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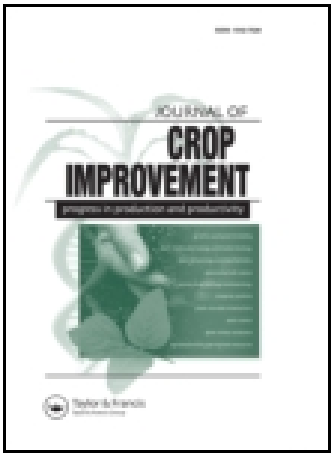

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Morphological and Cytomolecular Assessment of Intraspecific Variability in Scarlet Eggplant (Solanum aethiopicum L.)

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Morphological and Cytomolecular Assessment of Intraspecific Variability in Scarlet Eggplant (*Solanum aethiopicum* L.)

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Solanum aethiopicum L. *is native to sub-Saharan Africa but is now found in many parts of the world. It is used for food, medicinal, and ornamental purposes. It has also been used as a rootstock for tomato and common eggplant because of its resistance to certain pathogens. However, very little is known about its genetics, so the purpose of this work was to assess intraspecific variability in* S. aethiopicum *via morphological and cytomolecular characterization of 12 scarlet eggplant accessions. Cluster analysis was used for grouping the accessions using means of 27 variables. Four separate groups were found, with two groups each consisting of five accessions and two other groups each consisting of only one accession. Variability was high with flower- and fruit-associated descriptors among the accessions. Monoploid genome sizes (Cx-value), average chromosome sizes (C/n-value), and GC content were determined. Haploid genome size of S. aethiopicum*

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ranged from 1.312 pg/1C to 1.538 pg/1C., which is close to the genome size (1.2 pg/1C) of the common eggplant. Only PI 420226 (1.538 pg/1C) was significantly different from the rest, giving credence to the theory that PI 420226 is actually a S. macrocarpon *accession. GC content of S. aethiopicum accessions was about 40%. We used 18S-28S rDNA and 5S rDNA probes to study the distribution and physical position of these ribosomal genes in S. aethiopicum. These results help to better understand intraspecific variability in S. aethiopicum and can be important for the breeding and selection of this crop.*

KEYWORDS Fluorescence in situ *hybridization (FISH), Nuclear DNA content, 8S-28S, 5S rDNA, Ethiopian eggplant*

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INTRODUCTION

Solanum aethiopicum L., which belongs to the family Solanaceae and subfamily Solanoideae, is a cultivated eggplant indigenous to Africa. Cultivated *S. aethiopicum* is the result of a domestication process involving its wild ancestors, *Solanum anguivi* Lam. and semi-domesticated *Solanum distichum* Schumach. & Thonn, and that took place in Africa (Lester and Seck 2004). *S. aethiopicum* is commonly referred to as scarlet eggplant, Ethiopian eggplant, "pumpkin on a stick," or "mock tomato." The fruit, leaves, shoots, and roots of *S. aethiopicum* are used for both food and medicinal purposes, and the specific use depends on the geographic area and/or plant type. For example, immature fruits of *S. aethiopicum* are used as cooked vegetables or sometimes eaten raw, and the leaves and shoots can also be used as cooked vegetables whereas fruits, leaves, and roots of bitter cultivars are used as medicine in many African countries to treat ailments ranging from colic and high blood pressure to uterine complaints (Lester and Seck 2004; Sunseri et al. 2010; Adneniji et al. 2012).

According to some authors, *S. aethiopicum* has four cultivar groups including Gilo, Kumba, Shum, and Aculeatum groups (Lester and Seck 2004; Adneniji et al. 2012). These groups are defined by specific plant characteristics such as prickly leaves and stems, leaf shape, fruit shape and taste, and environmental growing requirements. Cultivars of the Aculeatum group are used as ornamentals (Daunay et al. 1995), and those of both the Gilo and Aculeatum groups have been used as rootstock or source of resistance for biotic or abiotic tolerance for tomato and common eggplant because of their resistance to certain pathogens such as *Fusarium oxysporum* f. sp. *melongenae,* a worldwide soil-borne disease of eggplant, and *Ralstonia solanecearum* (Hebert 1985; Daunay et al. 1991; Rizza et al. 2002; Toppino et al. 2008), *Pseudomonas solanacearum* EF Smith (Ano et al. 1991), and

higher tolerance to drought and heat (Lester et al. 1981). *S. aethiopicum* is a complex species consisting of groups that are morphologically very different. Indeed, these groups were previously treated as four separate species (Polignano et al. 2010). The purpose of this work was to contribute to the understanding of intraspecific variability in *S. aethiopicum* via morphological and cytomolecular characterization of 12 scarlet eggplant accessions.

MATERIALS AND METHODS

Plant Materials

Twelve *Solanum aethiopicum* accessions collected from various parts of the world were used in this study. Eleven of these accessions are from USDA's National Plant Germplasm System, and one accession, Tourimé, is a landrace variety from northeastern Senegal, in western Africa. Twenty seeds from each of the 12 *S. aethiopicum* accessions were sown in seed starter mix Sunshine #3 mix (Sun Gro Horticulture Inc., Bellevue, WA). As seedlings produced sufficient root systems, they were potted in a maintenance soil Pro Mix soil (Premier Horticulture Inc., Quakertown, PA) and subsequently transferred to the field during the months of April–August 2012. A new set of 20 seeds per accession was used during the same period in 2013.

Morphological Characterization and Pollen Viability

Traits evaluated for morphological characterization included petiole length (cm), cotyledon length (cm), and cotyledon width (cm). Cotyledon and petiole measurements were recorded for all 20 plants of each *S. aethiopicum* accession. For each seedling, the width and length were measured for the two cotyledons and the length was measured for the same cotyledon's petiole. Other traits examined included fruit number, fruit fresh weight (g), fruit dry weight (g), fruit solids (%), fruit length (cm), fruit diameter (cm), and fruit circumference (cm), as well as number of stomates or stomatal density, stomatal length (μm), and stomatal width (μm). In addition to morphological traits, pollen viability as assessed by *in vitro* pollen germination percentage was estimated.

Flower characteristics were also evaluated, and the list of evaluated flower traits were as follows: total flower length [the length from the base of the receptacle to the tip of the furthest floral structure (stigma, anther, or petal)]; stamen (anther and filament) number and length; petal number, length, and width; sepal number, length, and width; style and stigma number and length; and peduncle (stem of whole inflorescence) length. All length and width measurements for flower characteristics were expressed in centimeters and originated from a minimum of 10 different plants from each of the 12 accessions. Number of stomates as well as stomatal length and width

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were measured using a simple fingernail polish peel technique (Sakhanokho et al. 2009). For pollen viability as assessed by *in vitro* pollen germination, fresh pollen was collected from field-grown plants of all 12 scarlet eggplants. An *in vitro* pollen germination test was performed to evaluate pollen viability using an improved "hanging drop" technique (Sakhanokho and Rajasekaran 2010).

Experimental Design and Statistical Analysis

The experimental design was a randomized complete-block design with two replications and 20 plants per accession in both 2012 and 2013. Fruit measurements and weights were collected on mature fruit, i.e. when fruit color started turning red. Data were analyzed using SAS software (SAS Version 9.1.3), and means were compared using Tukey's test ($P = 0.05$). Cluster analysis was used to investigate potential groupings of accessions for similarity among the 27 phenotypic variables. Cluster analysis was conducted using standardized means and Ward's method with the CLUSTER and TREE procedures of SAS (version 9.3; SAS Institute Inc., Cary, NC); the number of clusters were determined using semipartial R^2 and between-cluster sum of squares statistics (Blythe and Merhaut 2007). Descriptive statistics and mean comparisons for the 27 variables for the four identified clusters were obtained using generalized linear mixed models with the GLIMMIX procedure of SAS, with multiple comparison adjustments made using the Holm-Simulated method.

Genome Size and GC Content Estimations

Genome size and the genomic percentage of guanine + cytosine (GC content) estimations were measured by flow cytometry. Flow cytometry and chromosome spread preparation were performed following the techniques described by Sakhanokho and Islam-Faridi (2013). Plant tissue was excised from growth chamber grown *Solanum aethiopicum* plants. In total, four separate plants were sampled, and *Pisum sativum* 'Ctirad' (2c = 9.09 pg DNA), kindly supplied by Dr. Jaroslav Doležel from the Experimental Institute of Botany, Czech Republic, was also grown in a growth chamber and used as an internal standard. Procedures were performed twice on each sample.

For nuclear DNA content, monoploid genome sizes, average chromosome sizes, and base pair composition, DAPI- and PI-stained nuclei were analyzed using a flow cytometer. Fluorescence ratios, calculated relative to the internal reference *Pisum sativum* 'Ctirad' (2c = 9.09 pg DNA), were converted to DNA content values and expressed in picograms following the formula: Sample 2C -value (picograms) = reference 2C-value × [(Sample 2C mean peak)/(Reference 2C mean peak)]. Monoploid genome size (Cx-value) was calculated following the method described by Greilhuber et al.

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(2005) and Šmarda et al. (2008) as the absolute 2C DNA content (2C-value) of the sample divided by the ploidy level. The average chromosome size (C/n-value) was calculated according to Šmarda et al. (2008) by dividing the somatic total DNA content (2C) by somatic chromosome number (2n). The percentage of AT in *S. aethiopicum* was measured in relation to the *P. sativum* 'Ctirad' standard by comparing the peaks of fluorescence of the DAPI-stained G_0/G_1 nuclei following the method described by Godelle et al. (1993): $AT_{sample} = AT_{standard} \times (R_{DAPI}/R_{PI})^{1/r}$, where $AT_{standard}$ (%) = 61.5, R is the ratio of fluorescence intensity from the peak of *S. aethiopicum* to that of *P.* 'Ctirad' (2c = 9.09 pg DNA) and r (binding length) = 3.5 (Meister and Barrow 2007; Portugal and Waring 1988). Analysis of variance was conducted using the GLM procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC), and mean separations were performed using Tukey's test at $\alpha = 0.05$. Pearson correlation coefficients (P = 0.001) among four genetic parameters, namely 2C nuclear DNA content (2C-value), monoploid genome sizes (Cx-value), average chromosome sizes (C/n-value), and GC content, were calculated to assess their relatedness for the 12 accessions.

Chromosome Spread Preparation and Fluorescence *In Situ* Hybridization

Flow cytometry was used to determine the ploidy level of *S. aethiopicum* accessions, and chromosome spread was performed on selected accessions to confirm flow cytometry results. Fluorescence *in situ* hybridization (FISH) procedure was conducted to determine the chromosomal location of 5S and 18S-28S rDNA genes in accession Tourimé using FISH technique following mostly the procedures described by Jewell and Islam-Faridi (1994) and Sakhanokho and Islam-Faridi (2013). Chromosome spreads from *S. aethiopicum* accession Tourimé were viewed under a 63X plan apo-chromatic objective and digital images were recorded using an epi-fluorescence microscope (Axio Imager M2, Carl Zeiss Microscopy GmbH, Göttingen, Germany) with suitable filter sets (Chroma Technology, Bellows Falls, VT) and a CoolCube 1 (MetaSystems Group Inc., Boston, MA) high performance charge-coupled device (CCD) camera.

RESULTS

Morphological Characterization

A great deal of phenotypic difference exists among accessions of *S. aethiopicum* for leaf shape and color, flower traits, and fruit shape and color (Figure 1). *S. aethiopicum* means of quantitative descriptors for seedling, stomates, flower, and fruit traits are reported in Tables 1, 2, 3, and 4. Similarities and differences were found among the 12 scarlet eggplant

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FIGURE 1 Representative leaves, flowers, and fruit of 12 morphologically different *S. aethiopicum* L. accessions. A = Grif 14165; B = PI 194166; C = PI 247828; D = PI 374695; E = PI 420226; F = PI 420230; G = PI 424860; H = PI 441851; I = PI 441885; J = PI 441912; K = PI 636107; L = Tourimé.

TABLE 1 Mean cotyledon length (cm), cotyledon width (cm), and petiole length (cm) from seedlings of 12 *Solanum aethiopicum* L. accessions

Accession	Cotyledon length (cm)	Cotyledon width (cm)	Petiole length (cm)
Grif 14165	1.09 c	0.43 c	0.20 b
PI194166	1.54 abc	0.62 abc	0.39 b
PI 247828	1.25 bc	0.64 abc	0.33 b
PI 374695	1.92 a	0.86 ab	0.43 ab
PI 420226	1.85 ab	0.75 abc	0.66 a
PI 420230	1.75 abc	0.83 abc	0.43 ab
PI 424860	1.74 abc	0.59 bc	0.46 ab
PI 441851	1.46 abc	0.65 abc	0.38 b
PI 441885	1.64 abc	0.60 abc	0.41 ab
PI 441912	1.71 abc	0.57 bc	0.44 ab
PI 636107	1.92 a	0.95 a	0.43 ab
Tourimé	1.36 abc	0.78 abc	0.30 B

*Means followed by same letters within a column are not significantly different (P = 0.05). The Schaffer-Simulated method was used for multiple mean comparisons.

accessions for the 27 quantitative descriptors. No significant differences were found among the accessions for the seedling and stomatal traits evaluated (Tables 1 and 2). Most of the variability among accessions was found among quantitative descriptors associated with flower and fruit traits (Tables 3 and 4). A dendrogram showing formation of four groups of *S. aethiopicum* accessions as obtained from a four-cluster solution selected using Ward's method of hierarchical cluster analysis based on 27 phenotype traits with the between-cluster sum of squares statistic at each step of cluster formation is shown in Figure 2. Included in Group 1 are 5 accessions, namely PI 374695, PI 420230, PI 636107, PI 194166, and PI 247828. Groups 2 (Tourimé) and 3 (PI420226) had each only one accession. The remaining 5 accessions (PI 424860, PI 144185, Grf14165, PI441885, and P I441912) constituted the fourth group.

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TABLE 2 Mean number of stomates, stomatal length (μm), and width (μm) from 12 *Solanum aethiopicum* L. accessions

Accession	No. stomates	Stomatal width	Stomatal length
Grif 14165	46.00 ab	13.16 bcd	18.76 abc
PI194166	50.17 ab	14.56 a	20.27 abc
PI 247828	50.83 ab	14.04 ab	20.94 ab
PI 374695	58.83 a	13.64 abc	19.47 abc
PI 420226	52.00 ab	13.85 ab	21.47 a
PI 420230	55.17 ab	13.31 abcd	18.54 abc
PI 424860	53.50 ab	12.14 d	17.90 bc
PI 441851	56.17 ab	12.56 cd	17.14 c
PI 441885	47.83 ab	12.28 d	17.82 bc
PI 441912	38.33 b	12.17 d	18.14 bc
PI 636107	44.17 ab	12.42 cd	18.87 abc
Tourimé	48.67 ab	14.24 ab	20.69 Ab

*Means followed by same letters within a column are not significantly different (P = 0.05). The Schaffer-Simulated method was used for multiple mean comparisons.

TABLE 3 Mean flower length (cm), number of stamens, stamen length (cm), number of petals, petal length (cm) and width (cm), and percentage of *in vitro* pollen germination from 12 *Solanum aethiopicum* L. accessions

Accession	Flower length	No. stamens	Stamen length	No. petals	Petal length	Petal width	Pollen germ. (%)
Grif 14165	1.63 bc	7.67 a	0.48 c	6.50 a	1.19 b	0.48 bc	72 ab
PI194166	1.40 bcde	5.33 a	0.59 ab	4.83 a	1.35 ab	0.65 b	63 bc
PI 247828	1.05 de	5.33 a	0.49 bc	5.33 a	0.92 b	0.37 c	52 d
PI 374695	1.24 bcde	5.17 a	0.51 bc	5.17 a	1.13 b	0.52 bc	76 a
PI 420226	2.48 a	5.00 a	0.58 abc	5.00 a	1.75 a	1.35 a	73 ab
PI 420230	1.48 bcd	5.00 a	0.57 abc	5.00 a	1.15 b	0.33 c	75 a
PI 424860	1.59 bc	7.00 a	0.50 bc	5.50 a	1.28 ab	0.42 bc	27 f
PI 441851	1.29 bcde	7.50 a	0.50 bc	6.67 a	1.67 b	0.38 c	7 g
PI 441885	1.72 b	8.50 a	0.67 a	7.00 a	1.35 ab	0.45 bc	35 e
PI 441912	1.57 bcd	7.33 a	0.66 a	6.17 a	1.28 ab	0.37 c	26 f
PI 636107	0.96 e	5.00 a	0.50 bc	5.17 a	0.88 b	0.40 c	61 cd
Tourimé	1.18 cde	6.17 a	0.49 bc	5.33 a	0.98 b	0.51 bc	58 Cd

*Means followed by same letters within a column are not significantly different (P = 0.05). The Schaffer-Simulated method was used for multiple mean comparisons.

Genome Size and GC Content Estimations

For flow cytometry analysis, a minimum of 3,000 nuclei was analyzed per sample. The CV provides information on the quality of the relative fluorescent peaks. In general, CVs below 3% are considered fully acceptable (Galbraith et al. 1998; Marie and Brown 1993). In our study, the mean coefficients of variation for DAPI and PI stained samples were 2.6% ± 0.3% and 2.6% ± 0.6%, respectively. All CV values for both PI and DAPI were well within the range defined by Doležel and Bartoš (2005).

Nuclear DNA content (2C-value), monoploid genome sizes (Cx-value), average chromosome sizes (C/n-value), and GC content were determined for

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TABLE 4 Mean fruit length (cm), circumference (cm), diameter (cm), freight weight (g), dry weight (g), number of stamens, stamen length (cm), number of petals, petal length (cm) and width (cm), and percentage of solids (%), and percentage of *in vitro* pollen germination (%) from 12 *Solanum aethiopicum* L. accessions

Accession	Length	Circumference	Diameter	Fresh weight	Dry weight	Solids (%)
Grif 14165	40.60 cd	138.08 bc	45.06 bc	32.85 bc	4.07 bc	12.59 cdef
PI194166	29.84 e	110.20 ef	34.37 fg	23.07 cde	2.51 cde	10.75 f
PI 247828	20.21 f	87.95 h	27.39 i	11.36 e	1.50 e	13.97 bcd
PI 374695	20.74 f	105.51 fg	32.33 gh	15.83 de	2.31 de	14.71 ab
PI 420226	38.71 cd	150.21 b	47.52 ab	39.20 b	6.53 a	16.75 a
PI 420230	31.33 e	119.21 e	37.94 de	22.75 cde	3.02 bcde	13.47 bcde
PI 424860	45.63 b	96.95 gh	30.14 hi	17.60 de	2.12 de	12.23 def
PI 441851	37.11 d	132.00 cd	41.18 cd	23.99 cd	3.24 bcd	13.67 bcd
PI 441885	42.68 bc	117.42 e	36.63 de	24.79 cd	3.49 bcd	14.14 bc
PI 441912	68.44 a	122.05 de	37.37 def	39.50 b	4.39 b	11.09 f
PI 636107	28.01 e	134.52 cd	41.21 cd	29.85 bc	3.41 bcd	11.39 f
Tourimé	29.04 e	171.57 a	51.95 a	54.88 a	6.32 a	11.44 Ef

*Means followed by same letters within a column are not significantly different (P = 0.05). The Schaffer-Simulated method was used for multiple mean comparisons.

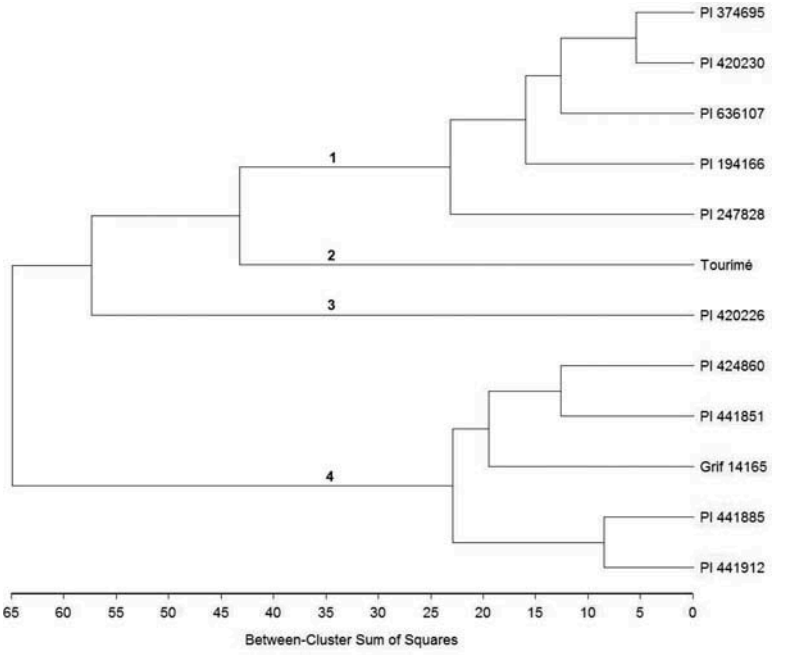

FIGURE 2 Dendrogram showing formation of four groups of *Solanum aethiopicum* accessions as obtained from a four-cluster solution selected using Ward's method of hierarchical cluster analysis based on 27 phenotype traits with the between-cluster sum of squares statistic at each step of cluster formation.

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TABLE 5 Mean values for 2C nuclear DNA content (2C-value), monoploid genome sizes (Cx-value), average chromosome sizes (C/n-value), and GC content of 4′,6-diamidino-2-phenylindole (DAPI)- and propidium iodide (PI)-stained samples and size standard obtained by flow cytometry analysis of 12 *Solanum aethiopicum* accessions using *Pisum sativum* 'Ctirad' (2c = 9.09 pg DNA) *Glycine* as internal reference standard

Accession	2C-value (pg)*	Cx-value (pg)*	C/n-value (pg)*	GC content (%)*
Grif 14165	2.644 d	1.322 d	0.128 a	39.68 ab
PI 194166	2.717 b	1.358 b	0.113 b	39.32 bc
PI 247828	2.653 cd	1.326 cd	0.111 cd	39.29 bc
PI 374695	2.657 cd	1.328 cd	0.111 cd	39.51 abc
PI 420226	3.076 a	1.538 a	0.128 a	38.91 c
PI 420230	2.623 d	1.312 d	0.109 d	40.07 a
PI 424860	2.660 cd	1.330 cd	0.111 cd	39.88 ab
PI 441851	2.661 cd	1.330 cd	0.111 cd	39.78 ab
PI 441885	2.646 cd	1.323 cd	0.110 cd	39.58 ab
PI 441912	2.695 bc	1.348 bc	0.112 bc	39.49 abc
PI 636107	2.662 cd	1.331 cd	0.111 cd	39.32 bc
Tourimé	2.695 bc	1.348 bc	0.112 bc	39.62 Ab

*Means followed by same letters within a column are not significantly different according to Tukey's test at < 0.05.

the 12 accessions (Table 5). No significant differences (P = 0.05 were found among the 12 accessions for GC content (Table 5). Nuclear DNA content of the 12 eggplant accessions evaluated ranged from 2.623 pg (PI 420230) to 3.076 pg (PI 420226), and with the exception of PI 420226, there was no significant difference ($\alpha = 0.05$) among the scarlet eggplants evaluated for their nuclear DNA content (Table 5). Similar results were obtained for monoploid genome sizes (Cx-value) and average chromosome sizes (C/n-value) for the 12 accessions. This was not surprising as the basic chromosome number (x = 12) was the same for all the accessions and Cx-value and C/n-value were derived from 2C-value, which also explains the fact that these values were linearly correlated (Table 6).

Chromosome Spread and Fluorescence *In Situ* Hybridization

Flow cytometry was used to estimate the ploidy level of all the accessions included in this study, and chromosome spread was performed following

TABLE 6 Correlation coefficients among four genetic parameters in 12 *Solanum aethiopicum* L. accessions

	2C-value	Cx-value	C/n-value	GC content
2C-value		1.000***	1.000***	−0.524***
Cx-value			1.000***	−0.524***
C/n-value				−0.524***

***Pearson correlation coefficients highly significant at P < 0.001.

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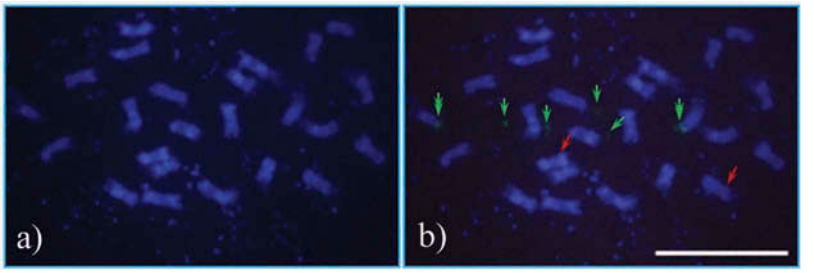

FIGURE 3 FISH probed with 18S-28S and 5S rDNA clones on somatic chromosome ($2n = 2x = 24$) spread of *Solanum aethiopicum* accession Tourimé. The chromosome spread (prometaphase) was counterstained with DAPI (a) and the FISH signals from 18S-28S rDNA (green signals) and 5S rDNA (red) shown in (b). Bar = 5 μm.

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the techniques described by Sakhanokho and Islam-Faridi (2013) on selected accessions to confirm the results obtained with flow cytometry. High-quality somatic chromosome spreads were prepared from actively growing root tips of *S. aethiopicum* accessions using modern protoplast technique as described by Jewel and Islam-Faridi (1994) and Sakhanokho and Islam-Faridi (2013) without squashing the tissue under a glass cover-slip. The spreads were mostly free from cell walls, nuclear membranes, and cytoplasmic debris, which are prerequisite for a successful FISH study. All accessions of *S. aethiopicum* accessions evaluated were found to have a diploid chromosome number of $2n = 2x = 24$ (Figure 3a), which is consistent with other chromosome number reports for this species (Anaso 1991; Lester and Seck 2004; Obute et al. 2006).

We used 18S-28S rDNA and 5S rDNA probes to study the distribution and physical position of these ribosomal genes in *S. aethiopicum*, accession Tourimé. One each of 18S-28S and 5S rDNA sites has been identified in this accession (Figure 3b). The 18S-28S rDNA locus is located toward the distal end of an arm of a homologous pair of chromosomes, and the 5S rDNA locus, on the other hand, is located interstitially in the median position of one arm of another pair of chromosomes as revealed by simultaneous FISH signals. Dispersed green FISH signals were observed (single green arrows, Figure 3b) from the 18S-28S rDNA probe, indicating that the rDNA is still in de-condensed stage and the chromosomes are in early pro-metaphase. Similar FISH results have also been reported for *Brassica* (Maluszynska and Heslop-Harrison 1993), alfalfa (Calderini et al. 1996), and *Populous trichocarpa* (Islam-Faridi et al. 2009).

DISCUSSION

The scarcity of genetic information is a hindrance to the development of improved cultivars of *S. aethiopicum*, particularly in Sub-Saharan Africa,

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center of origin of the species. A detailed description of morphological characteristics is useful in many respects; for example, it is a prerequisite for patenting or registering of new cultivars. Descriptive statistics and mean comparisons for the 27 variables for the 12 *S. aethiopicum* accessions were obtained using generalized linear mixed models with the GLIMMIX procedure of SAS, with multiple comparison adjustments made using the Holm-Simulated method. No significant differences were found among the 12 accessions for quantitative descriptors related to seedlings (petiole length, cotyledons length and width) and stomates, even though more stomates were consistently found on the abaxial (Figure 4A) surface of leaves for all 12 accessions. An increase in stomatal dimension, which results in reduced stomatal density, is often indicative of increased ploidy level in a species; therefore, the lack of variability among the accessions for stomatal density and size is not surprising since all 12 accessions were found to be diploid with a chromosome number of $2n = 2x = 24$ (Figure 3a).

On the other hand, most of the variation among the accessions was the result of variability in flower and fruit traits, which could provide breeders with a tool to improve other *S. aethiopicum* and related eggplant species for these traits. For example, plants of accession PI 420226, which is the sole constituent of group 3, tend to have bigger flowers with longer and wider petals (Table 3; Figure 1E), making them more visible to insects and thus helping with fertilization. Pollens of the same accession had a very good *in vitro* germination (Table 3; Figure 4B), which could also help with fertilization even though fertilization is better measured by in vivo pollen tube growth or vigor. Furini and Wunder (2004) argued that, based on its morphology, PI 420226 should be reclassified as *Solanum macrocarpon,* a domesticated species with edible fruits and leaves, cultivated throughout a large part of Africa. Furthermore, some *S. aethiopicum* cultivars are used for ornamental purposes. For example, PI 247828 (group 1), previously classified

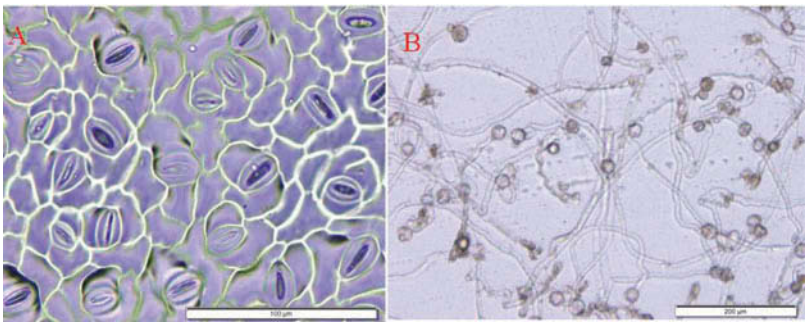

FIGURE 4 (A) Stomates from abaxial (lower) leaf surface of a *Solanum aethiopicum* L. accession (PI 420226). Magnification = 20 ×. Bar = 100 μm. (B) *In vitro* germination of pollen grains from *S. aethiopicum* (PI 374695) after 24 h. Magnification = 10 ×. Bar = 200 μm.

as *Solanum nodiflorum* and then *Solanum americanum*, has very beautiful purple leaves but with small flowers and fruit (Tables 3 and 4; Figure 1C). The ornamental value of this accession could be improved through introgression of genes for larger flower size. Cluster group 2 also has one accession, namely Tourimé, probably due to its larger fruit size. In northeastern Senegal, where this accession originated, scarlet eggplant is almost exclusively grown for its edible fruit; therefore, even though this accession is a landrace variety, it was probably selected for this trait. Moreover, the larger Kumba group to which Tourimé belongs is characterized by wide fruits, slightly acidic fruit, and good fruit taste (Adeniji et al. 2012). The five accessions (PI 424860, PI 144185, Grf14165, PI441885, and P I441912) comprising Group 4 are all members of the larger Gilo group.

In general, eggplant, including the commonly cultivated *S. melongena*, remains a "genomic orphan species" as very little investment has been made toward elucidating its molecular genetics, despite the fact that *S. melongena* is the third most important solanaceous crop after potato and tomato (Barchi et al. 2012). We have determined nuclear DNA content (2C-value), monoploid genome sizes (Cx-value), average chromosome sizes (C/n-value), and GC content for 12 *S. aethiopicum* accessions. We also undertook ploidy analysis of the accessions evaluated using both flow cytometry and chromosome spread and found all the accessions to be diploid with $2n = 2x = 24$ (Figure 3a), which is consistent with previous reports. This is also the same chromosome number reported for the diploid common eggplant (*Solanum melongena* L). To the best of our knowledge, our work is the first report on genome size, average chromosome size, and GC content determination in *S. aethiopicum*.

The DNA content for the 12 *S. aethiopicum* accessions included in our study ranged from 2.623 pg to 3.076 pg, which is close to the DNA content (2.4 pg/2C) of *S. melongena* as well as to those of both potato and tomato (Arumuganathan and Earl 1991). For the most part, there was very little variability in nuclear DNA content among the *S. aethiopicum* accessions studied; only PI 420226 had a DNA content significantly different from the rest of accessions evaluated (Table 5). Indeed, intraspecific genome size variability remains controversial as numerous earlier reports on genome size variability below the species level were dismissed due to, according to Greilhuber (2005), inaccurate methods leading to unreliable measurement results obtained in studies involving endogenous staining inhibitors (Price et al. 2000; Noirot et al. 2003; Beaulieu et al. 2007). Among the recent methods of nuclear DNA content estimation, the most precise results are routinely obtained with flow cytometry (Doležel and Bartoš 2005), in particular when non sequence-specific intercalating fluorescent dyes such as propidium iodide (as in our study), considered the "gold standard" for measuring genome size in plants, are used (Zaitlin and Pierce 2010). Recently, several authors have reported intraspecific genome size variability in several

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plant species including *Festuca, Sinningia*, and *Camellia* species (Šmarda and Bureš 2006; Zaitlin and Pierce 2010; Huang et al. 2013). Therefore, the fact that the accession PI 420226 had a DNA content significantly different than those of the other accessions is most likely not a fluke, but the extent and frequency of this intraspecific variability in *S. aethiopicum* remains to be elucidated as probably a larger sample size representing all four groups (Kumba, Shum, Aculeatum, and Gilo) than the one used in our study needs to be evaluated for that purpose. It is also likely that PI 420226, as suggested by Furini and Wunder (2004), is actually a *S. macrocarpon* accession instead of *S. aethiopicum*.

We also determined base composition (GC content) in *S. aethiopicum*. Base composition provides valuable information useful in characterizing plant species, and it is sometimes a useful parameter in identifying speciation relationships or heterochromatin evolution patterns (Godelle et al. 1993; Leitch and Bennett 2007). A negative correlation ($r = -0.524$, $P < 0.001$) was found between genome size and GC content among the 12 accessions (Table 6). Vesely et al. (2012) have suggested unimodal relationships between GC content and the entire genome spectrum of angiosperms in which a positive relationship exists for taxa with small genomes (< 18400 Mb or 18.8 pg/2C), no correlation for medium-sized genomes, and a negative correlation for taxa with extremely large genomes such as geophytic plants. Genome size in plants varies nearly 2,000-fold from 63 Mb in *Genlisea margaretae* to 124,852 Mb in *Fritillaria assyriaca* (Greilhuber et al. 2006; Leitch and Bennet 2007), and in our study, the 2C values ranged from 2.623 pg (2570.54 Mb) to 3.076 pg (3014.48 Mb). Although these values are smaller than the cutoff point suggested by Vesely et al. (2012), they suggest a negative trend between genome size and GC content in the evaluated *S. aethiopicum* accessions and, therefore, divert from the trend predicted by these authors. Conflicting results have been reported on the correlation between genome size and GC content. For example, GC content was positively correlated with genome size (Lipnerova et al. 2013), and similar results have been documented at a low taxonomic level (Šmarda et al. 2008), but this positive relationship has not been confirmed among seed plants (Barrow and Meister 2002).

FISH can be very useful when studying important details of structural organization of a given plant genome. Additionally ribosomal RNA gene families (18S-28S rDNA and 5S rDNA) provide valuable cytological landmarks for karyotyping and studying the relationships between species and genera as demonstrated by Jiang and Gill (1994) in their investigation of the evolution of polyploid wheats. Furthermore, detection of changes in positions of rDNA sites may be useful in identifying small chromosomal rearrangements (Castilho and Heslop-Harrsion 1995), which could play a role in speciation as suggested by a study of rDNA FISH pattern between two *Pinus*

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subgenera (Cai et al. 2006). Several studies of rDNA variation and structural positions have helped to provide an evolutionary and phylogenetic view of many plant species, including *Arabidopsis* and *Gossypium* (Maluszynska and Heslop-Harrison 1993; Hanson et al. 1996). One site each of 18S-28S and 5S rDNA has been identified in *S. aethiopicum* accession Tourimé (Figure 3b).

CONCLUSIONS

Cluster analysis was used to effectively classify the 12 scarlet eggplants into four distinct groups. A great deal of variability was found among the accessions, most of which was associated with flower and fruit-related traits. Also, this study demonstrated the genetic closeness of *S. aethiopicum* and *S. melongena* not only in terms of their chromosome number but also in terms of their genome size. We used 18S-28S rDNA and 5S rDNA probes to study the distribution and physical position of these ribosomal genes in *S. aethiopicum*, accession Tourimé, thus laying the groundwork for a comparative study of their distribution and physical location in other *S. aethiopicum* accessions and those of other solonaceous plants such as the common eggplant, tomato, and potato plants.

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