nightshade. Bioremediation Biodivers Bioavailab 7:6–13

Olet EA, Heun M, Lye KA (2005) African crop or poisonous nightshade; the enigma of poisonous or edible black nightshade solved. Afr J Ecol 43:158–161

Olet EA, Lye KA, Heun M (2011) Amplified fragment length polymorphisms (AFLPs) analysis of species of *Solanum* section *Solanum* (*Solanaceae*) from Uganda. Afr J Biotech 10:6387–6395

Page RDM (1996) TREEVIEW. An application to display phylogenetic tree on personal computers. Comput Appl Biosci 12:357–358

Pavlicek A, Hvda S, Flegr J (1999) Freeware program for construction of phylogenetic trees on the basis of distance data and bootsrap/jacknife analysis of the tree robustness. Application in the RAPD analysis of the genus *Frenkelia*. Folia Biol 45:97–99

Peakall R, Smouse PE (2012) GenAlex 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Poczai P, Hyvönen J (2011) On the origin of *Solanum nigrum*. Can network help? Mol Biol Reprod 38:1171–1185

Poczai P, Taller J, Szabo I (2008) Analysis of phylogenetic relationships in the genus *Solanum* (Solanaceae) as revealed by RAPD markers. Plant Syst Evol 275:59–67

Poczai P, Matyas K, Taller J, Szabo I (2010) Study of the origin of the rarely cultivated edible *Solanum* species: morphological and molecular data. Biol Plant 54:543–546

Poczai P, Cernak I, Varga I, Hyvonen J (2014) Nuclear intro-targeting markers in genetic analysis of black nightshade (*Solanum* sect. *Solanum,* Solanaceae) accessions. Genet Resour Crop Evol 61:247–266

Roldan-Ruiz J, Van Bockstaele E, Depicker A, De Loose M (2000) AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Mol Breed 6:125–134

Ronoh R, Linde M, Winkelmann T, Abukutsa-Onyango M, Dinssa F, Debener T (2018) Development of next generation sequencing (NGS) based SSRs in African nightshades; tools for analysis of genetic diversity in conservation and breeding. Sci Hortic 235:152–159

Salmi H, Bahar M, Mirlohi A, Talebi M (2016) Assessment of the genetic diversity among potato cultivars from different geographical areas using genomic and EST microsatellites. Iran J Biotechnol. https://doi.org/10.15171/isb.1280

San-Yi SJ, Atoi SA, Fujimura T, Yamanaka S, Watanabe J, Watanbe KN (2008) Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-center of origin. Plant Breed 127:189–196

Schippers RR (2002) African indigenous vegetables: an overview of cultivated species. Technical Centre for Agricultural and Rural Cooperation, University of Greenwich, Natural Resources Institute, London

Shukla V, Mattoo AK (2013) Developing robust crop plants for sustaining growth and yield under adverse climatic changes. In: Gill SS, Tuteja N (eds) Climate change and plant abiotic stress tolerance. Wiley-VCH, Weinheim, pp 27–56

Szpiech ZA, Jakobsson M, Rosenberg N (2008) ADZE; rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24:2498–2504

van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genet Resour 8:1–15

Vieira MLC, Santini L, Diniz AL, Munhoz CF (2016) Microsatellite markers: what they mean and why they are useful. Genet Mol Biol 39:312–328

Vilanova S, Hurtado M, Cardona A, Plazas M, Gramazio P, Herraiz FJ, Andujar I, Prohens J (2014) Genetic diversity and relationships in local varieties of eggplant from different cultivar groups as assessed by genomic SSR markers. Not Bot Horti Agrobot 42:59–65

Zapata JE, Cueras H, Zhu H, Steffen S, Senalik D, Zeldin D, McCown B, Harbut R, Simon P (2012) Using next generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. Am J Bot 99:193–208

Supplementary tables and figures

Table S1: Primer sequences and repeat motif of 16 microsatellite loci used in the study

Loci	Primer sequences	Expected fragment size	Repeat motif
Tet_Ole20163	F- GTAAAACGACGGCCAGTGCCACTGCTTTCTTATGATTTTCC	140	(TTTA)6
	R- GGAAAGTTCAATTTGACACGG		
Tet_Ole27092	F- GTAAAACGACGGCCAGTCTTGAGGAGCATGCAGAACA	161	(AAAT)6
	R- ATCTGTTACCTTCGAGGCGA		
Tet_Ole36530	F- GTAAAACGACGGCCAGTCGCGCTCAGAATTGGTTATT	150	(TTTC)8
	R- GGAAGAGATACATACGCATACCC		
Tet_Ole50895	F- GTAAAACGACGGCCAGTCGTGCCTAGTTTTACCCCAA	158	(TAAA)6
	R- TTAGTCCAACCCAAAACCCA		
Tet_Ole61583	F- GTAAAACGACGGCCAGTTTAAACATAGCCAACCCCCA	232	(AAAT)6
	R- TTCTTTGGCATGCTAGGATAGA		
Tet_Ole77901	F- GTAAAACGACGGCCAGTAGTCCGTTTCCTTTTGAACG	169	(TTTC)6
	R- GCAAATGCTGAATCATCACA		
Tet_Ole101141	F- GTAAAACGACGGCCAGTTTGATACAATGGGGGTGGTT	174	(GTAT)6
	R- TCCATCCATTAAAACGGAGC		
Tet_Ole106079	F- GTAAAACGACGGCCAGTCCTCTTGAGAAGCTCCAACG	168	(CAAA)6
	R- CGATGACCCAGATCCTGAAT		
Pen_Ole14507	F- GTAAAACGACGGCCAGTGAACCGTCCATCACCTGACT	165	(TTCTC)5
	R- TTGAGACGCACCTTTTGTTG		
Pen_Ole56306	F- GTAAAACGACGGCCAGTAGCAGATTACGCGGCTAGAA	251	(TTTTA)5
	R- TTTCCTTTAGAAGAATGGGACG		
Pen_Ole1865	F- GTAAAACGACGGCCAGTTGCTTAAGAGGTGCTTACGTG	258	(TAAAA)5
	R- GGAACAAATGCTTGTGAAGGA		
Pen_Ole69791	F- GTAAAACGACGGCCAGTTTGCATGCATTTCAACCAAC	219	(TCAAA)5
	R- TCATAGGTCCCCCACCACTA		
Hex_Ole56218	F- GTAAAACGACGGCCAGTCACCAAATAAATGGCTGCAT	260	(TTTCTT)4
	R- CCTGGATTGAACCTCTCCAA		
Hex_Ole111446	F- GTAAAACGACGGCCAGTGCTGCGAGGCTTTTAAACAA	121	(CTGAAA)4
	R- ACCAAACTACGCCAACGAAA		
Hex_Ole120086	F- GTAAAACGACGGCCAGTAATGGGCAAACGGTAGAGTG	258	(TTTTCG)4
	R- TGGAGAAGGAACAACATCTGG		
Hex_Ole51860	F- GTAAAACGACGGCCAGTCATCAATGGCTTCCTCATCA	162	(GAGTTG)4
	R- CCAGAAATTGAATGTCAGATGC		

Table S2: Qualitative traits of African nightshades assessed under field conditions

Entry	Ripe fruit color	Stem ridges	Leaf shape	Inflorescence type	Growth habit
ABUK1	purple	medium	elliptical	simple umbellate cyme	upright
ABUK2	purple	medium	ovate	simple umbellate cyme	upright
ABUK3	yellow	small	ovate	simple raceme-like cyme	prostrate
ACC.1	yellow	small	ovate	simple raceme-like cyme	prostrate
ACC.10	yellow	small	ovate	simple raceme-like cyme	prostrate
ACC.11	yellow	small	ovate	simple raceme-like cyme	prostrate
ACC.15B	purple	medium	ovate	simple+branched umbellate cymes	upright
ACC.16A	purple	medium	elliptical	simple umbellate cyme	bushy
ACC.16B	purple	medium	ovate	simple umbellate cyme	bushy
ACC.18	purple	large	ovate	simple umbellate cyme	upright
ACC.20	purple	large	ovate	simple umbellate cyme	upright
ACC.21	purple	large	ovate	simple umbellate cyme	upright
ACC.25	yellow	small	ovate	simple raceme-like cyme	upright
ACC.27	yellow	small	lanceolate	simple raceme-like cyme	upright
ACC.28	yellow	small	lanceolate	simple raceme-like cyme	upright
ACC.29	yellow	small	lanceolate	simple raceme-like cyme	upright
ACC.3	purple	medium	elliptical	simple umbellate cyme	bushy
ACC.30	yellow	small	lanceolate	simple raceme-like cyme	upright
ACC.31	yellow	small	lanceolate	simple raceme-like cyme	upright
ACC.32	yellow	small	ovate	simple raceme-like cyme	prostrate
ACC.4	purple	large	ovate	simple umbellate cyme	bushy
ACC.6	purple	medium	ovate	simple umbellate cyme	upright
ACC.7	purple	medium	ovate	simple umbellate cyme	upright
ACC.8B	purple	large	ovate	simple umbellate cyme	upright
ACC.9	yellow	small	ovate	simple raceme-like cyme	prostrate
BG01	red	small	ovate	simple raceme-like cyme	upright
BG03	red	medium	ovate	simple raceme-like cyme	upright
BG04	purple	small	elliptical	simple umbellate cyme	upright
BG07	purple	large	ovate	simple umbellate cyme	upright
BG13	purple	small	elliptical	simple umbellate cyme	bushy
BG14	purple	large	ovate	simple umbellate cyme	upright
BG16	purple	large	ovate	simple+branched umbellate cymes	upright
BG20	red	small	ovate	simple raceme-like cyme	upright
BG28	purple	medium	ovate	simple raceme-like cyme	upright
IP01	red	medium	ovate	simple raceme-like cyme	upright
IP02	purple	small	ovate	simple umbellate cyme	upright
IP03	red	medium	lobed	simple raceme-like cyme	upright
IP09	purple	large	ovate	simple umbellate cyme	upright
IP10	purple	medium	ovate	simple umbellate cyme	upright
IP21	purple	large	ovate	simple umbellate cyme	upright

MW07	purple	medium	ovate	simple umbellate cyme	upright
MW10	purple	large	ovate	simple umbellate cyme	upright
MW13	red	medium	lobed	simple raceme-like cyme	upright
MW17	red	medium	lobed	simple raceme-like cyme	upright
MW18	red	medium	ovate	simple raceme-like cyme	upright
MW22	purple	medium	ovate	simple umbellate cyme	upright
MW23	purple	medium	ovate	simple umbellate cyme	upright
MW25	purple	large	ovate	simple umbellate cyme	upright
MW28	purple	large	ovate	simple umbellate cyme	upright
SS04.2	purple	large	ovate	simple+branched umbellate cymes	upright
SS09	purple	large	ovate	simple umbellate cyme	upright
SS40	purple	large	ovate	simple+branched umbellate cymes	upright
SS49	purple	large	ovate	simple+branched umbellate cymes	upright
SS52	purple	large	ovate	simple+branched umbellate cymes	upright

Table S3: Separation of means for quantitative traits of *S. scabrum* entries

Entry	Height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Fruit mass (g)	Fruits number	Fresh mass (g)	Dry mass (g)	Peduncle length (mm)	Number of branches	Days to flowering
ABUK1	57.00^{gh}	13.00^{abc}	50.44^{ijk}	1.517^{abcde}	66.7^{jklm}	120.50^{cd}	16.18^{bcd}	5.667^{lmn}	28.17^{bcd}	76.33^{ijk}
ABUK2	78.67^{cdefgh}	14.17^{abc}	130.00^{defgh}	1.383^{abcde}	124.7^{fghijklm}	168.9^{bcd}	17.68^{abcd}	5.850^{klmn}	26.33^{bcdef}	89.33^{cde}
ACC.15B	98.83^{abcdef}	15.00^{abc}	46.31^{jk}	1.483^{abcde}	207.3^{cdefghi}	234.30^{abc}	28.45^{abc}	5.367^{mn}	37.00^{ab}	80.33^{fghij}
ACC.16A	64.33^{fgh}	13.25^{abc}	43.30^{jk}	1.400^{abcde}	55.5^{klm}	219.50^{abc}	26.22^{abcd}	7.633^{hijklm}	46.50^{a}	79.33^{ghijk}
ACC.16B	91.30^{abcdefg}	15.75^{abc}	20.98^{k}	1.433^{abcde}	186.8^{cdefghijk}	223.4^{abc}	27.58^{abcd}	4.850^{n}	42.17^{a}	81.33^{fghij}
ACC.18	63.83^{fgh}	13.08^{abc}	81.71^{hij}	1.467^{abcde}	50.3^{lm}	121.60^{cd}	12.35^{cd}	6.367^{jklmn}	12.00^{hi}	89.33^{cde}
ACC.20	89.17^{abcdefg}	15.58^{abc}	60.52^{ijk}	1.317^{bcde}	112.0^{ghijklm}	173.30^{bcd}	22.60^{abcd}	6.583^{jklmn}	26.00^{bcdefg}	89.00^{cde}
ACC.21	96.17^{abcdef}	17.75^{ab}	86.38^{ghij}	1.550^{abcde}	93.2^{ijklm}	345.9^{a}	39.37^{ab}	6.633^{jklmn}	24.67^{bcdefgh}	89.00^{cde}
ACC.3	55.17^{gh}	13.33^{abc}	47.24^{ijk}	1.600^{abcde}	60.2^{jklm}	164.4^{bcd}	20.45^{abcd}	5.583^{lmn}	21.00^{cdefgh}	85.00^{efgh}
ACC.4	75.33^{defgh}	18.50^{a}	172.40^{cd}	1.450^{abcde}	98.3^{hijklm}	314.9^{ab}	41.60^{a}	5.550^{lmn}	33.67^{abc}	90.00^{cde}
ACC.6	46.60 ^{h}	11.08^{c}	87.34^{fghij}	1.683^{abcde}	17.5^{m}	99.73^{cd}	13.52^{cd}	5.483^{lmn}	13.67^{efghi}	83.67^{efghi}
ACC.7	47.75^{h}	12.25^{bc}	104.96^{efghi}	1.517^{abcde}	16.8^{m}	31.02^{cd}	2.90^{d}	5.333^{mn}	5.50^{i}	81.33^{fghij}
ACC.8B	68.33^{efgh}	18.08^{ab}	145.29^{cdef}	1.233^{cdef}	82.5^{ijklm}	235.10^{abc}	23.62^{abcd}	11.817^{abcd}	26.67^{bcde}	89.00^{cde}
BG04	107.75^{abcd}	14.17^{abc}	140.44^{cdefg}	1.833^{ab}	347.5^{b}	113.17^{cd}	18.17^{abcd}	8.383^{ghij}	21.33^{cdefgh}	75.33^{jk}
BG07	95.83^{abcdef}	14.50^{abc}	87.82^{fghij}	1.482^{abcde}	312.0^{bc}	131.67^{cd}	21.50^{abcd}	7.750^{hijkl}	22.33^{cdefgh}	90.00^{cde}
BG13	117.00^{ab}	14.50^{abc}	50.243^{ijk}	1.578^{abcde}	271.0^{bcde}	116.50^{cd}	9.67^{cd}	7.433^{ijklm}	20.83^{cdefgh}	72.67^{kl}
BG14	122.42^{ab}	15.00^{abc}	146.45^{cde}	1.223^{cdef}	283.3^{bcde}	133.00^{cd}	11.83^{cd}	10.467^{abcdefg}	21.00^{cdefgh}	77.33^{ijk}
BG16	94.83^{abcdef}	17.67^{ab}	371.50^{a}	1.505^{abcde}	231.7^{bcdefgh}	173.00^{bcd}	15.35^{bcd}	11.617^{abcde}	16.67^{defghi}	92.67^{cd}
BG28	110.40^{abcd}	13.67^{abc}	152.04^{cde}	1.407^{abcde}	300.3^{bcd}	166.00^{bcd}	24.67^{abcd}	8.067^{hijk}	18.17^{defghi}	80.33^{fghij}
IP09	102.25^{abcde}	13.50^{abc}	147.45^{cde}	1.288^{bcde}	286.7^{bcd}	145.67^{cd}	20.00^{abcd}	9.083^{fghi}	20.67^{cdefgh}	92.67^{cd}
IP10	103.60^{abcde}	12.17^{bc}	141.64^{cdefg}	1.545^{abcde}	276.3^{bcde}	118.67^{cd}	16.67^{abcd}	5.867^{klmn}	19.50^{defgh}	94.67^{bc}
IP21	124.33^{ab}	15.00^{abc}	193.05^{c}	1.692^{abcd}	149.5^{efghijklm}	136.33^{cd}	19.00^{abcd}	9.883^{bcdefgh}	14.67^{efghi}	100.67^{b}
MW07	117.50^{ab}	15.83^{abc}	175.54^{cd}	1.820^{abc}	294.3^{bcd}	235.20^{abc}	33.00^{abc}	10.617^{abcdefg}	21.00^{cdefgh}	76.67^{ijk}
MW10	117.83^{ab}	13.67^{abc}	137.32^{cdefgh}	1.705^{abcd}	489.5^{a}	137.00^{cd}	16.17^{bcd}	8.700^{ghij}	22.67^{cdefgh}	80.00^{fghijk}
MW22	88.42^{bcdefg}	13.00^{abc}	160.57^{cde}	1.958^{a}	237.7^{bcdefg}	137.70^{cd}	21.83^{abcd}	11.417^{abcdef}	16.00^{defghi}	78.00^{hijk}
MW23	105.83^{abcd}	15.50^{abc}	151.24^{cde}	1.408^{abcde}	190.8^{cdefghij}	149.30^{bcd}	23.00^{abcd}	9.517^{defghi}	15.00^{defghi}	65.67^{l}
MW25	102.67^{abcde}	12.67^{abc}	139.53^{cdefgh}	1.202^{def}	238.2^{bcdefg}	184.33^{abcd}	28.67^{abc}	9.700^{cdefghi}	18.67^{defghi}	87.00^{def}
MW28	89.40^{abcdefg}	12.83^{abc}	175.09^{cd}	1.610^{abcde}	249.5^{bcdef}	112.67^{cd}	14.00^{cd}	9.383^{efghi}	19.17^{defgh}	85.00^{efgh}
SS04.2	114.75^{abc}	15.00^{abc}	169.10^{cd}	0.680^{f}	192.0^{cdefghij}	201.90^{abc}	18.97^{abcd}	12.017^{abc}	14.50^{efghi}	86.50^{defg}
SS09	113.83^{abc}	13.17^{abc}	141.07^{cdefg}	1.632^{abcde}	123.8^{fghijklm}	137.17^{cd}	18.33^{abcd}	7.767^{hijkl}	13.33^{fghi}	90.33^{cde}
SS40	125.25^{a}	16.00^{ab}	259.10^{b}	1.232^{cdef}	168.5^{defghijk}	241.10^{abc}	22.63^{abcd}	12.217^{ab}	22.67^{cdefgh}	92.67^{cd}
SS49	121.58^{ab}	14.83^{abc}	367.30^{a}	1.287^{bcde}	113.5^{ghijklm}	232.10^{abc}	21.68^{abcd}	12.417^{a}	12.83^{ghi}	116.50^{a}
SS52	116.67^{ab}	18.00^{ab}	174.36^{cd}	1.092^{ef}	122.0^{fghijklm}	244.40^{abc}	24.63^{abcd}	11.667^{abcde}	25.67^{bcdefg}	85.67^{defg}
Average	94.68	14.59	138.11	1.461	183.33	172.71	20.98	8.384	21.82	85.56

Turkey pairwise comparisons analysed at 95% confidence level. Means within columns that do not share a letter are significantly different.

Table S4: Separation of means for quantitative traits of *S. villosum* entries

Entry	Height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Fruit mass (g)	Fruit number	Fresh mass (g)	Dry mass (g)	Peduncle length (mm)	Number of branches	Days to flowering
ABUK3	34.00^{ef}	5.92^{cdefgh}	11.82^{de}	0.23^{b}	63.3^{c}	48.03^{cd}	7.25^{defg}	1.23^{h}	27.67^{b}	64.00^{efgh}
ACC.1	33.67^{ef}	8.17^{bcdefg}	38.90^{c}	0.28^{b}	78.8^{c}	55.45^{c}	5.30^{g}	4.47^{f}	3.83^{g}	62.00^{gh}
ACC.10	21.50^{f}	3.00^{h}	4.68^{e}	0.23^{b}	103.7^{c}	33.23^{d}	0.64^{h}	1.83^{gh}	8.17^{fg}	63.67^{efgh}
ACC.11	36.50^{ef}	5.42^{defgh}	8.92^{de}	0.30^{b}	106.0^{c}	41.55^{cd}	6.30^{efg}	1.68^{gh}	42.83^{a}	60.67^{h}
ACC.25	34.25^{ef}	5.67^{cdefgh}	16.25^{d}	0.20^{b}	134.0^{c}	46.63^{cd}	4.40^{gh}	1.97^{gh}	13.00^{def}	62.33^{fgh}
ACC.27	41.02^{def}	9.67^{abcd}	6.69^{de}	0.30^{b}	179.8^{bc}	98.65^{b}	13.67^{b}	2.42^{g}	21.00^{bc}	68.00^{de}
ACC.28	24.00^{f}	4.92^{fgh}	16.98^{d}	0.23^{b}	120.0^{c}	43.98^{cd}	4.37^{gh}	2.17^{g}	10.50^{efg}	75.67^{ab}
ACC.29	30.33^{ef}	4.42^{gh}	59.09^{a}	0.23^{b}	108.5^{c}	34.50^{cd}	0.48^{h}	2.07^{gh}	7.83^{fg}	72.33^{bcd}
ACC.30	62.00^{cd}	12.50^{a}	7.60^{de}	0.28^{b}	260.2^{ab}	92.80^{b}	19.28^{a}	2.28^{g}	7.67^{fg}	69.00^{cde}
ACC.31	51.17^{de}	8.17^{bcdefg}	5.46^{e}	0.25^{b}	112.2^{c}	34.48^{cd}	6.23^{efg}	1.92^{gh}	7.00^{fg}	61.00^{h}
ACC.32	29.67^{ef}	4.17^{gh}	11.03^{de}	0.23^{b}	79.0^{c}	29.67^{d}	6.73^{efg}	1.75^{gh}	5.83^{fg}	60.00^{h}
ACC.9	23.00^{f}	5.15^{efgh}	7.91^{de}	0.23^{b}	100.2^{c}	45.35^{cd}	5.38^{fg}	2.05^{gh}	17.67^{cde}	61.67^{gh}
BG01	84.83^{b}	9.33^{abcde}	49.95^{ab}	1.61^{a}	306.0^{a}	102.00^{ab}	8.33^{cdefg}	9.68^{a}	21.33^{bc}	67.33^{def}
BG03	119.17^{a}	10.83^{ab}	58.95^{a}	0.33^{b}	295.3^{ab}	103.17^{ab}	11.67^{bc}	6.35^{de}	21.17^{bc}	78.33^{a}
BG20	76.17^{bc}	9.83^{abc}	53.49^{a}	0.25^{b}	354.0^{a}	105.67^{ab}	11.17^{bcd}	5.50^{e}	26.17^{b}	75.00^{ab}
IP01	120.33^{a}	9.33^{abcde}	52.55^{a}	0.25^{b}	362.8^{a}	113.83^{ab}	9.67^{bcde}	6.37^{de}	20.00^{bcd}	69.00^{cde}
IP03	115.33^{a}	10.33^{ab}	39.82^{bc}	0.27^{b}	331.0^{a}	123.17^{a}	18.33^{a}	6.87^{cd}	22.50^{bc}	66.67^{efg}
MW13	121.58^{a}	11.67^{ab}	58.61^{a}	0.32^{b}	267.8^{ab}	110.17^{ab}	9.33^{cdef}	7.03^{bcd}	17.83^{cde}	74.33^{abc}
MW17	91.83^{b}	9.50^{abcd}	52.42^{a}	0.28^{b}	273.3^{ab}	122.50^{a}	18.83^{a}	7.80^{b}	21.00^{bc}	74.00^{abc}
MW18	82.50^{bc}	9.00^{abcdef}	53.81^{a}	0.25^{b}	338.3^{a}	103.00^{ab}	10.17^{bcde}	7.43^{bc}	21.83^{bc}	67.33^{def}
Average	61.64	7.85	30.75	0.33	198.7	74.39	8.88	4.14	17.24	67.62

Tukey pairwise- comparisons analysed at 95% confidence level. Means within columns that do not share a letter are significantly different.

Table S5: Coefficient of variance for quantitative traits of African nightshade entries

	CV (%) for;		
	all entries	*S. scabrum*	*S. villosum*
plant height	42.89	29.12	59.97
stem diameter	37.84	21.31	41.91
leaf area	86.56	59.76	72.64
fruit mass	64.00	22.66	93.94
fresh mass	67.00	54.48	47.90
fruit number	63.57	65.97	59.86
dry mass	74.34	61.25	63.06
peduncle length	49.11	31.03	64.25
branches number	50.77	46.10	57.19

Table S6: Genetic diversity analysis of 54 entries using 16 SSR markers

	Na	Ne	I	Ho	He	Ar	P
1. Developed entries							
S. scabrum							
ABUK1	1.69±0.12	1.65±0.12	0.46±0.08	0.64±0.12	0.33±0.06	1.06±0.06	0
ABUK2	1.75±0.11	1.73±0.11	0.51±0.08	0.73±0.11	0.37±0.05	1.06±0.05	0
BG04	1.75±0.14	1.71±0.12	0.49±0.09	0.69±0.12	0.35±0.06	1.06±0.06	1
BG07	2.56±0.22	1.98±0.15	0.60±0.09	0.66±0.11	0.44±0.05	1.94±0.14	0
BG13	1.81±0.16	1.75±0.14	0.51±0.09	0.68±0.12	0.35±0.06	1.12±0.08	0
BG14	1.69±0.12	1.69±0.12	0.48±0.08	0.69±0.12	0.34±0.06	1.00±0.00	0
BG16	1.75±0.11	1.70±0.12	0.49±0.08	0.70±0.11	0.35±0.06	1.06±0.06	0
BG28	1.75±0.14	1.73±0.13	0.50±0.09	0.66±0.12	0.35±0.06	1.06±0.06	0
IP09	1.62±0.12	1.62±0.12	0.43±0.09	0.62±0.12	0.31±0.06	1.00±0.00	0
IP10	1.69±0.15	1.64±0.13	0.45±0.09	0.62±0.12	0.32±0.06	1.06±0.06	0
IP21	1.62±0.12	1.62±0.12	0.43±0.09	0.62±0.12	0.31±0.06	1.00±0.00	0
MW07	1.69±0.12	1.69±0.12	0.48±0.08	0.69±0.12	0.34±0.06	1.00±0.00	0

MW10	1.69±0.12	1.69±0.12	0.48±0.08	0.69±0.12	0.34±0.06	1.00±0.00	0
MW22	1.69±0.12	1.69±0.12	0.48±0.08	0.69±0.12	0.34±0.06	1.00±0.00	0
MW23	1.69±0.12	1.69±0.12	0.47±0.08	0.69±0.12	0.34±0.06	1.00±0.00	0
MW25	1.81±0.19	1.76±0.15	0.51±0.09	0.56±0.13	0.35±0.06	1.12±0.12	0
MW28	1.62±0.12	1.62±0.12	0.43±0.09	0.62±0.12	0.31±0.06	1.00±0.00	0
SS042	1.81±0.14	1.78±0.12	0.54±0.08	0.72±0.11	0.38±0.06	1.12±0.08	0
SS09	1.62±0.12	1.62±0.12	0.43±0.09	0.62±0.12	0.31±0.06	1.00±0.00	0
SS40	1.62±0.13	1.63±0.13	0.43±0.09	0.62±0.12	0.31±0.06	1.00±0.00	0
SS49	1.69±0.12	1.64±0.12	0.45±0.08	0.63±0.12	0.32±0.06	1.06±0.06	0
SS52	1.94±0.19	1.79±0.14	0.55±0.09	0.69±0.11	0.38±0.06	1.25±0.14	0
Mean±SE	1.75±0.29	1.7±0.15	0.48±0.09	0.66±0.11	0.34±0.06	1.09±0.21	1
S. villosum							
BG01	1.56±0.18	1.55±0.18	0.41±0.1	0.56±0.13	0.29±0.06	1.13±0.12	0
BG03	2.50±0.32	1.92±0.21	0.65±0.11	0.53±0.11	0.39±0.06	2.12±0.33	3
BG20	2.25±0.25	1.74±0.19	0.54±0.08	0.54±0.12	0.37±0.06	1.75±0.17	0
IP01	2.25±0.27	1.91±0.18	0.62±0.11	0.66±0.11	0.39±0.06	1.62±0.15	1
IP03	1.50±0.16	1.50±0.16	0.39±0.09	0.56±0.13	0.28±0.06	1.00±0.00	0
MW13	1.50±0.16	1.50±0.16	0.39±0.09	0.56±0.13	0.28±0.06	1.00±0.00	0
MW17	1.69±0.17	1.61±0.17	0.47±0.09	0.57±0.13	0.33±0.06	1.25±0.14	0
MW18	1.56±0.16	1.56±0.16	0.43±0.09	0.62±0.12	0.31±0.06	1.00±0.00	0
Mean±SE	1.85±0.29	1.66±0.18	0.49±0.09	0.58±0.11	0.34±0.05	1.36±0.43	4
2. Cultivars							
S. scabrum							
ACC.3	1.75±0.14	1.71±0.13	0.49±0.88	0.64±0.11	0.35±0.06	1.19±0.10	0
ACC.4	1.69±0.15	1.68±0.15	0.46±0.09	0.61±0.12	0.32±0.06	1.13±0.12	0
ACC.6	1.75±0.14	1.68±0.14	0.47±0.09	0.62±0.12	0.33±0.06	1.12±0.08	0
ACC.7	1.75±0.14	1.69±0.12	0.49±0.09	0.64±0.12	0.34±0.06	1.06±0.06	1
ACC.8B	1.69±0.12	1.6±0.12	0.43±0.08	0.59±0.12	0.31±0.06	1.12±0.08	0
ACC.15B	1.63±0.12	1.62±0.12	0.43±0.09	0.61±0.12	0.31±0.06	1.12±0.08	0
ACC.16A	1.75±0.11	1.75±0.11	0.50±0.08	0.7±0.11	0.36±0.05	1.12±0.08	1
ACC.16B	1.75±0.14	1.73±0.13	0.50±0.09	0.68±0.12	0.35±0.06	1.06±0.06	0
ACC.18	1.69±0.12	1.69±0.12	0.48±0.08	0.69±0.12	0.34±0.06	1.00±0.00	0
ACC.20	1.87±0.15	1.79±0.14	0.54±0.09	0.7±0.11	0.37±0.06	1.19±0.10	0
ACC.21	1.81±0.14	1.74±0.13	0.51±0.08	0.69±0.11	0.36±0.06	1.13±0.08	1
Mean±SE	1.74±0.2	1.7±0.12	0.48±0.08	0.65±0.11	0.34±0.06	1.11±0.05	3
S. villosum							
ABUK3	1.81±0.16	1.67±0.13	0.48±0.09	0.63±0.12	0.33±0.06	1.19±0.10	1
ACC.25	2.06±0.28	1.93±0.22	0.63±0.12	0.69±0.11	0.41±0.06	1.69±0.20	2
ACC.29	2.00±0.24	1.82±0.21	0.59±0.10	0.70±0.11	0.39±0.06	1.44±0.18	0

Mean±SE	1.95±0.33	1.8±0.26	0.56±0.12	0.67±0.11	0.37±0.06	1.44±0.25	3
3. Local cultivars							
ACC.1	2.25±0.25	1.98±0.20	0.65±0.11	0.60±0.11	0.41±0.06	1.56±0.17	0
ACC.9	2.06±0.25	1.87±0.18	0.57±0.01	0.63±0.11	0.37±0.07	1.56±0.24	1
ACC.10	2.56±0.32	2.16±0.21	0.73±0.12	0.61±0.11	0.44±0.07	2.12±0.33	3
ACC.11	2.25±0.25	2.25±0.25	0.66±0.11	0.68±0.11	0.42±0.06	1.62±0.20	1
ACC.27	1.68±0.15	1.67±0.15	0.52±0.08	0.69±0.11	0.37±0.05	1.18±0.13	0
ACC.28	2.56±0.29	2.14±0.17	0.76±0.09	0.68±0.11	0.48±0.05	2.00±0.29	1
ACC.30	1.88±0.24	1.77±0.19	0.55±0.10	0.68±0.11	0.37±0.07	1.43±0.22	1
ACC.31	2.69±0.29	2.15±0.21	0.76±0.11	0.66±0.11	0.45±0.06	2.12±0.24	0
ACC.32	1.94±0.21	1.87±0.19	0.55±0.11	0.60±0.12	0.37±0.07	1.56±0.26	0
Mean±SE	2.21±0.35	1.98±0.27	0.64±0.11	0.65±0.1	0.41±0.05	1.71±0.35	7
All entries for;							
S. scabrum	1.75±0.32	1.70±0.15	0.48±0.09	0.66±0.11	0.34±0.06	1.09±0.60	4
S. villosum	2.00±0.38	1.81±0.27	0.56±0.09	0.63±0.10	0.37±0.04	1.50±0.42	14
Entry							
IP02	0.81±0.13	0.81±0.13	0.04±0.04	0.06±0.06	0.03±0.03	1.00±0.00	12

Na-mean number of alleles; Ne- effective number of alleles; I-Shannon's diversity index; Ho-observed heterozygosity; He- expected heterozygosity; Ar-allelic richness; P-private alleles.

Table S7: Genetic differentiation (Fst) in different groups of African nightshade entries

Locus	Local cultivars	Released cultivars	Accessions (*S. scabrum*)	Accessions (*S. villosum*)
Pen_Ole14507	0.038	0.000	0.003	0.008
Tet_Ole61583	0.136	0.000	0.198	0.013
Tet_Ole50895	0.345	0.194	0.387	0.130
Pen_Ole56305	0.345	0.009	0.239	0.374
Hex_Ole120080	0.083	0.098	0.119	0.111
Hex_Ole51860	0.217	0.000	0.119	0.020
Hex_Ole11446	0.151	0.000	0.158	0.000
TetO_le77901	0.554	0.000	0.160	0.159
TetO_le27092	0.182	0.000	0.000	0.163
Pen_Ole1865	0.358	0.178	0.071	0.313
Tet_Ole106079	0.107	0.000	0.001	0.177
Tet_Ole101141	0.029	0.004	0.001	0.007
Pen_Ole69791	0.312	0.000	0.001	0.087
Tet_Ole20163	0.939	0.000	0.000	1.000
Hex_Ole56218	0.347	0.000	0.000	0.795
Tet_Ole36530	0.207	0.000	0.051	0.164
mean	0.27±0.1	0.04±0.2	0.11±0.03	0.22±0.07

Table S8: Analysis of molecular variance (AMOVA) based on SSR data across populations of African nightshade entries

Source of variation	df	SS	MS	Variance components	% variation	Fixation indices	P-value
1. Species: *S. scabrum* and *S. villosum*							
Among species	1	529.937	574.17	1.750	34.04	0.379	0.001
Among entries	51	344.381	1.88	0.305	5.94	0.090	0.001
Among individuals	583	1799.750	5.18	3.087	60.02	0.340	
2. Groups: Local cultivars, released cultivars, accessions							
Among groups	2	251.12	106.72	0.584	13.13	0.307	0.001
Among entries	50	623.19	2.801	0.781	17.55	0.202	0.001
Among individuals	583	1799.75	5.175	3.087	69.32	0.131	
3. *S. scabrum*							
Among entries	32	120.66	3.872	0.065	2.12	0.021	0.09
Among individuals	363	1085.92	5.551	2.991	97.88		
4. *S. villosum*							
Among entries	19	303.18	15.957	0.816	20.07	0.28	0.001
Among individuals	220	715.50	6.254	3.252	79.93		

Significance at P<0.05

Table S9: Characteristics of AFLP markers used in 162 genotypes of African nightshades

Primer combination	total scored bands	polymorphic bands	PIC
H-AAC/M-AAC	88	59	0.42
H-AAC/M-CTA	98	47	0.35
H-AAC/M-ACA	84	25	0.33
H-AAC/M-CCA	67	9	0.33
H-AAC/M-ACT	110	62	0.56
Total	447	202	1.99
Average	89.4	40.4	0.4

PIC- polymorphic information content

Figure S1: Plant growth habits of *S. scabrum* entries SS49 (A) and ACC.16A (B); and *S. villosum* entries ACC. 25 (C) and ACC.11 (D).

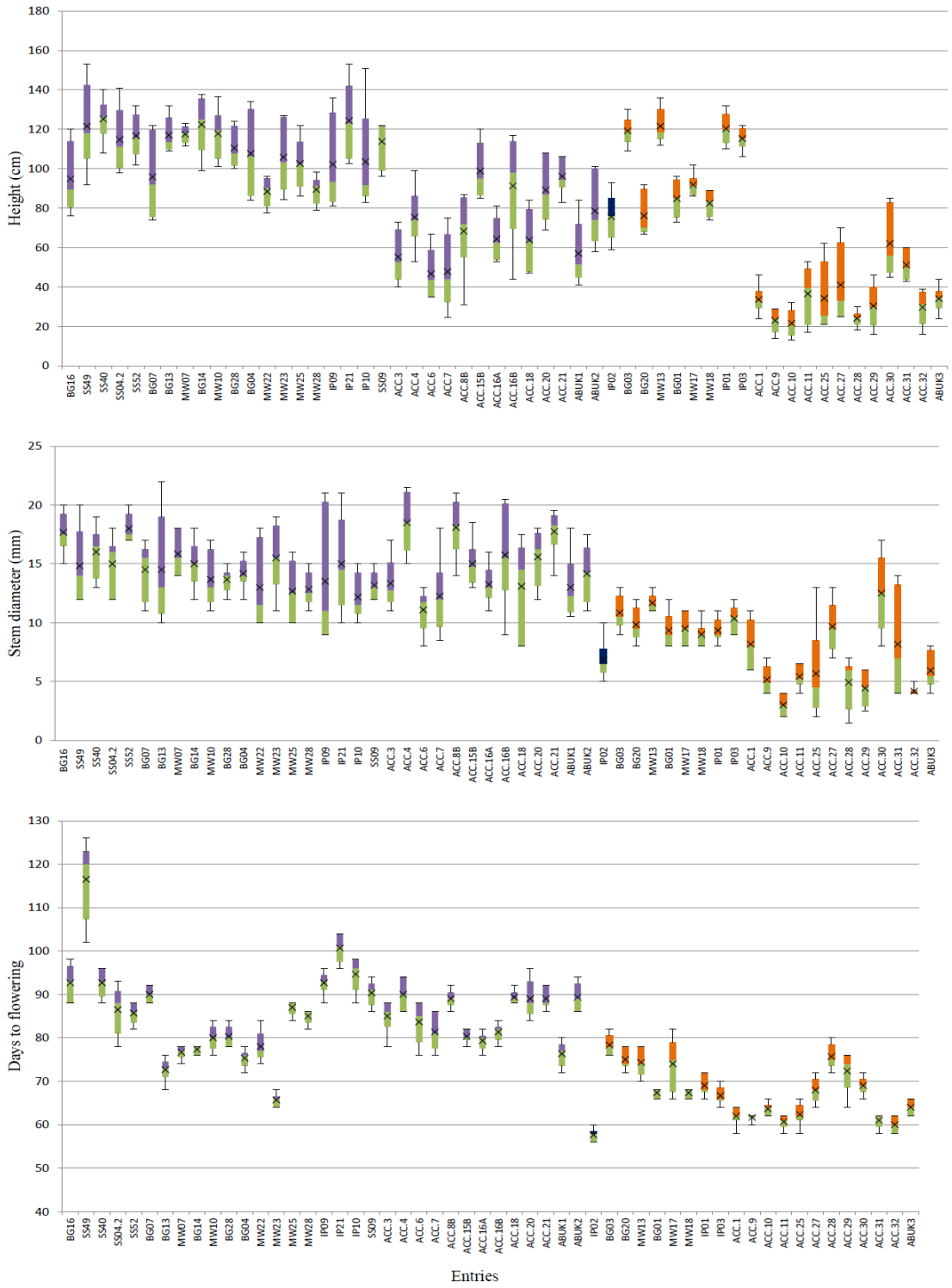

Figure S2: Descriptive statistics for height, stem diameter and days to flowering of *S. villosum* and *S. scabrum* entries. *S. scabrum* entries are labelled in purple while *S. villosum* entries are labelled in orange.

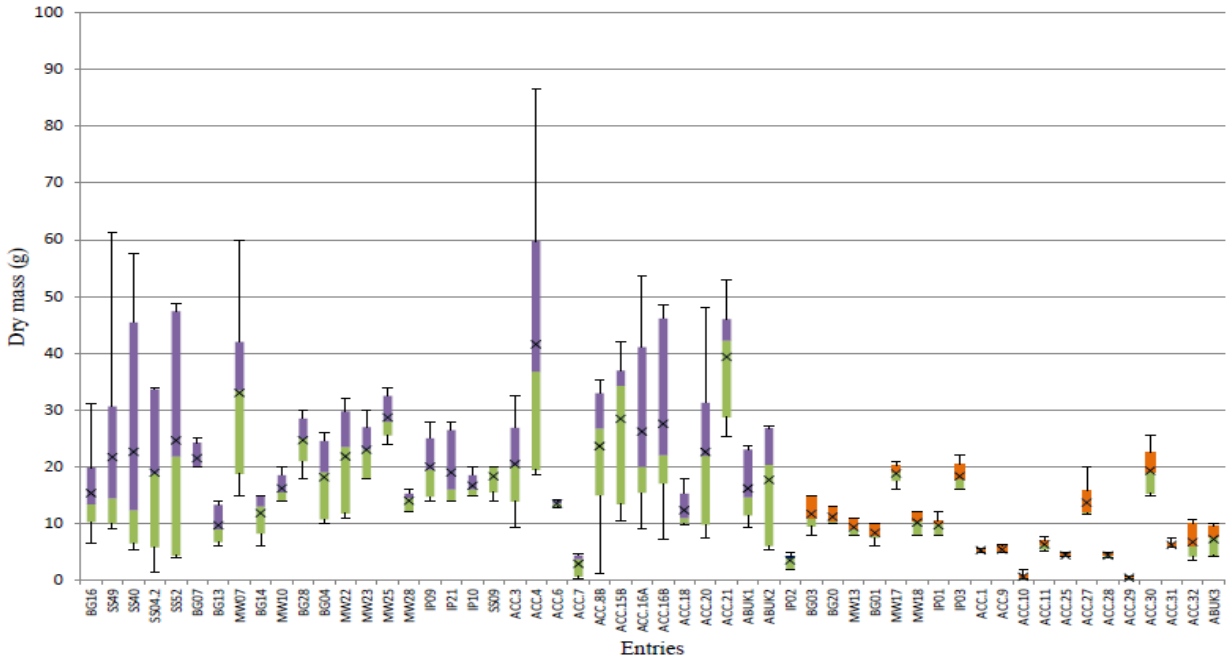

Figure S3: Descriptive statistics of dry mass of African nightshade entries. Entries labelled in purple are *S. scabrum* and entries labelled in orange are *S. villosum*.

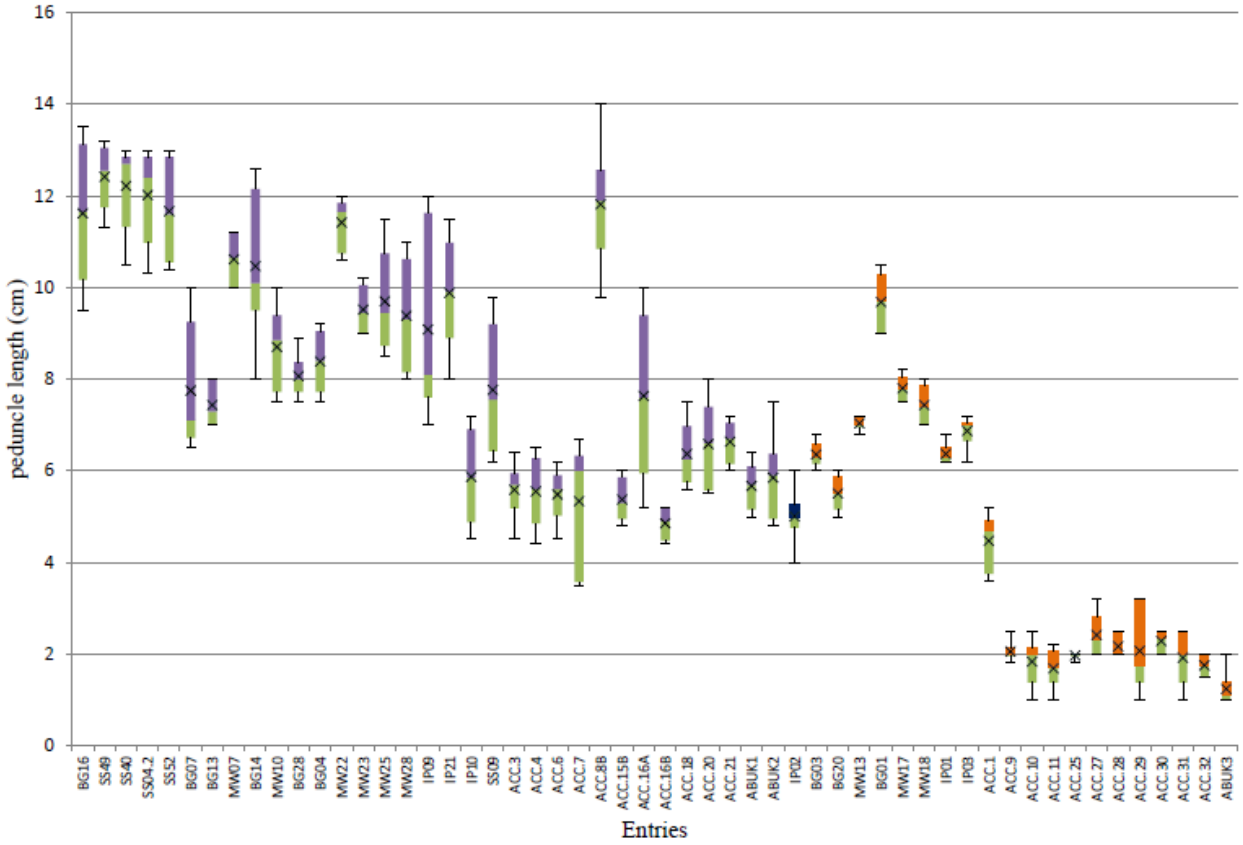

Figure S4: Descriptive statistics of peduncle length of African nightshade entries. Entries labelled in purple are *S. scabrum* and entries labelled in orange are *S. villosum*.

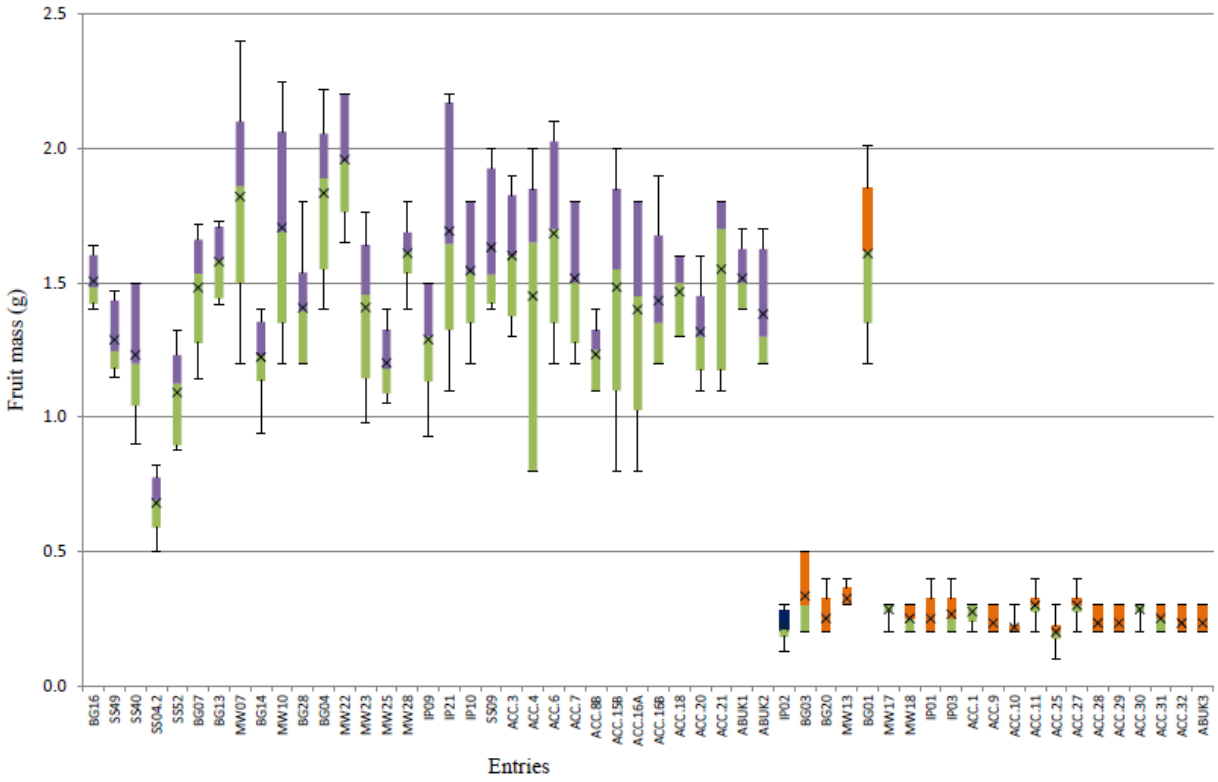

Figure S5: Descriptive statistics of fruit mass of African nightshade entries. Entries labelled in purple are *S. scabrum* and entries labelled in orange are *S. villosum*

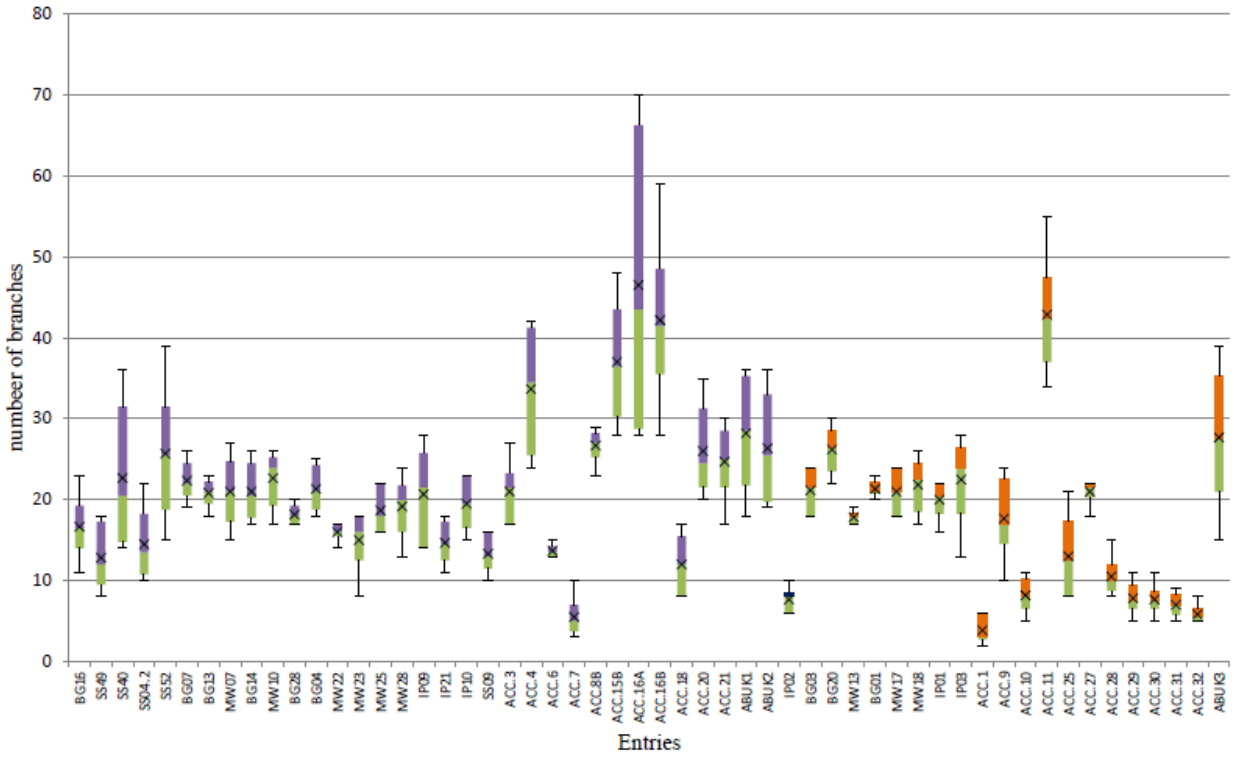

Figure S6: Descriptive statistics of number of branches of African nightshade entries. Entries labelled in purple are *S. scabrum* and entries labelled in orange are *S. villosum.*

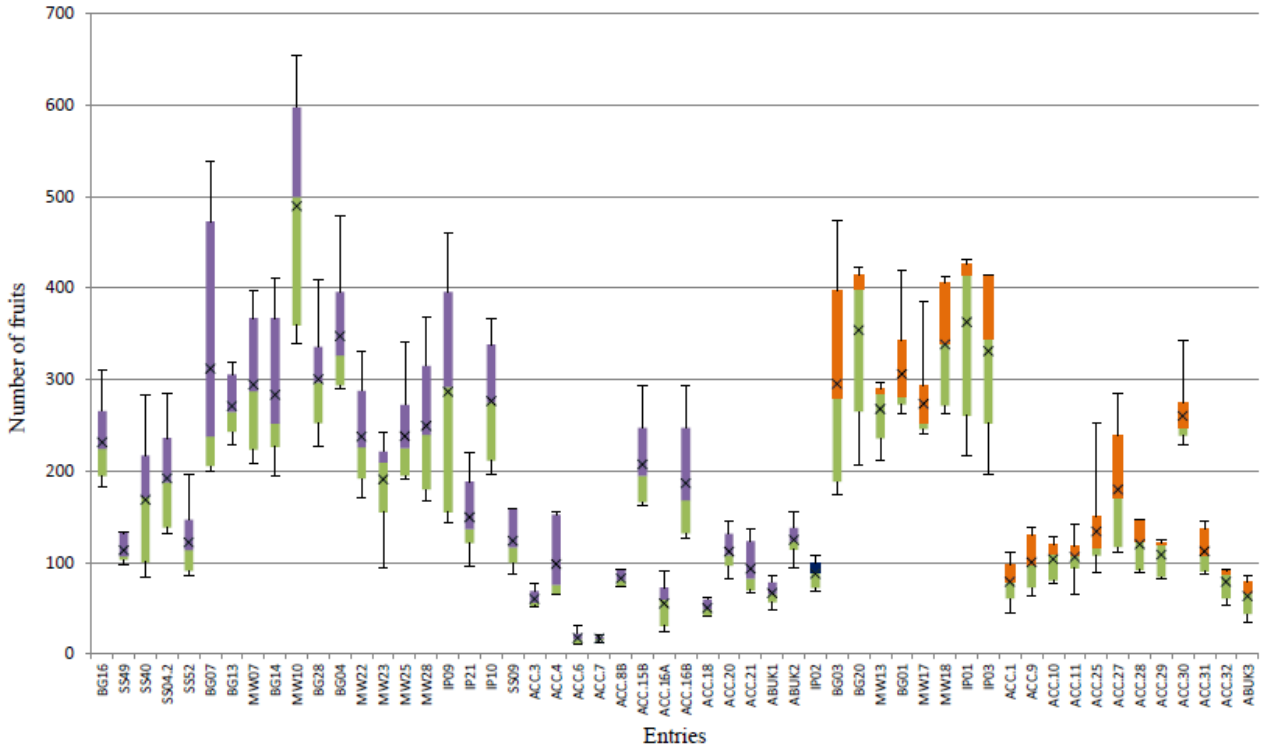

Figure S7: Descriptive statistics of the number of fruits of African nightshade entries. Entries labelled in purple are *S. scabrum* and entries labelled in orange are *S. villosum.*

3 General discussion, conclusions, recommendations

3.1 General discussion

The section *Solanum* is known for its taxonomic complexity in the genus *Solanum*, and although a number of studies using molecular markers have provided data on the interspecific relationships as summarized in Ronoh et al., (2017) very little information about genetic diversity within species or within accessions is available. Locus-specific markers, such as SSR, for diversity studies or even marker-assisted selection in African nightshade are lacking. Information about genetic diversity offers an opportunity to breeders to develop improved cultivars with farmers' preferences such as high yield and breeders preferred traits such as pest and disease resistance (Gorvindaraj et al., 2015).

Molecular marker technology has a potential to improve efficiency and precision of plant breeding in marker assisted selection and also maximize the probability of success in breeding for traits relating to yield stability and sustainability for example disease resistance, abiotic stress tolerance and nutrient/water use efficiency (Peleman and Van der Voort, 2003). DNA markers are more precise and faster than conventional breeding because target genotypes can be effectively selected and certain traits can be fast tracked resulting in quicker line development and cultivar release (Li et al., 2013). Use of markers for selection is also cost effective since they allow selection of desirable gene combinations in early generations and only high priority breeding material is maintained (Zapata et al., 2012). Plants can be selected for at seedling stage thus allowing early selection for traits which may be expressed in adult plants only. Target alleles that are difficult and/or time consuming to score phenotypically, for example environmentally sensitive traits, can be selected with the assistance of markers since they are neutral to

environmental variation (Jena and Mackill, 2008). With DNA markers recessive genes can be maintained without the need for progeny tests in each generation, as homozygous and heterozygous plants can be distinguished with the aid of co-dominant markers such as SSRs (Vieira et al., 2016).

Molecular markers, especially AFLPs and SSR are reported as appropriate tools for distinguishing plant varieties or lines (Bonow et al., 2009). Another potential application of AFLP or SSR markers is the identification of essentially derived varieties (EDV) in the context of variety registration (Heckenberger et al., 2003). Apart from cultivar identification, markers are also important in assessing genetic diversity present in the germplasm which gives detailed information to assist in parental selection and also in hybrids confirmation (Sakiyama et al., 2014; Yada et al., 2010). During seed handling seeds of different accessions may get mixed when handling large samples in breeding programs, and markers can be used to confirm the true identity of individual plants since DNA markers can be obtained from any plant tissue instead of growing the plants to maturity and then assessing them morphologically (Xu and Crouch, 2008). Markers also make it possible for introgression of beneficial traits from wild relatives and landraces during crop improvement (Hajjar and Hodgkin, 2007).

The concern that crop improvements lead to erosion of genetic diversity has been observed in several crops such as wheat (Christiansen et al., 2002; Figliuolo et al., 2005; Fu and Somers, 2009), rice (Steele et al., 2009; Sun et al., 2001; Wei et al., 2009), maize (Leclerc et al., 2005; Reif et al., 2005), sorghum (Casa et al., 2005; Deu et al., 2010) and tomato (Albrecht et al., 2010; Carelli et al., 2006; Yi et al., 2008). On the whole, substitution of local varieties by newly bred varieties has been seen as one of the main reasons for genetic biodiversity loss. Many cultivars in breeding programs are derived from crosses between genetically related varieties while wild

relatives are rarely included in crossings (Fu, 2006). The genetic base of the breeding material will eventually narrow down due to recurrent selection more so when fewer genotypes are selected as parents (Pereira et al., 2008; Vilela et al., 2008). Genetic uniformity often increases vulnerability of crops to environmental shocks such as diseases and pest outbreaks (Keneni et al., 2012). Molecular markers can provide new insights into questions regarding the loss of allelic variation through plant breeding practices (Rauf et al., 2010). To test the hypothesis that modern plant breeding narrows crop diversity over time AFLPs or SSR markers appear most useful to examine changes in the diversity. In most studies where SSR markers were used to measure decrease in genetic diversity it was noted that there was a decrease in either number of alleles per locus or reduction in gene diversity when landraces were compared to varieties (Donini et al., 2005; Vilela et al., 2008).

The SSR and AFLP markers already discussed in chapter two were able to discriminate entries between and within species. The discrimination power of these markers is an important aspect in variety identification and genetic assessment which is a step towards MAS. SSRs with high PIC have been found to be the most useful for application in marker assisted selection (Karakousi et al., 2003). Most of the SSR markers used in this study were highly informative with the highest PIC value of 0.71. SSRs may play an even more important role in ensuring diversity at all levels in breeding whereby cultivars are identified and those with high diversity can be used to develop core collections and therefore offer a broader genetic base for future crop improvement. An example is in rice where AFLP and SSR markers were utilized to develop rational strategies to broaden their genetic basis through plant introduction in order to improve yield (Mantegazza et al., 2008; Xu et al., 2004). Wild relatives and landraces are often inferior to the cultivated varieties with respect to quality or yield but they can be superior in terms of resistance.

Molecular markers offer the possibility of utilizing these resistances in plant breeding as has been the case for bacterial blight in rice, maize, and potato (Dwivedi et al., 2007). In the section *Solanum*, *S. nigrum* is known to carry resistance genes to *Phythopthora infestans* that infect most *Solanaceae* plants (Lebecka, 2009).

Entries from different countries of origin were grouped in the same clusters for both *S. scabrum* and *S. villosum*. This may be due to exchange of germplasm across geographical regions resulting in sharing of common genes. Gene bank accessions clustered together and had low genetic distance within cluster compared to farmer cultivars which also formed a separate cluster. Clustering patterns resulting from genetic distance may be used to select the best heterotic parents for constituting a core sample. It has been found that there is a positive correlation between genetic distance and hybrid performance calculated from AFLP data (Franco et al., 2005). This correlation may be used to predict performance of progeny resulting from hybridization of core samples.

Knowledge about the pollen viability is useful in identifying favourable genotypes in controlled hybridizations and, consequently, support breeding initiatives (Paula et al., 2014). A pollen viability study is commonly used in plant breeding for diverse species due to its ease, fast and low financial cost and the reliability of the technique (Saha and Datta, 2014). Analysis of *in vitro* germination of pollen is a popular method for assessing viability in breeding programs to ensure higher success rates of crosses and also to determine pollen vigor by assessing rate of germination and/or length of pollen tubes (Abdelqadir et al., 2011; Huang et al., 2013). To ensure success in the use of selected superior individuals, and especially production of new cultivars by means of recombination of traits by controlled hybridization, it is important that the pollen to be used has good viability (Criollo-Escobar and Dominguez, 2018). High meiotic

instability, associated with genetic abnormalities and/or chromosome aberrations that result in the formation of atypical or male-sterile plants, or those unable to form pollen grains, may hinder achieving the minimum standards required for seed production as well as affecting pollination (Pogorzelec et al., 2014). Pollen viability has been found to be directly proportional to seed and fruit set (Animasaun et al., 2014).

Pollen is also a useful source of rich allelic genepool and can be a reliable propagule for gene banks (Volk, 2011). The potential for immediate use of pollen, ease of storage and shipment provide researchers with increased options when designing their breeding programs. *In vitro* germination assays provide reliable viability assessments. Gene banked pollen ensures availability year-round and can be made available to breeders upon request (Shivanna, 2003). Pollen captures diversity within small sample sizes, and documentation may be available for long-term survival of pollen. Pollen can also be shipped internationally, often without threat of disease or pest transfer (Volk, 2011).

Nuclear DNA amount and genome size are important biodiversity characters, whose study provides comparative analysis of genome evolution in plants (Bennett and Leitch, 2011). It has been suggested that genome size have more general effect on multi-locus genetic fingerprinting techniques such as AFLP that assay the nuclear genome. In polyploids the genome size may be the factor effecting quality of AFLP as total nuclear DNA content increases. The use of the genome size (rather than nuclear DNA content) is therefore a more useful indicator of the utility of different AFLP protocols for polyploids (Fay et al., 2005). Genome size has also been used as a reliable guide in the separation of closely related species and their hybrids (Mahelka et al., 2005).

The flow cytometry data in this study was shown to support tetraploidy in *S. villosum* and hexaploidy in *S. scabrum*. Based on the mean DNA content of all the measured entries, *S. scabrum* had a 2C content of 6.14 pg (genome size =2.04 pg/2C) and 4.37 pg/2C for *S. villosum* (genome size =2.18 pg/2C). Both *S. villosum* and *S. scabrum* genotypes produced a maximum of three alleles per locus using the SSR markers. African nightshades have been thought to be allopolyploids since they show bivalent formation at metaphase (Ojiewo et al., 2007) whereas the SSR data in this study seem to support autopolyploidy. A study on karyotypes of *S. villosum* and *S. americanum* by Sultana and Alam (2007) also support autotetraploidy of *S. villosum* and considered it to be an ancient autotetraploid of *S. americanum*. Although the karyotypes showed bivalent formation at metaphase I and regular segregation at anaphase I, the symmetric karyotype showed *S. villosum* to be a primitive species. In general symmetrical karyotypes are regarded primitive and only show bivalent formation (Shan et al., 2003). Total DAPI-banding patterns, location and percentage of CMA-banded regions and karyotypes also indicated exact genomic duplication of *S. americanum*.

All the morphological traits studied showed significant variations ($P < 0.05$) indicating the presence of sufficient amount of variability both within and between the entries for all the traits. Significant positive correlations between fresh mass (which is equivalent to yield) and leaf area, plant height, branches number and days to flowering, indicate that in selecting high yielding genotypes these characters should be given more emphasis as the best selection criteria. Fruit mass and number always showed negative correlations with other desirable yield traits for *S. scabrum* entries. This indicates that the increase in one trait would result in the reduction of the other (Ganguly and Bhat, 2012). The negative correlation of fruit traits with other yield traits indicated that it would be very difficult to identify a genotype having higher fruit numbers

simultaneously with higher fresh mass (Mazid et al., 2013). Direct selections with a view to develop yield may be complicated because complex plant characters like yield are quantitatively inherited and influenced by genetic effects as well as by the interaction between genotype and environment and therefore indirect selection using highly correlated characters would be preferable (Ahmadikhah et al.,2008).

3.2 Conclusions and recommendations

Both AFLP and SSR markers identified entries that were previously misclassified. This was supported by DNA content measurements and some morphological traits such as colour of ripe berries. Further analysis of these entries however should be done by including clearly described accessions from the species they were originally classified.

The genetic diversity present in African nightshade entries support further selection of superior cultivars. Furthermore it would be desirable to obtain molecular markers linked to key traits such as fresh mass while keeping genetic diversity as high as possible, for example by integrating farmer cultivars. Morphological traits showed significant variation between entries also supporting a potential for breeding superior cultivars. Parameters such as taste, nutritional values and shelf-life should be addressed in future research as they determine the quality of an accession.

4 References

Abdelqadir, H.A., Johnson, S.D., Van Staden, J. (2011). Pollen viability, pollen germination and pollen tube growth in biofuel seed crop Jatropha curcas (*Euphobiaceae*). *South African Journal of Botany*, 79,132-139.

Afari-Sefa, V., Tenkouano, A., Ojiewo, C.O., Keatinge, J. D. H., Hughes, J. d'A. (2012). Vegetable breeding in Africa, constraints, complexity and contributions toward achieving food and nutritional security. *Food Security*, 4,115–127.

Ahmadikhah, A., Nasrollanejad, S., Alishah, O. (2008). Quantitative studies for investigating variation and its effect on heterosis of rice. International Journal of Plant. *International Journal of Plant Production*, 2, 297–308.

Albrecht, E., Escobar, M., Chetelat, R.T. (2010). Genetic diversity and population structure in tomato-like nightshades *Solanum lycopersicoides* and *S. sitiens*. *Annals of Botany*, 105, 535-554.

Alexander, D.H., Novembre, J., Lange, K. (2009). Fast model based estimation of ancestry in unrelated individuals. *Genome Research*, 19, 1655-1664.

Animasaun, D.A., Oyedeji, S., Azeez, M.A., Onyegwukwu, F. (2014). Evaluation of growth and pollen viability in relation to fruit set among five variaties of tomatoes grown in Nigeria. *Agronomski Glasnik*, 4, 203-218.

Arif, I.A., Bakir, M.A., Khan, H.A., Farhan, A.H., Homaidan, A.A., Bahkali, A., Sadoon, M., Shobrak, M. (2010). A brief review of molecular techniques to asses plant diversity. *International Journal of Molecular Science*, 11,2079-2096.

Ayua, E., Mugalavai, V., Simon, J., Weller, S., Obura, P., Nyabinda, N. (2016). Ascorbic acid content in leaves of nightshade (*Solanum* spp.) and spider plant (*Cleome gynandra*) varieties grown under different fertilizer regimes in Western Kenya. *African Journal of Biotechnology*, 15,199-206.

Bennett, M.D., Leitch, I.J. (2011). Nuclear DNA amounts in angiosperms, targets, trends and tomorrow. *Annals of Botany*, 107, 467–590.

Berinyuy, J.E., Fontem, D.A., Schippers, R.R. (2002). Morphological diversity of *Solanum scabrum* accessions in Cameroon. *Plant Renetic Resources Newsletter*, 131, 42-48.

Bonow, S., Von Pinho, E., Vieira, M., Vosman, B. (2009). Microsatellite markers in and around rice genes, applications in variety identification and DUS testing. *Crop Science*, 49, 886-880.

Boydston, R.A., Mojtahedi, H., Crosslin, J.M., Brown, C.R., Aderson, T. (2008). Effects of hairy nightshade (*Solanum sarrachoides*) presence on potato nematodes, disease and insect pests. *Weed Science*, 56, 151-154.

Caballero, A., Rodrıguez-Ramilo, S.T., Avila, V., Fernandez, J. (2010). Management of genetic diversity of subdivided populations in conservation programmes. *Conservation Genetics* 11, 409–419.

Carelli, B.P., Greald, L.T.S., Grazziotin, F.P., Echeverrigary, S. (2006). Genetic diversity among Brazillian cultivars and landraces of tomato *Lycopersicaon esculentum* Mill. revealed by RAPD markers. *Genetic Resources and Crop Evolution*, 53, 395-400.

Casa, A.M., Mitchell, S.E., Hamblin, M.T., Sun, H., Bowers, J.E., Aquadro, C.F. (2005). Diversity and selection in sorghum; simultanous analyses using simple sequence repeats. *Theorertical and Applied Genetics*, 111, 23-30.

Christiansen, M. J., Andersen, S. B., Ortiz, R. (2002). Diversity changes in an intensively bred wheat germplasm during the 20th century. *Molecular Breeding*, 9, 1-11.

Chweya, J.A., Eyaguirre, P.B. (1999). The Biodiversity of Traditional Leafy Vegetables. International Plant Genetic Resources Institute, Rome, Italy.

Costa, R., Pereira, G., Garrido, I., Tavares-de-Sousa, M., Espinosa, F. (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchard grass (*Dactylis glomerata* L.) germplasm variations. *PLoS ONE*, 11(4), e0152972.doi,10.1371/journal.pone.0152972.

Criollo-Escobar, H., Dominguez, J.J. (2018). Germinability and pollen viability of four improved cultivars of palm oil under laboratory conditions. *Revista Facultata Nacional Agronomia Medellin*, 71, 8395-8405.

Depres, L., Gielly, L., Redoulet, B., Taberlet, P. (2003). Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. *Molecular and Phylogenetic Evolution*, 27,185-196.

Deu, M., Sagnard, F.,Chantereau, J., Catalayud, C., Vigouroux, Y., Pham, J.L., Mariac, C., Kapran, I., Mamadou, A., Gerald, B., Ndjeunge, J., Bezancon, G. (2010). Spatio-temporal dynamics of genetic diversity in *Sorghum bicolor* in Niger. *Theoretical and Applied Genetics*, 120,1301-1313.

Dinssa, F.F., Stoilova, T., Rouamba, A., Tenkouano, A., Ebert, A.W., Hanson, P., Afari-Sefa, V., Keatinge, J.D.H., and d'A.Hughes, J. (2013). Prospects and challenges for preserving and mainstreaming underutilized traditional African vegetables. 3rd International Conference on Neglected and Underutilized Species, Accra, Ghana.

Dinssa, F., Ebert, A., Tenkouano, A. (2014). The diversity of African leafy vegetables, agromorphological characterization of germplasm collection. *Acta Horticulturae*, Doi 10.17660/ActaHortic.2015.1102.7.

Dinssa, F.F., Stoilova, T., Nenguwo, N., Aloyce, A., Tenkouano, A., Hanson, P., Hughes, J. d'A., Keatinge, J.D.H. (2015). Traditional vegetables, improvement and development in sub-Saharan Africa at AVRDC – The World Vegetable Center. *Acta Horticulturae*, DOI 10.17660/ActaHortic.2015.1102.2.

Donini, P., Law, J.R., Koebner, R.M.D., Reeves, J.C., Cooke, R.J. (2005). The impact of breeding on genetic diversity and erosion in bread wheat. *Plant Genetic Resources, Characterization and Utilization*, 3, 391-399.

Dwivedi, S.L., Stalker, H.T., Blair, M.W., Bertioli, D.J., Nielen, S., Ortiz, R. (2008). Enhancing crop gene pools with beneficial traits using wild relatives. *Plant Breeding Reviews*, 30, 179-230.

Edmonds, J.M., Chweya, J.A. (1997). Black nightshades: *Solanum nigrum* L. and related species. Promoting the conservation and use of underutilized and neglected crops. Institute of Plant Genetics and Crop Plant Research. Rome Italy.

Excoffier, L., Smouse, P.E., Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distence among DNA haplotypes; application to human mitochondrial DNA restriction data. *Genetics*, 131, 479-491.

Fay, M.F., Cowan, R.S., Leitch, I.J. (2005). The effects of nuclear DNA content (C-value) on the quality and utility of AFLP fingerprints. *Annals of Botany*, 95, 237–246.

Figliuolo, G., Mazzeo, M., Greco, I. (2007). Temporal Variation of diversity in italian durum wheat germplasm. *Genetic Resources and Crop Evolution*, 54, 615-626.

Foulley, J., Ollivier, L. (2006). Estimating allelic richness and its diversity. *Livestock Science*, 101, 150–158.

Franco, J., Crossa, J., Taba, S., Shands, H. (2005). A sampling strategy for conserving genetic diversity when forming core subset. *Crop Science*, 45, 1035-1044.

Fu, Y. B. (2006). Impact of plant breeding on genetic diversity of agricultural crops, searching for molecular evidence. *Plant Genetic Resources*, 4, 71-78.

Fu, Y.B., Somers, D.J. (2009). Genomewide reduction of genetic diversity in wheat breeding. *Crop Science*, 49, 161-168.

Ganguly, P.R., Bhat, K.V. (2012). Analysis of agronomically important morphological traits diversity in *Vigna radiate*. *DHR International Journal of Biomedical and Life Sciences*, 3, 84-92.

Govindaraj, M., Vetriventhan, M., Srinivasan, M. (2015). Importance of genetic diversity assesement in crop plants and its recent advances, An overview of its analytical properties. *Genetic research international*, vol.2015, article ID 431487, 14 pages, 2015.doi.10.1155/2015/431487.

Greenbaum, G., Templeton, A.R., Zarmi, Y., Bar-David, S. (2014). Allelic richness following population founding events – a stochastic modeling framework incorporating gene flow and genetic drift. *PLoS ONE*, 9(12), e115203. doi,10.1371/journal.pone.0115203.

Hajjar, R., Hodgkin, T. (2007). The use of wild relatives in crop improvement, a survey of developments over the last 20 years. *Euphytica*, 156, 1-13.

Heckenberger, M., Van Der Voort, J. R., Melchinger, A. E., Peleman, J., Bohn, M. (2003). Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties: II. genetic and technical sources of variation in AFLP data and comparison with SSR data. *Molecular Breeding*, 12, 97-106.

Huang, H., Tong, Y., Zhang, Q-J, Gao, L-Z. (2013). Genome size variation among and within *Camellia* species by using flow cytometic analysis. *PLoS ONE* 8(5),e64981, doi,10.1371/journal.pone.0064981.

Indrees, M., Irshad, M. (2014). Molecular markers in plants for analysis of genetic diversity; a review. *European Academic Research*, 2,1513-1540.

Jared, J.J., Murungi, L.K., Wesonga, J., Torto, B. (2016). Steroidal glycoalkaloids; chemical defence of edible African nightshades against the tomato red spider mite, *Tetranychus evansi* (*Acari, Tetranychidae*). *Pest Management Science*, 72, 828–836.

Jena, K.K., Mackill, D.J. (2008). Molecular markers and their use in marker-assisted selection in rice. *Crop Science*, 48, 1266-1276.

Juma, P., Turoop L.T., Murungi, L.K., Meyhöfer, R. (2017). Management of damping off disease and enhancement of African nightshade (*Solanum scabrum*) growth through the

use of *Bacillus subtilis* and *Trichoderma asperellum*. *Asian Journal of Natural and Applied Sciences*, 6, 1-9.

Kalia, R.K., Kalia, S., Rai, M.K., Dhawa, A. (2011). Microsatellite markers: An overview of the recent progress in plants. *Euphytica*, 177, 309-334.

Kamga, R.T., Kouame, C., Atangana, A.R., Chagomoka, T., Ndango, R. (2013). Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research*, 21, 99-106.

Karakousi, A., Barr, AR., Chalmers, K.J., Ablett, G.A., Holton, T.A., Henry, R.J., Lim, P., Langridge, P., (2003). Potential of SSR markers for plant breeding and variety identification in Australian barley germplasm. *Australian Journal of Agricultural Research*, 54, 1197-1210.

Keneni, G., Bekele, E., Imtiaz, M., Dagne, K. (2012). Genetic variability of modern crop cultivars: causes, mechanism and remedies. *International Journal of Plant Research*, 2, 69-79.

Khan, M.M.A., Samiullah, S.H., Afridi, R.M. (2000). Response of black nightshade (*Solanum nigrum* L.) to phosphorus application. *Journal of Agronomy and Crop Science*, 184, 157–163.

Kosman, E., Leonard, K.J. (2005). Similarity coefficient for molecular markers in studies of genetic relationships between individuals for haploid, diploid and polyploid species. *Molecular Ecology*, 14, 415-424.

Le Clerc, V., Bazante, F., Baril, C., Guiard, J., Zhang, D. (2005). Assessing temporal changes in genetic diversity of maize varieties using microsatellite markers. *Theoretical and Applied Genetics*, 110, 294-302.

Lebecka, R. (2009). Inheritance of resistance in *Solanum nigrum* to *Phytophthora infestans*. *European Journal of plant pathology*, 124,345-348.

Lehmann, C., Biela, C., Stefan, T., Jansen, G., Vogel, R. (2007). *Solanum scabrum*—a potential source of a coloring plant extract. *Euphytica*, 158,189–199.

Li, L., Tacke, E., Hofferbert, H.R.,Lübeck, J., Strahwald, J., Draffehn, A.M. (2013). Validation of candidate gene markers for marker- assisted selection of potato cultivars with improved tuber quality. *Theoretical and Applied Genetics*, 126, 1039-1052.

Luoh, J.W., Begg, C.B., Symonds, R., Ledesma, D., Young, R. (2014). Nutritional yield of African indigenous vegetables in water-defficient and water-sufficient conditions. *Food and Nutritional Sciences*, 5, 812-822.

Mahelka V, Suda J, Jarolımova V, Travnıcek P, Krahulec F. (2005). Genome size discriminates between closely related taxa *Elytrigia repens* and *E. intermedia* (*Poaceae, Triticeae*) and their hybrid. *Folia Geobotanica,* 40, 367–384.

Mantegazza, M., Biloni, M., Grasi, F., Basso, B., Lu, B.R. (2008). Temporal trends of variation in Italian rice germplasm over the past two centuries revealed by AFLP and SSR markers. *Crop Science*, 48,1832-1840.

Masinde, P.W., Stutzel, H., Agong, S.G., Fricke, A. (2006). Plant growth, water retentions and transpiration of two species of African nightshade (*Solanum villosum* Mill. ssp. *miniatum* and *S. sarrachoides* Sendtn.) under water-limited conditions. *Scientia Horticulturae* 110, 7–15.

Masinde, P.W., Wesonga, J.M., Ojiewo, C.O., Agong, S.G., Masuda, M. (2009). Plant growth and leaf N content of *Solanum villosum* genotypes in response to nitrogen supply. *Dynamic Soil Dynamic Plant*, 3, 36–47.

Maundu, P., Achigan-Dako, E., Mprimoto, Y. (2009). Biodiversity of African vegetables. In: African Indigenous Vegetables in Urban Agriculture. Eds. Shackleton, C.M., Pasquini, M.W., Drescher, A.W.. London, UK., Earthscan, pp 65-104.

Mazid, M.S., Rafii, M.Y., Hanafi, M.M., Rahim, H.A., Shabanimofrad, M., Latif, M.A. (2013). Agro-morphological characterization and assessment of variability, heritability, genetic advance and divergence in bacterial blight resistant rice genotypes. *South African Journal of Botany*, 86, 15-22.

Mcglaughlin, M., Baley, L., Helenurm, K. (2009). Isolation of microsatellite loci from endangered plant *Galium catalinense* subspecies *acrispum* (*Rubiaceae*). *Molecular Eology Resources*, 9, 984-986.

Miah, G., Rafii, M. Y., Ismail, M.R., Puteh, A.B., Rahim, H.A., Islam, K., Latif, M.A. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences*, 14, 22499-528. doi:10.3390/ijms141122499.

Mohyuddin, A., Khan, Z., Ahmad, M., Kashmir, M.A. (2010). Chemotaxonomic value of alkaloid in *Solanum nigrum* complex. *Pakistan Journal of Botany*, 42, 653–660.

Morgante, M., Hanafey, M., Powell, W. (2002). Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics*, 30,194–200.

Mureithi, .D.M., Fiaboe, K.K.M., Ekesi, S., Meyhöfer, R. (2017). Important arthropod pests on leafy Amaranth (*Amaranthus viridis*, *A. tricolor* and *A.blitum*) and broad-leafed African

nightshade (*Solanum scabrum*) with a special focus on host-plant ranges. *African Journal of Horticultural Sciences*, 11, 1-17.

Mwai, G.N., Onyango, J.C., Abukusta-Onyango, M. (2007). Taxonomic identification and characterization of African nightshades (*Solanum* Section *Solanum* L.). A*frican Journal of Food and Agriculture Nutrition and Development* 7, 1-16.

Noda, A., Nomura, N., Mitsui, Y., Setoguchi, H. (2009). Isolation and characterization of microsatellite loci from *Calystegia soldanella* (*Convolvulaceae*), an endangered coastal plant isolated in Lake Bwa, Japan. *Conservation genetics*, 10, 1077-1079.

Ojiewo, C.O., Murakami, K., Masinde, P.W., Agong, S.G. (2007). Polyploid breeding of African nightshade (*Solanum* section *Solanum*). *International Journal of Plant Breeding*, 1, 10-21.

Ojiewo, C.O., Mwai, G.N., Abukutsa-Onyango, M., Agong, S.G., Nono-Womdim, R. (2013a). Exploiting the genetic diversity of vegetable African nightshades. *Bioremediation, Biodiversity and Bioavailability*, 7, 6-13.

Ojiewo, C.O., Mbwambo, O., Swai, J., Samali, S., Tilya, M.S., Mnzara, R.N., Mrosso, L., Minja, R., Oluoch, M. (2013b). Selection, evaluation and release of varieties from genetically diverse African nightshade germplasm. *International Journal of Plant Breeding*, 7, 76-89.

Ondieki, M. J., Aguyo, J.N., Opiyo, A. (2011a). Variations in growth and yield characteristics of three black nightshade species grown under high altitude conditions. *Agriculture and Biology Journal of North America*, 2, 401-406.

Ondieki, M.J., Aguyoh, J.N., Opiyo, A.M. (2011b). Fortified compost manure improves yield and growth of African nightshades. *International Journal of Science and Nature*, 2, 231-237.

Opiyo, A.M. (2004). Effect of nitrogen application on leaf yield and nutritive quality of black nightshade (*Solanum Nigrum* L.) . *Outlook on Agriculture*, 33, 209 - 214.

Paula, C.M.P., Techio, V.H., Benites, F.R.G., Sobrinho, F. (2014). Intra-inflorescence pollen viability in accessions of *Brachiara ruziziensis*. *Acta Scientiarum*, 36, 209-213.

Peleman, J.D., Van Der Voort, J.R. (2003). Breeding by design. *Trends in Plant Science*, 8, 330-334.

Pereira, L.k., Scapim, C.A., Mangolin, C.A., Machado, F.P., Pacheco, C.A.P., Mora, F. (2008). Heterozygosity following half-sib recurrent selection in popcorn using isozyme markers. *Electronic Journal of Biotechnology*, 11, 41-49.

Petit, R., Mousadik, A., Pons, O. (1998). Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* 12, 844–855.

Pickrell, J.K., Pritchard, J.K. (2012). Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS* Genet.8,e1002967.

Pogorzelec, M., Glebocka, K., Nowak, B., Parymies, M. (2014). Reproduction and diversity of endangered *Salix lapponum* L. populations in Eastern Poland. *Turkish Journal of Botany*, 38, 1239-1247.

Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E. Shadick, N.A. (2006). Principal component analysis corrects for stratification in genome-wide association studies. *Nature Genetics*, 38, 904-909.

Pritchard, J.K., Stephens, M., Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.

Rauf, S., Jaime, A., da Silva, T., Khan, A., Naveed, A. (2010). Consequences of plant breeding on genetic diversity. *International Journal of Plant breeding*, 4, 1-21.

Reed, D.H., Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservertion Biology* 17, 230-237.

Reif, J., Hamrit, S., Heckenberger, M., Schipprack, W., Maurer, H., Bohn, M., Melchinger, A. (2005). Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. *Theoretical and Applied Genetics*, 111, 838-845.

Ronoh, R., Ekhuya, N., Linde, M., Winkelmann, T., Abukutsa-Onyango, M., Dinssa , F.F., Debener, T. (2017). African nightshades, genetic, biochemical and metabolite diversity of an underutilised indigenous leafy vegetable and its potential for plant breeding. *The Journal of Horticultural Science and Biotechnology*, DOI,10.1080/14620316.2017.1358112.

Saha, M., Datta, B.K. (2014). Reproductive biology of *S. viarum* Dunal (*Solanaceae*) in Northeast India. *Pleion*, 8, 258-266.

Sakiyama, N.S., Ramos, H.C.C., Caixeta, E.T., Pereira, M.G. (2014). Plant breeding with marker-assisted selection in Brazil. *Crop Breeding and Applied Biotechnology*, 14, 54-60.

Schippers, R.R. (2000). African indigenous vegetables. An overview of the cultivated species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK.

Semagn, K., Bjørnstad, A., Ndjiondjop, M.N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, 5, 2540-2568.

Shackleton, C.M., Pasquini, M.W., Drescher, A.W. (2009). African Indigenous Vegetables in Urban Agriculture. Sterling VA, London.

Shan, F., Yan, G., Plummer, J. (2003). Karyotype evolution in the genus *Boronia* (*Rutaceae*). *Botanical Journal of the Linnean Society*, 142, 309-320.

Sherwin, W.B., Jabot, F., Rush, R., Rossetto, M. (2006). Measurement of biological information with applications from genes to landscapes. *Molecular Ecology*, 15, 2857–2869.

Shivanna, K.R. (2003). Pollen biology and biotechnology. Science Publishers, Enfield, New Hampshire.

Steele, K.A., Gyawali, S., Joshi, K.D., Shrestha, P., Sthapit, B.R., Witcombe, J.R. (2009). Has the introduction of modern rice varieties changed the genetic diversity in high altitude region of Nepal? *Field Crop Research*, 113, 24-30.

Sultana, S.S., Alam, S.S. (2007). Diffential fluorescent chromosome banding of *Solanum nigrum* L. and *Solanum villosum* L. from Bangladesh. *Cytologia*, 72, 213-219.

Sun, C.Q., Wang, X.K., Li, Z.C., Yoshimura, S., Iwata, N. (2001). Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa*) using RFLP markers. *Theoretical and Applied Genetics*, 102, 157-162.

Szulkin, M., Bierne, N., David, P. (2010). Heterozygosity-fitness correlations, a time for reappraisal. *Evolution*, 64, 1202–1217.

Teyer, F.S, Salazar, M.S., Esqueda, M., Barraza, A., Robert, M.L . (2009). Genetic variability of wild *Agave angustifolia* populations based on AFLP, a basic study for conservation. *Journal of Arid Environment*, 73, 611–616.

Tuwei, C., Opala, P.A., Omami, E.N., Opile, W.R. (2013). Response of the African nightshade to phosphate fertilizer application in Western Kenya. *Archives of Applied Science Research*, 5, 195-201.

Varshney, R.K., Graner, A., Sorrells, M.E. (2005). Genic microsatellite markers in plants,features and applications. *Trends in Biotechnology*, 23, 48–55.

Varshney, R.K., Thudi, M., Aggarwal, R., Börner, A. (2007). Genic molecular markers in plants, development and applications. In: Genomics-assisted crop improvement, genomics approaches and platforms. Eds. Varshney, R.K., Tuberosa, R.. Dordrecht, Springer, pp 13–29.

Vieira, M.L.C., Santini, L., Diniz, A.L., Munhoz, C.F. (2016). Microsatellite markers, what they mean and why they are useful. *Genetics and Molecular Biology*, 39, 312-328.

Vilela, F.O., Amaral, A.T., Pereira, M.G. (2008). Effects of recurrent selection on the genetic variability of the UNB-2U popcorn populations using RAPD markers. *Acta Science Agronomica*, 30, 25-30.

Volk, G.M. (2011). Collecting pollen for genetic resources conservation. In: Collecting plant genetic diversity, technical guidlines. Eds. Guarino, L., Rao, V.R., Reid, R. CAB International, 1-10.

Wagner, A. (2008). Robustness and evolvability, a paradox resolved. *Proceedings. Biological Sciences*, 275, 91–100.

Wei, X., Yuan, X., Yu, H., Wang, Y., Xu, Q., Tang, S. (2009). Temporal changes in SSR allelic diversity of major rice cultivars in China. *Journal of Genetics and Genomics*, 36, 363-371.

Xu, Y., Crouch, J.H. (2008). Marker-Assisted selection in plant breeding, from publications to practice. *Crop Science*, 48, 391-407.

Xu, Y., Beachell, H. Mccouch, S.R.A. (2004). Marker-based approach to broadening the genetic base of rice in the USA. *Crop Science*, 44, 1947-1959.

Yada, B., Tukamuhabwa, P., Wanjala, B., Kim, D.J., Skilton, R.A., Alajo, A. (2010). Characterization of Ugandan sweet potato germplasm using fluorescent labeled simple sequence repeat markers. *Horticultural Science*, 45, 225–230.

Yi, S.S., Jatoi, S.A., Fujimura, T., Yamanaka, S., Watanabe, J., Watanabe, K.N. (2008). Potential loss of unique genetic diversity in tomato landraces by genetic colonization of modern cultivars at a non-centre of origin. *Plant Breeding*, 127, 189-196.

Zapata, J.E., Cueras, H., Zhu, H., Steffen, S. Senalik, D., Zeldin, D., McCown, B., Harbut, R., Simon P. (2012). Using next generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. *American Journal of Botany*, 99, 193-208.

Zhang, L.D., Yun, D.J., Yu, S.W., Li, Z.G., Cao, Y.F., Miao, Z.Q., Qian, H.M., Tang, K.X. (2004). Preference of simple sequence repeats in coding and non-coding regions of *Arabidopsis thaliana*. *Bioinformatics*, 20, 1081-1086.

Zhang, Q.Q., Wu, C.L., Ren, F.L., Li, Y., Zhang, C.Q. (2012). Association analysis of important agronomical traits of maize inbred lines with SSRs. *Australian Journal of Crop Science*, 6, 1131-1138.

5 Appendices

5.1 Buffers and working solutions used

10x RL Buffer

100 mM Tris HCL
100 mM MgAc
500 mM KAc
50 mM DTT (77 mg/10 mL)
pH 7.5

10 x Williams buffer (100 ml)

(ml)		Final concentration
10	Tris/HCL, pH 8,3 (1M)	100 mM
50	KCL(1M)	500 mM
2	$MgCl_2$ (1M)	20 mM
10	Gelatine (0.1 %)	0.01%
28	H_2O	

Loading dye for the Licor Sequencers

49 ml of 98% Formamide
0.5 ml of 10 mM EDTA
25 mg of Pararosanilin (0.05%)

10x DNA loading buffer

0.25 % (w/v) Orange G (Merck Millipore, Darmstadt, Germany)
30.00 % (w/v) Glycerin
1mM EDTA pH 8.0

1x TAE buffer

40 mM Tris
1mM EDTA
pH 8.0 (adjust with concentrated acetic acid)

1x TBE buffer (pH 8.0)

89 mM Tris
89 mM Boric acid
2 mM Na_2EDTA

5.2 Single M13 SSR PCR reaction (in tubes)

	(µl)
H2O	5.65
10x Williams Buffer	1
dNTPs (2mM) [µl]	0.75
Taq-Polymerase DCS (5U/µl)	0.1
M13 tailed forward primer (0,5pmol/µl = 1:200)	0.5
M13 forward labelled (2,5pmol/µl = 1:40)	0.5
reverse primer (5,0 pmol/µl = 1:20)	0.5
Reaction Volume	9.00
DNA (10ng/µl)	1.0

5.3 Oligonucleotides sequences

DNA Adapter	**Sequences**
MseI O	5’-GACGATGAGTCCTGAG-3’
MseI U	5’-TACTCAGGACTCAT-3’
HindIII O	5’-CTCGTAGACTGCGTACC-3’
HindIII U	5’-AGCTGGTACGCAGTCTAC-3’

AFLP-Primer	**Sequences**
MseI+0	5'-GACGATGAGTCCTGAGTAA-3'
HindIII+0	5'-AGACTGCGTACCAGCTT-3'
MseI+XXX	5'-GATGAGTCCTGAGTAA XXX-3'
HindIII+XXX (IRD 700 or 800)	5'-GACTGCGTACCAGCTT XXX-3'

5.4 Preparation of 6% acrylamide gels

	Loading SSR	Loading AFLP
Monomer Solution	12 ml	16 ml
Complete Buffer	3 ml	4 ml
APS (10 %)	120 µl	160 µl

Curriculum Vitae

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PUBLICATIONS

Ronoh, R., Linde, M., Winkelmann, T., Abukutsa, M.O., Dinssa, F.F., Debener, T. (2018). Morphological characterization, genetic diversity and population structure of African nightshade entries (section *Solanum* L.). *Genetic Resource and Crop Evolution* (submitted).

Ronoh, R., Linde, M., Winkelmann, T., Abukutsa, M.O., Dinssa, F.F., Debener, T. (2018). Development of next-generation sequencing (NGS) based SSR in African nightshades; Tools for analyzing genetic diversity for conservation and breeding. *Scientia Horticulturae* 235:152-159.

Ronoh, R., Ekhuya, N.A, Winkelmann, T., Linde, M., Abukutsa, M.O., Dinssa, F.F., Debener, T. (2017). African Nightshade: genetic, biochemical and metabolite diversity of an underutilized indigenous leafy vegetable and its potential for plant breeding. *Journal of Horticultural Science and Biotechnology* DOI: 10.1080/14620316.2017.1358112.

Ronoh, R., Budambula, N.L., Mwirichia, R.K., Boga, H.I. (2013). Isolation and characterization of Actinobacteria from Lake Magadi, Kenya. *African Journal of Microbiology Research* **7**:4200-4206.

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