



## Research article

Intraspecific crossability and compatibility within *Solanum aethiopicum*Ruth Buteme<sup>a,\*</sup>, Mary Nakajiri<sup>a</sup>, Newton Kucel<sup>a</sup>, Pamela Nahamya Kabod<sup>a</sup>, Godfrey Sseremba<sup>b</sup>, Elizabeth Balyejusa Kizito<sup>a</sup><sup>a</sup> Department of Agricultural and Biological Sciences, P.O.Box 4, Uganda Christian University, Mukono, Uganda<sup>b</sup> National Agricultural Research Organization-NACORRI, P. O. Box 185, Kituza, Mukono, Uganda

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## ABSTRACT

Understanding hybridization barriers is relevant for germplasm conservation and utilization. The prezygotic barriers to hybridization include floral morphological differences like pistil and stamen length, pollen characteristics and pollen-pistil interactions. This study sought to elucidate the reproductive biology of *Solanum aethiopicum*; its mating systems and compatibility barriers. Eight genotypes of *Solanum aethiopicum* were examined for differences in floral morphology, phenology and cross compatibility in a full diallel mating design, with assessment of fruit set, seed set and seed viability. *In-vivo* pollen tube growth was observed for failed crosses at 24, 48 and 72 h after pollination. All genotypes had heterostyly flowers, with predominantly small white petals. Incompatibility was observed in five out of 39 combinations. All selfed genotypes displayed compatibility implying the genotypes are self-compatible. Pollen-pistil incompatibility, which was exhibited in four out of the five failed cross combinations, occurred on the stigma, upper style and lower style, a phenomenon typical in Solanaceae. *Solanum aethiopicum* is self-compatible and majorly self-pollinating but has features that support cross-pollination.

## 1. Introduction

*Solanum aethiopicum* is a herbaceous vegetable belonging to section Oliganthes, sub family Solanoideae, family Solanaceae (Adeniji et al., 2012). *Solanum aethiopicum* also known as the scarlet eggplant or garden egg, originated from tropical Africa (Plazas et al., 2014) and is said to have evolved from *Solanum anguivi* (Sseremba et al., 2017). The *Solanum aethiopicum* complex has four morphological groups, which include; shum, gilo, kumba and aculeatum, all cultivated for consumption of a particular part or for ornamental purposes in the case of aculeatum (Dinssa et al., 2016). The Shum and Gilo group are nutrient rich leafy and fruit vegetables commonly grown in Uganda and parts of Sub-Saharan Africa (Sseremba et al., 2018). In Central Uganda, Shum is proving to be a more important cash crop than coffee, which is the most important export crop for Uganda (Ssekabembe and Odong, 2008). Over 200t of Shum, trade weekly in major markets fetching at least US\$104 per value chain player on a weekly basis in Uganda (Jagwe et al., 2016). This crop therefore has the potential to contribute towards poverty alleviation, reduced hunger and improved food security (Ebert, 2014; Cernansky, 2015).

*Solanum aethiopicum* is diploid ( $2n = 24$ ) with a wide range of genetic and morphological diversity (Adeniji et al., 2013; Sseremba et al., 2017).

This genetic diversity provides a platform for varietal improvement through hybridization. Several external (e.g mechanical, geographical, temporal) or internal barriers however, are known to hinder crop improvement through breeding. Internal/plant tissue barriers can either be pre-zygotic, occurring before fertilization or post-zygotic, occurring after fertilization (Martins et al., 2015). Pre-zygotic barriers include flower morphological differences such as petal color and length (Sakhanokho et al., 2014); pistil and stamen length (Castro et al., 2009); pollen characteristics such as low pollen production and pollen sterility (Vieira et al., 2015); pollen-pistil interactions such as non-germination of pollen grains on the stigma of the receptor plant (McClure et al., 2011); or incomplete pollen tube growth towards the ovule (Asatryan and Tel-Zur, 2014). The latter two are genetically controlled systems categorized as self-incompatibility (SI) which promotes allogamy and cross-incompatibility (CI) that limits production of superior progeny (Camadro and Peloquin, 1981; De Nettancourt, 2001; Ferrer et al., 2009; Acquah, 2012).

Wide-ranging studies in economically viable species in Solanaceae have shown the contribution of a highly polymorphic loci known as the S-locus whose effects can be classified as Sporophytic Self Incompatibility (SSI) and Gametophytic Self Incompatibility (GSI) (Acquah, 2012;

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Eijlander et al., 2000). SSI occurs where the S-haplotype is present in a pollen grain and is determined by the pistil of pollen parent genotype or its own genotype (De Nettancourt, 2001; McClure et al., 2000). GSI which is a common phenomenon in Solanaceae, occurs where the pistil of a female parent and pollen of the male express the same S-haplotype causing inhibition of pollen germination in either the upper, middle or bottom part of the style (Camadro et al., 2012; Gao et al., 2015) contrary to SSI where incompatible pollen may be inhibited on the stigma surface (Acquaah, 2012). However, mutations of the SI genes and the expression and action of S-locus inhibitors in the GSI system have been recorded which have enabled GSI polyploids to produce seed after selfing (De Nettancourt, 2001; Li and Chetelat, 2010).

CI on the other hand is classified as unilateral (incompatible in only one direction) or bilateral (incompatible in both crossing directions) (Maune et al., 2018). In both cases pollen tubes growth can be prohibited at either the stigma; upper, middle or bottom part of the style or in the ovary, as observed in tomatoes (Baek et al., 2015; Maune et al., 2018), eggplants (Afful et al., 2018) and peppers (Onus and Pickersgill, 2004). It has also been reported that in unilateral incompatibility, pollination and eventual fertilization is successful when viable pollen from a self-incompatible (SI) plant is used on a self-compatible (SC) plant while pollen tubes are inhibited in the stigma, style or ovary of the SI plant in a reciprocal cross also known as the SI xSC rule (McClure et al., 2011; Lewis and Crowe, 1958). However, a number of exceptions have been observed, for example in *Solanum tuberosum* where compatibility was seen in SIxSC combinations (Eijlander et al., 2000) and incompatibility in *Solanum lycopersicum* SCxSI combinations (Baek et al., 2015; Li and Chetelat, 2010).

In cases where pollination has been successful, research has identified post-zygotic barriers like embryo death caused by endosperm degeneration (Dickinson et al., 2012; Tonosaki et al., 2016); where no seed is formed and flowers abort before fruit formation (Premabati Devi et al., 2015). Pollen-pistil interaction studies within the Solanaceae family have been able to categorize species as either self-compatible, self-incompatible or mutants from either (Bedinger et al., 2011; Martins et al., 2015; Afful et al., 2018). This has guided breeding programs in *S. lycopersicum* (Baek et al., 2015), *S. melongena* (Premabati Devi et al., 2015), *Solanum tuberosum* (Maune et al., 2018) and *Capsicum annum* (Jae et al., 2006), recording levels of crossability within and between species. *Solanum aethiopicum* is known to be a rich source of genes for resistance to bacterial wilt (Collonnier et al., 2001), therefore hybridization studies involving it have mainly been interspecific with socio-economically and experimentally economic species like *Solanum melongena* and *Solanum lycopersicum* (Behera and Singh, 2002; Afful et al., 2018). Some of these studies have recorded cases of incompatibility for example between *Solanum aethiopicum* Shum and *S. melongena* with no fruit set, parthenocarp and seedling death (Afful et al., 2018). No studies however, have documented the nature and levels of intraspecific crossability within *Solanum aethiopicum*, and there is scanty information on the floral biology of the crop. Therefore, in this study, we provide evidence of crossability within *S. aethiopicum* Shum and Gilo, we identify some of the barriers that underpin incompatibility and for the first time, record the predominant mating systems within the crop. We collected data on the floral morphological characteristics, crossing within its genotypes and subsequent pollen tube growth in this crop.

## 2. Materials and methods

### 2.1. Experimental site

We conducted the experiments at the Department of Agricultural and Biological Sciences at Uganda Christian University, Mukono between December 2017 and April 2020. Uganda Christian University is located in Mukono (0°21'27.0"N, 32°44'29.0"E (Latitude: 0.357500; Longitude: 32.741389)) approximately 22 km east of Uganda's capital city, Kampala.

### 2.2. Plant material

In this study, eight genotypes of *Solanum aethiopicum* were used from the breeding program in the Department of Agricultural and Biological Sciences (Sseremba, 2019). The characteristics of these genotypes are as shown in Table 1.

### 2.3. Design

#### 2.3.1. Floral morphological characteristics

The eight genotypes were planted in a Randomized Complete Block Design (RCBD) with 3 replicates on 4 × 4m experimental plots. A spacing of 35 × 35cm was used between plants and rows.

Data was collected on days to first flowering, days to 50 % flowering, number of flowers per plant on 30 plants per genotype; sepal length, petal length and color, flower breadth, style length, stamen length, and style exertion (coded) on 300 flowers per genotype (10 flowers per plant).

To assess pollen quantity, two dehiscent flowers were collected per plant between 7:00 a.m. to 8:30 a.m. from 10 plants per plot, yielding 20 flowers per plot and 60 flowers per genotype. In total, 480 flowers were assessed. The anthers from each flower were crushed in a vial containing 2 ml of distilled water and a uniform suspension of pollen grains was obtained by shaking. Using a micropipette, both chambers of a haemocytometer were filled with the mixture and viewed under a light microscope at 10x magnification to count number of pollens. The number of pollen grains were counted from the 10 main squares (equivalent to 1 μl) and multiplied by 2000 calculate the total number of pollen grains per flower in 2 mls.

Using the hanging drop method (Rathod et al., 2018), *in vitro* pollen viability was assessed. To do this, twenty dehiscent flowers were collected from each plot at 8:00am bringing the number of flowers assessed per genotype to 60 flowers and 480 flowers for all genotypes.

Using a dropper, 2 drops of a media composition of 15 % sucrose, 0.025 % boric acid, 0.1M of Calcium Nitrate and 0.1M Potassium nitrate was placed on a glass slide (Brewbaker and Kwack, 1963; França et al., 2009). Pollen grains were extracted from three anthers per flower and placed them in the media. The glass slide was covered with a cavity slide, inverted, and then incubated for 24 h at 27 °C. The slide was viewed under a light microscope at a magnification of x10. We considered pollen grains with pollen tube length at least equal to or greater than the pollen grain diameter as germinated/viable. Pollen viability was therefore determined by dividing the number of germinated pollen grains by the total number of pollens per field of view and expressed as a percentage.

#### 2.3.2. Crossing design and technique

To assess crossing success between *Solanum aethiopicum*, seven-selfed genotypes (N2, N4, N11, N14, N15, N18 and G10) were grown under controlled conditions in a screen house. We effected cross pollination using a diallel mating design where each of the genotypes was crossed with another, each having a chance to be either a male or female parent (Kuligowska et al., 2015). However, no crosses were attempted for the

Table 1. List of genotypes used in the study.

Genotype Code	Species	Pedigree	Drought tolerance
N2	<i>Solanum aethiopicum</i> Shum	SAS183/G/2015	Susceptible
N4	<i>Solanum aethiopicum</i> Shum	SAS163/G/2015	Moderate
N11	<i>Solanum aethiopicum</i> Shum	SAS148/2015	Moderate
N14	<i>Solanum aethiopicum</i> Shum	SAS184/G/2015	Susceptible
N15	<i>Solanum aethiopicum</i> Shum	SAS137/2015	Moderate to good
N16	<i>Solanum aethiopicum</i> Shum	SAS184/P/2015	Good
N18	<i>Solanum aethiopicum</i> Shum	SAS108/P/2015	Moderate to good
G10	<i>Solanum aethiopicum</i> Gilo	SAG303/2015	-

Source: (Sseremba, 2019).

**Table 2.** Quantitative floral morphological characteristics of parent genotypes.

Genotype	DFL	DFFL	NFLPI	NFPP	SL	PL	FB	STYL	STML
G10	84 ± 0	101 ± 0	6 ± 2.67 <sup>b</sup>	82 ± 49.7 (1.77 <sup>a</sup> )	0.9 ± 0.60 <sup>c</sup>	1.1 ± 0.19 <sup>d</sup>	2.4 ± 0.29 (0.37 <sup>c</sup> )	0.7 ± 0.11 <sup>c</sup>	0.52 ± 0.07 (0.72 <sup>d</sup> )
N2	75 ± 0	95 ± 0	5 ± 1.41 <sup>b</sup>	216 ± 85.5 (2.33 <sup>cd</sup> )	0.44 ± 0.08 <sup>a</sup>	0.64 ± 0.09 <sup>a</sup>	1.61 ± 0.21 (0.21 <sup>b</sup> )	0.46 ± 0.11 <sup>a</sup>	0.38 ± 0.09 (0.61 <sup>ab</sup> )
N4	71 ± 0.12	95 ± 0	4 ± 0.50 <sup>a</sup>	123 ± 36.55 (2.09 <sup>b</sup> )	0.53 ± 0.07 <sup>d</sup>	0.71 ± 0.12 <sup>b</sup>	1.77 ± 0.22 (0.247 <sup>cd</sup> )	0.52 ± 0.10 <sup>bc</sup>	0.39 ± 0.07 (0.62 <sup>ab</sup> )
N11	69 ± 0	95 ± 0	9 ± 0.79 <sup>c</sup>	156 ± 61.9 (2.29 <sup>cd</sup> )	0.4 ± 0.05 <sup>ab</sup>	0.72 ± 0.10 <sup>a</sup>	1.73 ± 0.19 (0.20 <sup>b</sup> )	0.56 ± 0.14 <sup>ab</sup>	0.44 ± 0.11 (0.66 <sup>c</sup> )
N14	72 ± 0	92 ± 0	9 ± 2.15 <sup>c</sup>	210 ± 110 (2.36 <sup>d</sup> )	0.45 ± 0.08 <sup>ab</sup>	0.72 ± 0.11 <sup>a</sup>	1.78 ± 0.16 (0.23 <sup>bc</sup> )	0.56 ± 0.11 <sup>ab</sup>	0.4 ± 0.08 (0.63 <sup>b</sup> )
N15	71 ± 0	93 ± 0	7 ± 1.78 <sup>c</sup>	247 ± 77.9 (2.29 <sup>c</sup> )	0.52 ± 0.10 <sup>abc</sup>	0.77 ± 0.10 <sup>c</sup>	1.86 ± 0.90 (0.27 <sup>d</sup> )	0.52 ± 0.11 <sup>c</sup>	0.40 ± 0.09 (0.63 <sup>bc</sup> )
N16	76 ± 0	70 ± 0	8 ± 2.35 <sup>d</sup>	342 ± 208.19 (2.68 <sup>c</sup> )	0.51 ± 0.09 <sup>cd</sup>	0.64 ± 0.12 <sup>b</sup>	1.47 ± 0.21 (0.17 <sup>a</sup> )	0.51 ± 0.09 <sup>bc</sup>	0.37 ± 0.07 (0.61 <sup>a</sup> )
N18	72 ± 0	87 ± 0	6 ± 1.52 <sup>b</sup>	167 ± 67.5 (2.153 <sup>b</sup> )	0.52 ± 0.10 <sup>bcd</sup>	0.74 ± 0.12 <sup>c</sup>	1.78 ± 0.66 (0.26 <sup>d</sup> )	0.58 ± 0.08 <sup>d</sup>	0.39 ± 0.07 (0.63 <sup>b</sup> )
Lsd			0.28	0.045	0.026	0.030	0.019	0.028	0.015
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figures in brackets present results from transformed data. Figures with the same letters indicate values with no significant differences between them. DFL = Days to first flowering, DFFL = Days to 50 % flowering, NFLP = No. of flowers per inflorescence, NFPP = No. of flowers per plant, SL = Sepal length (cm), PL = Petal length (cm), FB = Flower breadth (cm), STYL = Style length (cm), STML = Stamen length (cm).

combinations G10xN4, G10xN11 and G10xN15 due to limited number of flowers in G10. Twenty plants per genotype were planted. In total, 39 direct and reciprocal crossing combinations and 7 selfed pollinations were made.

At full bloom, flower buds from the base, middle and upper part of the female plants were emasculated and covered with a paper bag, one day prior to anthesis and then pollinated early in the morning the following day with pollen from dehiscent flowers of the male genotypes. Pollination was effected by tapping pollen onto a clean glass slide, which was rubbed against the stigma of the female parent and then re-bagged. Pollination was considered successful when the fertilized flower stayed intact (did not abort) and developed fruit and viable seed. Fruits with very small or underdeveloped seeds were considered as "seedless". We collected data on the number of fruits produced from each of the successful crosses and the seed number per fruit. We calculated fruit set (%) by dividing the number of developed fruits by the total number of pollinated buds and expressed as percentage.

### 2.3.3. In vivo pollen tube growth

*In vivo* pollen tube growth was used to assess the cause of the failed fruit and seed set. We conducted this experiment at the tissue culture laboratory at the National Crop Resources Research Institute in Namulonge. We modified and used a protocol by Martin (1958). Ten (10) flowers from each failed combination were pollinated on the same day and bagged, then later harvested at 24hrs, 48hrs and 72hrs after pollination. These were then fixed in Acetic acid and 70 % ethanol, then softened in 8M sodium hydroxide for 4 h, washed in 0.1 M K<sub>3</sub>PO<sub>4</sub> for 10 min and stained in a solution of 0.1 % aniline blue diluted in 0.1 M K<sub>3</sub>PO<sub>4</sub> for 8hrs. The gynoecia were mounted on slides in glycerol, smashed with a cover slip and observed with a fluorescence microscope. Images of the gynoecia were taken using a camera head (Nikon DS-L3) at a magnification of 4X and 10X. Pollen tube growth was considered as successful when pollen tubes were seen in the ovary and around the ovules.

**Table 3.** Qualitative floral morphological characteristic of parent genotypes.

Accession Code	Petal Color (n = 300)	Style exertion (n = 300)
G10	White yellow (100 %)	Inserted (3.3 %), Intermediate (83 %), Exerted (13 %)
N2	White yellow (100 %)	Inserted (18 %), Intermediate (80 %), Exerted (2 %)
N4	White yellow (100 %)	Inserted (14 %), Intermediate (78 %), Exerted (9 %)
N11	White yellow (100 %)	Inserted (11 %), Intermediate (82 %), Exerted (7 %)
N14	White yellow (100 %)	Inserted (4 %), Intermediate (96 %), Exerted (2 %)
N15	White purple (100 %)	Inserted (13 %), Intermediate (86 %), Exerted (2 %)
N16	White purple (100 %)	Inserted (7 %), Intermediate (77 %), Exerted (16 %)
N18	White purple (100 %)	Inserted (1 %), Intermediate (88 %), Exerted (11 %)

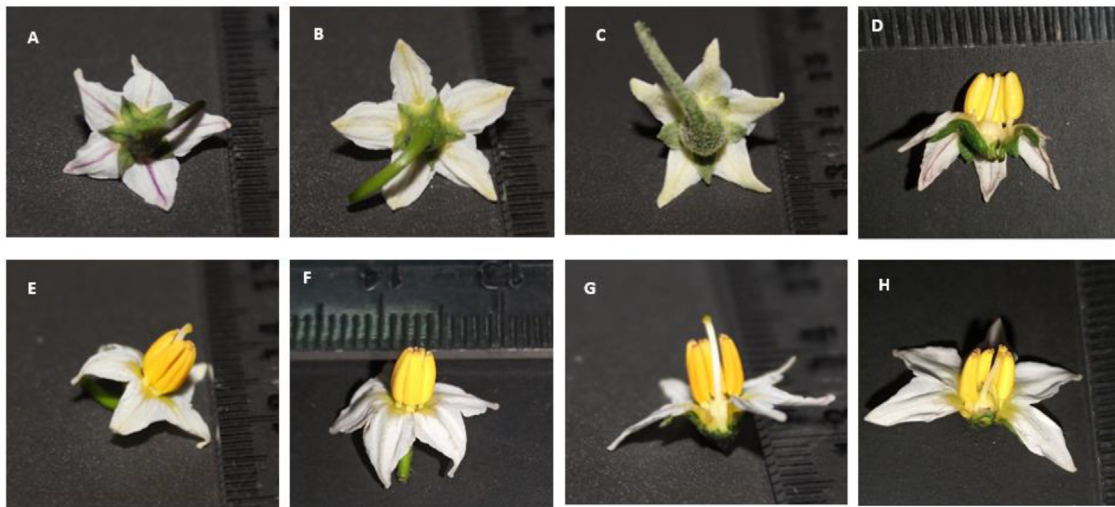
## 2.4. Data analysis

To ascertain the differences in morphological crossability differences within the species, data was analyzed using Genstat Version 14 where means and standard deviations were generated for the quantitative data, frequencies, and percentages for the qualitative traits. Analysis of variance (ANOVA) at P (0.05) was used to test for differences between genotypes. Log (base 10) transformation was used on data that was skewed (e.g number of flowers per plant, flower breadth, stamen length and mean fruit set by parental genotype). The ANOVA was followed with a Bonferroni test to separate means. For variables like days to first flowering and days to fifty percent flowering, which remained skewed even after transformation, a kruskal-wallis nonparametric test was done to determine statistical significant differences between genotypes for these traits.

## 3. Results

### 3.1. Floral morphological traits

We found significant differences between genotypes for all the quantitative morphological characteristics (Table 2). G10 had the highest number of days to first and 50 % flowering while N11 had the shortest time to first flowering and N16 had the shortest time to 50 % flowering. N11 and N14 had the highest number of flowers per inflorescence while N4 had the least. Flowers per plant varied from 82 to 342 where N16 had the highest number of flowers per plant and G10 had the least. For all genotypes, petals were longer than the sepals and G10 had the widest flowers compared to the rest of the genotypes while N16 flowers had the smallest width. All the genotypes had long styles >0.5cm with G10 having the longest and N2 with the shortest (Table 2). When we examined the qualitative traits of flowers, we found that six genotypes, G10, N11, N14, N4, N2 and N14 had white-



**Figure 1.** Variation in floral morphological traits among genotypes. Pictures A-C show the differences in petal color white-purple (A), white-yellow (B and C). Pictures D-H show the differences in style exertion; intermediate (D), exerted style (E and G), inserted style (F and H).

yellow petals, N18, N15, N16 had white-purple petals (Table 3). Style exertion between and within accession varied as shown in Table 3. All the genotypes had majority of their flowers with intermediate style exertion. Each genotype had a varying number of exerted and inserted styles. Aside having majority of their flowers with intermediate style exertion, N16, G10 and N18 had a considerably high percentage of flowers with exerted styles (16 %, 13 % and 11 % respectively) while N2, N4, N15 and N11 had a higher percentage of flowers with inserted styles over the exerted ones (18 %, 14 %, 13 % and 11 % respectively). The pictures in Figure 1 shows style exertion observed in the genotypes.

Studying the number of flowers open at anthesis at different times of the day provides a guide on the optimal time to carry out crossing. Our results showed significant differences between the genotypes in terms of the number of flowers at anthesis per plant within each of the times; 8am, 12pm and 4pm ( $p < 0.001$ ) and significant differences in number of flowers open per plant for each genotype at each of the different times  $p (<0.001)$  (see Table 4). All genotypes showed

**Table 4.** ANOVA table showing variation in number of flowers at anthesis at 8am, 12pm and 4pm by genotype by time.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	7	34877.9	4982.6	164.04	<.001
Time	2	64875.7	32437.8	1067.49	<.001
Genotype Vs Time	14	2618.61	187.04	6.16	<.001
Residual	2376	72168.8	28.83		
Total	2399	174540.95			

**Table 5.** Number of open flowers at anthesis per genotype at 8am, 12pm and 4pm.

Genotype	8AM	12PM	4PM
G10	11 ± 3	7 ± 3	2 ± 2
N2	16 ± 6	11 ± 5	5 ± 4
N4	21 ± 7	14 ± 6	6 ± 4
N11	14 ± 4	9 ± 3	4 ± 2
N14	15 ± 5	11 ± 5	4 ± 3
N15	21 ± 8	15 ± 7	6 ± 4
N16	28 ± 11	21 ± 9	11 ± 5
N18	19 ± 8	15 ± 6	5 ± 4

a gradual reduction in number of flowers at anthesis per plant from 8am to 4pm. The highest number of flowers at anthesis was seen at 8am followed by 12pm and then 4pm when most flowers were closed (Table 5). At 8am when number of flowers at anthesis per plant was highest, N16 had the highest number of flowers open followed by N15 and N4. G10 on the other hand had the fewest number of flowers open per plant at 8am. At 4pm when number of flowers at anthesis

**Table 6.** Average number of pollen grains per flower in *Solanum aethiopicum*.

Genotype	Pollen Quantity	Min	Max
G10	146,000 ± 92,000 (1.770 <sup>a</sup> )	28,000	390,000
N2	112,000 ± 44,000 (1.708 <sup>a</sup> )	44,000	190,000
N4	126,000 ± 38,000 (1.783 <sup>a</sup> )	60,000	206,000
N11	142,000 ± 60,000 (1.815 <sup>a</sup> )	62,000	270,000
N14	114,000 ± 30,000 (1.742 <sup>a</sup> )	52,000	178,000
N15	124,000 ± 30,000 (1.768 <sup>a</sup> )	54,000	192,000
N16	106,000 ± 28,000 (1.708 <sup>a</sup> )	48,000	176,000
N18	132,000 ± 54,000 (1.787 <sup>a</sup> )	56,000	270,000
Isd	0.092		
p-Value	0.239		

Figures in brackets present results from transformed data. Figures with the same letters indicate values with no significant differences between them.

**Table 7.** Pollen viability in *Solanum aethiopicum*.

Genotype	Pollen viability (%)	Min	Max
G10	66.5 ± 3.27 <sup>e</sup>	55.8	72.5
N2	62.2 ± 3.21 <sup>cd</sup>	56.3	68
N4	58.0 ± 3.15 <sup>ab</sup>	50.9	64.9
N11	59.9 ± 3.26 <sup>bc</sup>	54.2	66.5
N14	56.4 ± 2.67 <sup>a</sup>	52.1	62.7
N15	58.6 ± 3.37 <sup>ab</sup>	51	66.5
N16	63.3 ± 2.29 <sup>d</sup>	58.8	66.7
N18	59.1 ± 2.97 <sup>b</sup>	53.8	65.1
Isd	1.5		
p-Value	<.001		

Figures with the same letters indicate values with no significant differences between them.

per plant was lowest, N16 had the highest number flowers still open (Table 5).

### 3.2. Pollen quantity

We found evidence that pollen quantity did not vary between genotypes ( $p > 0.05$ ) (Table 6). It ranged from 106,000–146,000 pollen grains

**Table 8.** Crossing and selfing success within *Solanum aethiopicum* genotypes.

Cross	No. of pollinations	Fruit set%	No. of seeds per fruit	%Germination
N2xN4	115	33	31	35
N4xN2	164	45	51	29
N2xN11	139	10	4	33
N11xN2	155	36	64	66
N2xN14	140	25	67	20
N14xN2	180	19	11	52
N2xN15	170	17	27	26
N15xN2	140	40	43	62
N2xN18	175	19	34	43
N18xN2	182	16	32	22
N2xG10	124	8	43	80
G10xN2	8	50	87	100
N4xN11	184	7	29	68
N11xN4	142	90	85	36
N4xN15	135	10	25	12
N15xN4	129	12	7	69
N4xN14	123	17	34	87
N14xN4	165	35	27	33
N4xN18	121	0	N/A	N/A
N18xN4	177	26	18	32
N4xG10	115	0	N/A	N/A
N11xN14	160	93	42	11
N14xN11	111	31	20	47
N11xN15	172	33	32	21
N15xN11	146	7	21	36
N11xN18	158	34	46	24
N18xN11	98	4	34	38
N11xG10	131	90	2	52
N14xN15	104	20	49	50
N15xN14	177	4	35	90
N14xN18	141	23	8	0
N18xN14	154	30	17	67
N14xG10	10	0	N/A	N/A
G10xN14	6	0	N/A	N/A
N15xN18	145	29	15	18
N18xN15	86	33	14	78
N15xG10	129	3	0	N/A
N18xG10	106	87	29	73
G10xN18	8	0	N/A	N/A
Total	5025	28		
Selfs	No. of pollinations	Fruit set%	No. of seeds per fruit	%Germination
G10	62	45	48	17
N2	102	42	39	17
N4	91	80	116	16
N11	167	63	195	51
N14	120	79	8	75
N15	150	76	36	38
N18	93	91	20	61
Total	785	69		

per flower. The highest maximum number of pollen grains per flower was seen in G10 (390,000), followed by N11 and N18 with 270,000 pollen grains per flower.

### 3.3. Pollen viability in vitro

Pollen viability is an indicator of pollen grain ability to germinate under favorable conditions in terms of temperature and nutrition. Pollen viability for all the genotypes ranged from 56.4 % to 66.5 %. Results in Table 7 show highly significant differences in pollen viability between the genotypes ( $p < 0.001$ ).

### 3.4. Crossability

Our data on crossing success in terms of fruit and seed set showed that out of the 39 intraspecific cross combinations, five produced no fruit and consequently no seed; 5025 flower buds were pollinated; out of these, 1408 (28 %) produced fruit (Table 8). Fruit set was highest in N11xN14, followed by N11xG10, N11xN4 and N18xG10. Lowest fruit set was obtained in N15xG10, N15xN14 (4 %) and N18xN11. There was no fruit set at all in G10xN18, G10xN14, N4xG10, N4xN18, and N14xG10 (Table 8).

Unilateral incompatibility in terms of fruit set was seen when N4 was used as a female with N18 as male (N4xN18), when G10 was a female and N18 was male (G10xN18) (Table 8). Bilateral failure in fruit set on the other hand, was observed in N14xG10-G10xN14 combinations (Table 8).

The number of crosses performed depended on the number of flower buds available. In this respect, it was observed that G10 produced few flowers (Table 2) hence the number of hand pollinations made on it were not as many as the rest of the genotypes.

Seed set from the various cross combinations also varied (Table 8). Mean seed set per fruit was highest in G10xN2 (87 seeds), N11xN4 (85 seeds). It was lowest in N11xG10 (2 seeds per fruit), followed by N2xN11 with 4 seeds per fruit and N15xN4 with 7 seeds per fruit. All fruits produced from the N15xG10 cross had no seed.

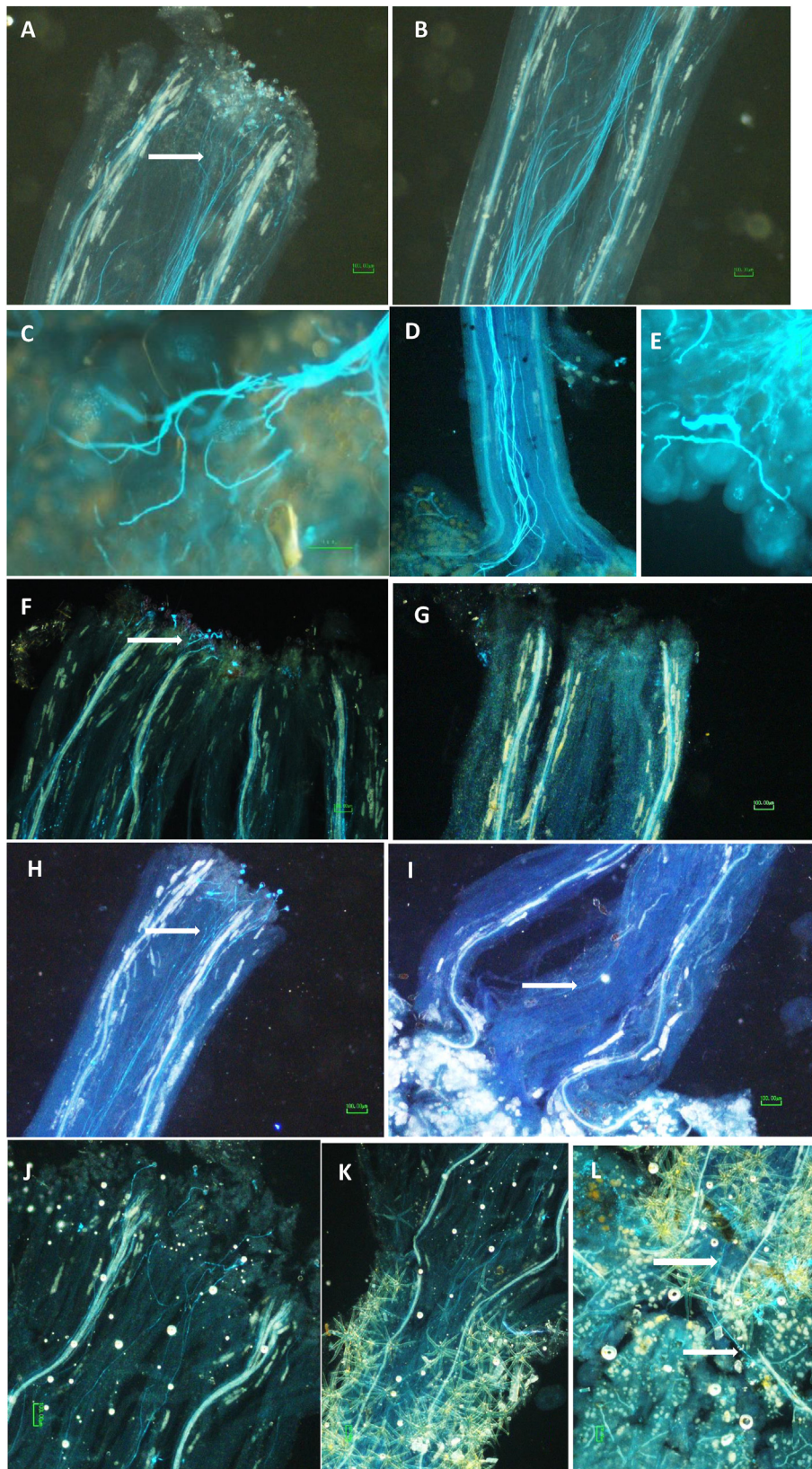
The seeds from the successful crosses were planted and tested for germination viability. Results show that the highest seed germination percentage was observed in G10xN2 with 100 %, followed by N15xN14 (90 %), N4xN14 (87 %) and N2xG10 (80 %). No incompatibility was recorded for the self-pollination events (Table 8).

Results also showed that overall there were significant differences among the parental genotypes for fruit set. Female parents such as N11 had the highest fruit set followed by N18, while lowest fruit set was observed in G10 (Table 9). However, fruit set did not vary among genotypes when used as male parents ( $p = 0.417$ ). There was also no significant variation in seed set when parents were used as either female ( $p = 0.215$ ) or male ( $p = 0.291$ ) parents across combinations.

**Table 9.** Mean fruit set by female parental genotype.

Female parent	Mean fruit set (%)
G10	7.1 ± 18.9 (1.691 <sup>b</sup> )
N11	60.1 ± 29.1 (1.734 <sup>b</sup> )
N14	18.9 ± 12.9 (1.263 <sup>ab</sup> )
N15	15.0 ± 14.1 (1.01 <sup>a</sup> )
N18	30.6 ± 26.7 (1.345 <sup>ab</sup> )
N2	17.9 ± 8.8 (1.206 <sup>ab</sup> )
N4	14.6 ± 15.8 (1.218 <sup>ab</sup> )
lsd	0.5417
p-value	0.018

Figures in brackets present results from transformed data. Figures with the same letters indicate values with no significant differences between them.



**Figure 2.** Shows germination of pollen in-vivo. In an example of the selfed cross N11xN11 (A–C), pollen tubes germinated from stigma (A) into the style (B), and into the ovule (C) after 24hrs. Pollen tubes were also in the style (D) and to the ovules (E) after 24hrs for cross N4xN18. Picture F shows failure of pollen tubes to move past the upper part of the style after 72hrs in G10xN18 while (G) shows no pollen grains attached to the stigma in cross N14xG10. Picture H shows the case of cross N4xG10; pollen grains germinated on the stigma and into the style (H), but after 72hrs, the pollen tubes were arrested in the bottom part of the style (I) in. J-L show pollen tubes moving from the stigma (J) in the style (K) and in the ovary (L) of cross G10xN14 but trichomes obstructed the ability to see any penetration into an ovule.

### 3.5. Pollen-pistil interaction-In vivo pollen tube growth

Fluorescent microscope examination of *in vivo* pollen tube growth showed the different levels of success in pollen tube growth (Figure 2).

Out of the five failed crosses (G10xN18, G10xN14, N4xG10, N4xN18, and N14xG10), only one showed pollen pistil compatibility. This was N4xN18 whose pollen grains germinated down the whole length of style into the ovary and penetrated the ovules 24hrs after pollination. The

failure of fruit set for N4xN18 cross could be due to post fertilization barriers (Afful et al., 2018), which was not assessed in this study. G10xN14 also showed pollen tubes in the ovary after 72hrs, although a mass of trichomes made it difficult to tell whether they had penetrated the ovules. The crosses between G10xN18, N4xG10 and N14xG10 showed incompatibility in different ways. For G10xN18, germinated pollen tubes did not grow beyond the upper mid style 72hrs after pollination. In the cross N4xG10, pollen grains germinated and penetrated the full length of the style 24hrs after pollination, but progressed no further after 72hrs and did not penetrate the ovary. An interesting scenario was observed with N14xG10 crosses where no pollen was observed on the stigmas of the pistils at all times of examination.

In the self-pollination event, which were used as controls, no incompatibility was detected; pollen tubes had penetrated the ovules 24hrs after pollination. It was however difficult to see how far the pollen tubes penetrated into the ovary in G10 because of the presence of trichomes, which disrupted clarity.

#### 4. Discussion

The hybridization barriers examined in this study included pre-fertilization heteromorphic incompatibility barriers such as floral morphological and phenological variations. In open field conditions, plant's morphological structures and phenology need to be able to facilitate cross-pollination of plants for cross-fertilization to be successful (Karapanos et al., 2008). Angiosperms have adapted the following mechanisms to aid cross pollination; unisexuality, where one sex organ exists on a flower, heterostyly nature, where the stigma is above the anthers, male sterility where anthers or pollen grains are absent or pollen grains are not viable, self-sterility, where pollen from the same flower cannot fertilize its own ovary and lastly, synchrony in flowering and anthesis with pollen donors (Acquaah, 2012). Results showed that *Solanum aethiopicum* is bisexual, produces many flowers per plant, an average of 193, a substantial number of pollen grains per flower (overall average of 126,000 pollen grains) and pollen viability ranged from 56.4 % to 66.5 %, which is considered as good viability according to (França et al., 2009).

For all genotypes, the highest number of flowers at anthesis was seen at 8am (18) which sequentially dropped by 12pm (13) and to 5 at 4pm. This could mean that the peak of flower opening in *Solanum aethiopicum* may be at 8am or probably slightly before (Sekara and Bieniasz, 2012). Full bloom anthesis during the morning hours between 6:45 and 9:45am has been reported in *S. melongena* (Das et al., 2017; Sekara and Bieniasz, 2012) and in *Capsicum annum* with a peak at 8:00am (Aleemullah et al., 2000). N2, N4 and N11 had the same number of days to 50 % flowering (95) with only a two and three days difference with N15 and N14 respectively.

In terms of style exertion, parental genotypes in this study were heterostyly in nature, with flowers with intermediate styles (stigma at the same level with anthers) as majority, followed by exerted styles (stigma above anthers) and inserted styles (stigma below anthers). These results mean that in favorable open field conditions, morphological features and phenology of *Solanum aethiopicum* can support both self and cross-pollination (Christopher et al., 2020; Conner and Rush, 1996). Studies have reported that variation in style exertion affects fruit set in open field conditions (Kowalska, 2003). These studies report that high fruit and seed set is achieved more in flowers with exerted and intermediate styles compared to those with inserted styles. This is because they are more exposed to the anthers, have larger stigmas, with styles rich in polysaccharides that provide nutrition to pollen tubes, and the downward orientation of the flowers that favors self-pollination (Kowalska, 2003, 2008).

To assess the presence of self-sterility and cross incompatibility, hand pollinations were made between the genotypes in all directions with self-

pollinations used as controls. Out of the 39 intraspecific cross combinations, five (12 %) produced no fruit and consequently no seed. On the other hand, all selfed parents produced fruit. All the cross and self-pollinations that produced fruit had seed except N15xG10. The cross combinations that produced no fruit were G10xN18, G10xN14, N4xG10, N4xN18, and N14xG10. To identify the cause of failure in fruit set in these crosses, *in-vivo* pollen tube growth was studied. Results showed pollen pistil compatibility in N4xN18 and incompatibility in the rest. Incompatibility was expressed in three ways; failure of pollen to stay attached on the stigma (N14xG10), pollen tubes trapped in the upper or lower parts of the style (G10xN18 and N4xG10 respectively). These results indicate incompatibility between pollen or pollen tubes of donor plants and the pistils of the female plants (Baek et al., 2015; Martins et al., 2015; Onus and Pickersgill, 2004). All the selfed crosses expressed self-compatibility. Pollen tubes reached the ovules 24 h after pollination indicating that the crosses made in this study were between self-compatible plants (SCxSC crosses). A number of studies have reported gametophytic incompatibility within Solanaceae and have also reported evidence of incompatibility occurring between two Self Compatible plants (Covey et al., 2010; McClure et al., 2011). This phenomenon has been attributed to the possibility that self-compatible species may have evolved from self-incompatible ones; mutating in an SI Sc SC' SC sequence (McClure et al., 2000). Other studies also showed the presence of S-locus encoding S-ribonucleases (*S-RNase*) in styles of some self-compatible species in *Solanacea* (Bedinger et al., 2017; Eijlander et al., 2000; Zhao et al., 2011). This may imply that a homologous Sc Sc species would be self-compatible and produce pollen, which would be compatible on SI styles but would display incompatibility of SI pollens in its own styles. Mutation from Sc to Sc' which is the intermediate stage of self-compatible style would not inhibit the pollen of SC but produce pollen compatible with SI styles; whereas mutation from Sc' to SC could lead to a self-compatibility phenotype which would produce pollen that is inhibited by SI species. However, other factors besides the presence of *S-RNase* in the pistil of the SC plants that affect compatibility (Li and Chetelat, 2010). For example, the presence or absence of HT-proteins and *S-RNase* binding protein NaTTS (Na-transmitting tract specific) in the style and S-locus F box proteins and SCF ubiquitin ligase components like Cullin 1 in pollen (Cruz-Garcia et al., 2003; Li and Chetelat, 2010). The failure of N4xN18 to produce fruit and N15xG10 to set seed despite successful fertilization could be indicators of post fertilization barriers like allelic incompatibility or endosperm breakdown (Bushell et al., 2003). Further research is needed to elucidate whether there is a relationship between pollen size, stigma depth and style length and the presence of SI genes in styles of the female flowers for the combinations that showed pollen-pistil incompatibility. Molecular tools could be used to elucidate on whether sporophytic incompatibility could be at play in N14xG10.

#### 5. Conclusion

Results from this study show that *Solanum aethiopicum* is majorly self-pollinating though some floral morphological features may support cross-pollination for example style exertion, and pollen quantity. The pollen tube growth studies showed that the genotypes are self-compatible. Both pre-fertilization barriers hampered intraspecific hybridization where pollen grains failed to attach or germinate past the stigma or pollen grains fail to reach the ovules and post fertilization barriers where fertilization takes place but no fruit is formed or if fruit and seed are formed or the seed fails to germinate. Further studies can be done to elucidate the presence of S-genes and HT proteins in the styles of N4 and G10. The poor/no seed germination recorded in some of the crosses can be resolved using embryo rescue, ovule culture and seed culture techniques, which have been successfully used to regenerate hybrids. The

information attained from this hybridization study will contribute towards the production  $F_1$  hybrids and successive generations in the development of new cultivars with superior quality traits.

## Declarations

### Author contribution statement

Ruth Buteme: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mary Nakajiri; Kucel Newton: Performed the experiments.

Pamela Nahamya Kabod: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Godfrey Sseremba: Conceived and designed the experiments; Analyzed and interpreted the data.

Elizabeth Balyejusa Kizito: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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