UGANDA CHRISTIAN UNIVERSITY

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DEPARTMENT OF AGRICULTURE

CROSS-COMPATIBILITY AND COMBINING ABILITY BETWEEN *SOLANUM AETHIOPICUM* WITH ITS CLOSE RELATIVES

NAMUTOSI WINNIE

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ABSTRACT

This study elucidated the reproductive barriers and combining ability effects for yield and yield related traits in crosses between *S. aethiopicum* and its relatives. Six parental lines of *S. aethiopicum* (Shum and Gilo) and its relatives (*S. incanum, S. macrocarpon, S. anguivi*) were examined. Anthesis, anther dehiscence, stigma receptivity, pollen viability, fruit, seed set and F_1 viability were assessed. Determining mode of gene action and combining ability, 15 successful F_1 hybrids obtained from a full diallel mating design were evaluated. Overall mean number of open flowers was highest (13) for N11 and least (3) for E12, though, differed significantly ($p<0.001$). Stigma receptivity differed significantly among genotypes and seasons ($p<0.001$) and was highest for N11 at 3.31± 1.32 and least for In1 at 2.24±1.27. All genotypes had high pollen viability ($\mu>$ 80%) though differed significantly ($p<0.01$). All genotypes exhibited self-compatibility to varying degrees with N11 showing the highest fruit success (67.93%), seed per fruit (n=82) and F1 germination (79%). Interspecific crosses where *S. macrocarpon, S. anguivi* and *S. incanum* were used as females showed poor or no fruit and seed. N11was the top performer as a female with the cross with S. *macrocarpon* (N11xE12) showing highest fruit success (65.89±26.48), *S. anguivi* (N11xA1) showing the highest seed set (n=56±34.23) and *S. incanum* (N11xIN1) showing the highest F_1 germination (64±18.72). High broad sense heritability (above 80%) was observed in all successful crosses for leaf and fruit traits. Specific combining ability effects were highly significant for all the traits measured ($P<0.01$); showing that traits were conditioned by non-gene additive action. *S. aethiopicum* can be selected as a female parent in crosses involving *S. incanum* and *S. macrocarpon* for raising fertile hybrids. Species with good breeding value and promising specific crosses provide a guide in establishment of breeding program for the crop.

DECLARATION

I Namutosi Winnie hereby declare to my full consent that this research is my original work, is not plagiarized and has not been submitted to any other institution for any award.

Signature: .....Date: 16th/08/2023.....

NAMUTOSI WINNIE

APPROVAL

This dissertation has been approved by the undersigned person as a requirement for the award of a degree of Masters of Science in Agriculture by Research.

As the candidate's supervisor, I agree to the submission of this dissertation.

SUPERVISORS

Signature..... Date..... 16 August 2023

ASSOC. PROF. ELIZABETH BALYEJUSA KIZITO (PHD)

Signature..... Date..... 16th Aug-2023

DR. BULYABA ROSEMARY

DEDICATION

I do dedicate this piece of work to my beloved family, my parents (Mr. Nabitu Patrick and Mrs. Oliver Nabitu), my brothers and sisters.

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GLOSSARY OF ACRONYMS

UCU	Uganda Christian University
FAS	Faculty of Agricultural Sciences
ANOVA	Analysis of Variance.
CI	Cross compatibility
SI	Self- incompatibility
SC	Self- Compatibility
UI	Unilateral- Incompatibility.
GCA	General Combining Ability
SCA	Specific Combining Ability
4WAT	Four Weeks after Transplanting
6WAT	Six Weeks after Transplanting

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Solanum aethiopicum (2n) is an important vegetable amongst farmers in Africa, especially in Uganda because of its high cultural, nutritional, medicinal and market values (Abukutsa-Onyango, 2014; Sseremba *et al.*, 2018; Nakyewa *et al*., 2021). The crop is reported to have originated from tropical Africa (Sseremba *et al*., 2018; Buteme *et al*., 2021). *S. aethiopicum* is a herbaceous vegetable which belongs to *Solanaceae* family (Plazas *et al*. 2016; Sseremba *et al*., 2018; Buteme *et al*., 2021). The crop has various forms of species which are morphologically different. These are Gilo, Kumba, Shum and Aculeatum (Csintalan *et al*., 2000). The leaves and fruits of *Solanum aethiopicum* are normally consumed apart from Aculetum which is used for ornamental purposes (Csintalan *et al*., 2000).

Despite of its high value, *Solanum aethiopicum* production has encountered various challenges such as drought stress, pests and diseases, poor seed viability, low yields among others (Sękara *et al*., 2007; Sseremba *et al.*, 2018; Buteme *et al*., 2021). These challenges can be addressed through hybridization which supports introgression of desirable genes between and with species hence improving crop output, resistance to diseases and pest, and harsh climate adaptations (Ghani *et al.*, 2020). Interspecific hybridization, which includes incorporating desirable genes from the close relatives for crop enhancement, is a crucial breeding strategy for *Solanum* species (Collonnier *et al*., 2001). Wild and other close relatives are good sources of variation for developing new cultivars of the crop with improved leaf and fruit yield, quality as well as the ability to adapt to unfavorable and changing climatic conditions (Prohens *et al*., 2013). The close relatives of the crop are rich in genes for resistance to pests and diseases, drought resistance and tolerance. For

instance, *S. incanum* was reportedly used in interspecific hybridization with other crops such as *S. melongena* (Kumchai *et al*., 2013; Afful *et al*., 2018). Some genes were transferred successfully for verticillium and fusarium wilt resistance in eggplants, and new hybrids for *S. melongena* were generated (Prohens *et al*., 2013; Plazas *et al.*, 2016). The potential for improvement of *S. aethiopicum* by crossing with close relatives has been underexplored by breeders in contrast to other vegetables like *S. lycoperscon* (tomatoes), *S. Melongena* (eggplants) and *S. tuberosum* (Irish potato) (Hajjar & Hodgkin, 2007; Díez & Nuez, 2008; Maune *et al.*, 2018).

Solanum aethiopicum improvement is hindered by limited information on incompatibility mechanisms (self and cross- incompatibilities) which limit utilization of wild relatives (Plazas *et al*., 2016; Afful *et al.*, 2018; Ghani *et al*., 2020). Self- Incompatibility (SI) hinders self-fertilization and promotes out crossing and allogamy. Investigations have identified SI mechanisms that prevent pollen on stigmas from germinating or pollen tubes elongation in styles (Bedinger *et al*., 2011; Baek *et al*., 2016). Cross Incompatibility (CI) also arises due to the absence of genetical material in the one of the species during hybridization (Hogenboom & Mather, 1975). Cross incompatibility can be unilateral or bilateral, depending on whether interspecific crosses were compatible in just one direction (Self incompatibility and Self-compatibility rule (SIxSC rule)) or even in both directions of the cross, respectively (Camadro & Peloquin, 1981; Onus & Pickersgill, 2004; Hayes *et al*., 2005; Baek *et al*., 2015). Interspecific compatibility barriers can be expressed before (pre-zygotic barriers) or after fertilization (post-zygotic barriers) (Rieseberg & Wendel, 2007; Bedinger *et al*., 2011; Buteme *et al*., 2021).

Pre-zygotic barriers are the initial factors which hinder gene flow between and within the species (Baek *et al*., 2016). These barriers can involve geographic isolation, variations in floral morphology and traits that lead to pollinator shifts (Baek *et al*., 2016; Buteme *et al*., 2021).

Geographic segregation between different taxa of plant species prevents the contact of two species hence reducing the transfer of genes (Sweigart *et al.*, 2006; Baek *et al*., 2016). Ecological differences of crop species occurring in sympatry is related to time to flowering (phenology) where some species produce flowers in dry periods and others in wet periods/seasons (Baek *et al*., 2016). Morphological traits of *Solanum aethiopicum* can also reduce mating chances (Levin, 1971; Buteme *et al*., 2021). Floral features including floral size (style length), shape and color also prevent hybridization between species (Lee *et al*., 2008; Baek *et al.*, 2015, 2016). The pollen grains of short- styled species for instance cross to the long styled species leading to failure of pollen transfer and growth (Lee *et al*., 2008; Baek *et al.*, 2016). Denkyirah, (2013) also found out that eggplants with long styled flowers had high number of fruits set compared to those with short styles. Short-styled flowers had small, decreased gynoecium which are frequently infertile, stigmas which are small having immature papillae as well as a spatial departure of anther pores and low sugar amounts, which hinder pollen grain germination and growth (Passam *et al*., 1997; Chen, 2001; Sekara & Bieniasz, *2008*). The differences in floral features drive the crops/ plants to be more self- pollinated or cross pollinated (Bedinger *et al*., 2011; Baek *et al.*, 2015; Premabati Devi *et al*., 2015). For instance, small flowers attract few pollinators than larger flowers (Quesada-Aguilar *et al*., 2008; Baek *et al*., 2016; J. Chen *et al*., 2018; Goodwillie & Weber, 2018).

Furthermore, the type of inflorescence and flower types also affect pollination. For instance, the drop of flowers is lower in solitary flowers compared to clustered flower types of *Solanum* species where flowers drop off up to 80% due to low pollination (Bose & Som, 1986; N. Patel *et al*., 2018; Han *et al*., 2021). The amount of out crossing is significantly influenced by the size of the flower and the stigma's exertions, or the distance the stigma extends beyond the anther (Bedinger *et al*., 2011, 2017). Species having inserted flowers receive more self-pollen than pollen from other

species (Chen *et al*., 2018; Goodwillie & Weber, 2018). The wet stigma surfaces which are highly receptive can also allow/ inhibit the germination of pollen tubes in the style (Fang *et al*., 2013; Souza *et al*., 2016). The stigmas are sites for pollen grain compatibility and assist pollen hydration and germination (Edlund *et al*., 2004). Stigmatic sensitivity also minimizes intersexual interference and prevents intra-floral selfing (Neeraja Puthiamadom, 2017; Rech *et al*., 2018; Peña-Yam *et al*., 2019). Once pollen lands on the stigma during pollination, post mating pre-zygotic barriers act to prevent crossing (Maune *et al*., 2018). Pollen germination failure on the stigma shows unilateral incompatibility; pollen from Self-compatible species cannot grow on pistils of SI species however success is seen in reciprocal crosses (Baek *et al*., 2015, 2016; Maune *et al*., 2018).

Post-zygotic barriers are also very significant barriers to hybridization in *Solanum* species and these include failure to develop fruit and seed, failure of hybrid seeds to germinate, hybrid necrosis and sterility which result into epigenetic incompatibility (Onus & Pickersgill, 2000, 2004; Maune *et al*., 2018; Buteme *et al.*, 2021). Even though some pollen grains can grow and reach the ovules to form a fruit, seed development can fail due to formation of abnormal embryos (Lester & Kang, 1998; Martins *et al*., 2015; Afful *et al*., 2018). Seeds of hybrids can fully develop and germinate, but later the F_1 off springs can express necrosis or sterility (Kondo *et al*., 2002; Laporta, 2005; Baek *et al.*, 2016). The occurrence of sterile hybrids is caused by genetic incompatibility (Kondo *et al*., 2002; Laporta, 2005). Hybrid sterility is related to chromosomal rearrangements and translocations (Baek *et al*., 2016). Production of highly fertile, partially fertile and even infertile hybrids in interspecific crosses of *S. melongena* is also attributed to which gene pool the wild relatives belong to (primary, secondary and tertiary gene pool, respectively) (Lester & Kang, 1998; Knapp *et al*., 2013; Davidar *et al*., 2015; Kouassi *et al*., 2019).

Even if the hybrids produced are fertile, often defects where some hybrids express intermediate phenotypic traits such as yield and yield related traits (plant height, leaf area, number of leaves and fruits, fruit size); resulting in low or high fitness can be noticed in the F_1 or next generations when parental conditions are provided (Rhode and Cruzan, 2005; Baack *et al*., 2015; Baek *et al*., 2016). Therefore, Combining ability (CA) analysis is vital phenomenon in predicting the genetic potential or fitness of parental lines and crosses (Liu *et al*., 2021). Combining ability (General combining abilities (GCA) and Specific combining abilities (SCA)) is important for selection of species for developing superior hybrids (Fasahat, 2016; Liu *et al.,* 2021). The parents with high GCA effects give off springs with low SCA effects and parents with low GCA produce off springs with high SCA (Viana, 2007; Pavan *et al*., 2022). During selection of parents for making crosses, understanding of the relationship between GCA of parents, SCA of crosses and knowing the dominating type of combining ability is key in improving breeding efficiency (Sseremba, 2019; Liu *et al.,* 2021). General combining ability is determined by additive gene actions and Specific combining ability is controlled by non-additive genes (dominance and epistasis) (Saleem *et al.,* 2013; Liu *et al*., 2021). Investigations on combining ability are widely undertaken in several interspecific crosses of other vegetables such as *S. melongena, S. lycopersicum* and *S. tuberosum* (Singh & Asati, 2011; Ansari & Singh, 2014). Studies on combining ability examined in intraspecific crosses of *S. aethiopicum* for drought tolerant traits identified GCA and SCA for genotypes for different traits (Sseremba, 2019). Highly significant specific combining ability was obtained in the intraspecific hybrids of *S. aethiopicum* for leaves per plant and leaf area (Buteme *et al*., 2021). Limited information regarding combining ability for traits in interspecific crosses between *S. aethiopicum* and its relatives is known.

Despite being aware of the challenges and potential of hybrids developed during interspecific hybridization of *S. melongena, S. lycopersicum, Capsicum spp and S. tuberosum*; information regarding interspecific hybridization of *S. aethiopicum* with related species, as well as the nature and levels of incompatibility barriers in interspecific crosses is still limited. There is a dearth of knowledge on inheritance and combining abilities which are necessary and relevant in exploring the principles, methods and strategies of *S. aethiopicum* cultivar development. Therefore, this study investigated the nature of incompatibility barriers and combining ability between *S. aethiopicum* and its relatives.

1.2 PROBLEM STATEMENT

Due to existing reproductive barriers, gene flow between *Solanum aethiopicum* and its relatives is prevented (Afful *et al*., 2018; Plazas *et al.*, 2016; Buteme *et al.*, 2021). Several studies on interspecific incompatibilities among other *Solanum* species abound, but information on *Solanum aethiopicum* and its related counterparts is rather scanty (EL & SJ, 1981; Onus & Pickersgill, 2004; Bedinger *et al*., 2011; Martins *et al*., 2015). Information on floral morphology and crossability in intraspecific crosses of *Solanum aethiopicum* is known (Buteme *et al.*, 2021). The investigations showed that 86% of intra-specific crosses produce fruits and only12 % of crosses fail to produce fruits due to failure of pollen to stay on the stigma and trapped pollen tubes in the upper and lower regions of the style (Buteme *et al*., 2021). In interspecific crosses of *S. melongena* and the relatives (*S. aethiopicum, S. anguivi, S. torvum and S. macrocarpon),* 84% of crosses showed incompatibility (Afful *et al*., 2018). The cross compatibility and production of interspecific hybrids in many other vegetables like *S. lycopersicum, S. tuberosum, capsicum spp* and *S. melongena* are extensively explored (Karapanos *et al*., 2008; Martins *et al*., 2015; Plazas *et al*., 2016; Yermishin *et al*., 2017). Some genes were transferred successfully for verticillium and fusarium wilt

resistance from wild relatives in eggplants, and new hybrids for *S. melongena* were generated (Prohens *et al*., 2013; Plazas *et al.*, 2016). The potential for breeding *Solanum aethiopicum* with close relatives has not received as much attention from breeders compared to other vegetable crops like the tomato, egg plants and potatoes (Mabhaudhi *et al*., 2017; Sseremba *et al*., 2017; Han *et al.*, 2021).

Although *S. aethiopicum* has a large number of valued close relatives which are good sources of genetic variation (Lester and Hasan, 1991; Daunay, 2008; Knapp *et al*., 2013), for development of cultivars to address challenges posed by biotic and abiotic stress (Voronsova *et al., 2013,* Mutegi *et al*., 2015, Kouassi *et al*., 2016), the relatives remain underutilized in hybridization studies (Jackson and Hanneman, 1999; Afful *et al*., 2018). This is due to the existence of interspecific barriers which affect introgression of genes between relatives during crossing (Lester & Kang, 1998; Eijlander *et al.*, 2000; McClure *et al*., 2000; Plazas *et al.*, 2016; Afful et al., 2018). Whereas in other *Solanum* species, these interspecific reproductive barriers and the generation of interspecific hybrids are extensively investigated and addressed (Qiao *et al.*, 2004; Kaushik *et al*., 2016; Afful *et al*., 2018; Maune *et al*., 2018). There is dearth information regarding the nature and degree of success of interspecific hybridization between *Solanum aethiopicum* (Shum and Gilo) and its close relatives. Yet, there is urgent need to develop new genomics of desired attributes of adaptability such as resistance to pests and diseases, drought tolerant, and high yielding varieties. Therefore, the study evaluated the cross-compatibility barriers and combining ability in *Solanum aethiopicum* with its close relatives.

1.3 OBJECTIVES

The main objective of this study was to investigate the nature of cross incompatibilities and gene action between selected *Solanum aethiopicum* genotypes and its wild relatives. The specific objectives are:

1. To identify the major pre-zygotic reproductive barriers in *Solanum aethiopicum* and its close relatives
2. To determine the level of crossing success between *Solanum aethiopicum* and its relatives
3. To determine the combining ability of yield and yield related traits in F_1 hybrids of *Solanum aethiopicum* and its close relatives.

1.4 HYPOTHESIS

1. Anthesis is the major contributor to incompatibility in *S. aethiopicum* and its close relatives
2. *S. aethiopicum* and its relatives will show crossing failure in 84% of the crosses as in S. *melongena* and its relatives (Afful *et al*., 2018)
3. Inheritance of yield and yield related traits in hybrids is conditioned by additive gene action

1.5 SIGNIFICANCE

Solanaceae crops is a widely consumed and utilized vegetables worldwide especially in Uganda and it contributes to improving nutrition and income especially among youth and women (Sseremba *et al*., 2017; PAEPARD, 2018; Nakyewa *et al*., 2021). In Uganda, *Solanum aethiopicum* is a major commercial and subsistence crop grown in both peri-urban and urban areas with central Uganda having the highest production and marketability (Ssekabembe & Odong, 2008; Pincus, 2015; Sseremba *et al*., 2017; PAEPARD, 2018). The *Solanum* crops are widely distributed in all regions of Uganda with 43.4% production (PAEPARD, 2018). *Solanum*

aethiopicum being the highest in production with 12.4% and other *Solanum* crops take up 31% (Sseremba *et al*., 2017; PAEPARD, 2018). Due to increasing demand for *Solanum aethiopicum* in Uganda, its production has potential to significantly contribute to household nutrition and incomes. Research shows that about 47% of households consume vegetables at least 3-5 times a week (PAEPARD, 2018). Additionally, Over four million people in urban and peri-urban areas earn from the Shum group and more than 200 tons of Shum are traded weekly, with each value chain player earning at least US$104 each week in Uganda (Ssekabembe & Odong, 2008; Jagwe *et al*., 2016; Nakyewa *et al*., 2021). Consequently, the crop plays an important role in alleviating poverty, hunger reduction and improving food and nutritional security in different households in the country (Abukutsa-Onyango, 2014; Cernansky, 2015; Nakyewa *et al*., 2021). Information generated from this research on incompatibility barriers and combining ability between the selected *S. aethiopicum* genotypes and wild relatives, *S. anguivi, S. macrocarpon* and *S. incanum* will provide a better understanding of mechanisms for further crop improvement alongside insights to unlock incompatibilities in African *Solanum* species for the improvement of nutrition, health, and incomes. Further, this study will be useful to breeders in developing high yielding varieties (hybrids) which are more adapted to diverse environments and climate change. As a result, the possible interspecific F_1 hybrids produced for *S. aethiopicum* and its relatives can benefit profit-making seed companies and ultimately smallholder farmers in Uganda and across Africa.

1.6 JUSTIFICATION

Reports on incompatibility barriers and combining ability in interspecific crosses of *S. aethiopicum* and its relatives is very vital for guiding the future breeding efforts (Buteme *et al*., 2021; Maune *et al*., 2018). With identification of the nature of interspecific incompatibility barriers associated with *Solanum aethiopicum and* its wild relatives, breeders will have insights and diverse means of

unlocking the barriers. Consequently, this necessitates continued research efforts on incompatibility mechanisms in *Solanum aethiopicum* and the wild relatives that will create awareness and deepens understanding of mechanisms for further crop improvement. The findings will guide breeders in developing comprehensive breeding approaches to overcome these barriers (Plazas *et al*., 2016; Maune *et al*., 2018). It is also important to categorize the relatives which are compatible with *S. aethiopicum* that will generate superior hybrids of high vigor with desirable characteristics.

1.7 CONCEPTUAL FRAMEWORK FOR CROSS-COMPATIBILITY AND COMBINING ABILITY

Crossability in *Solanum aethiopicum* means capability of the crop species to introgress with or between each other (Afful *et al*., 2018). Two parents must be compatible for a cross between *S. aethiopicum* and its relatives to result in high vigor hybrids. Interspecific hybridization is hindered by various reproductive and environmental factors. The reproductive barriers are both pre- zygotic (stigma insensitivity, time to anthesis, time to anther dehiscence and pollen viability) and post-zygotic barriers including hybrid sterility, hybrid inviability (seed abortion or reduced germination rates), reduction in hybrid vigor (reduced growth), and hybrid breakdown (Dell'Olivo *et al*., 2011; Plazas *et al*., 2016; Buteme *et al*., 2021). Unfavorable environmental factors can also influence the extent of reproductive barriers such as bud abscission and pollen viability. For instance, high temperatures can increase abscission in flowers whereas cool temperatures may also enhance male sterility (Bethke and Jansky, 2021). According to reports, mechanisms of incompatibility in Solanum species arise because one parent lacks genetic information that could affect the other parent's structure or physiology, or it could be the pistil's inability to recognize pollen from a

foreign source (Fu & Yang, 2014; Song *et al*., 2019). Therefore, to cross between *S. aethiopicum* and its relatives to generate high vigor hybrids, there is need to for two parents to be compatible.

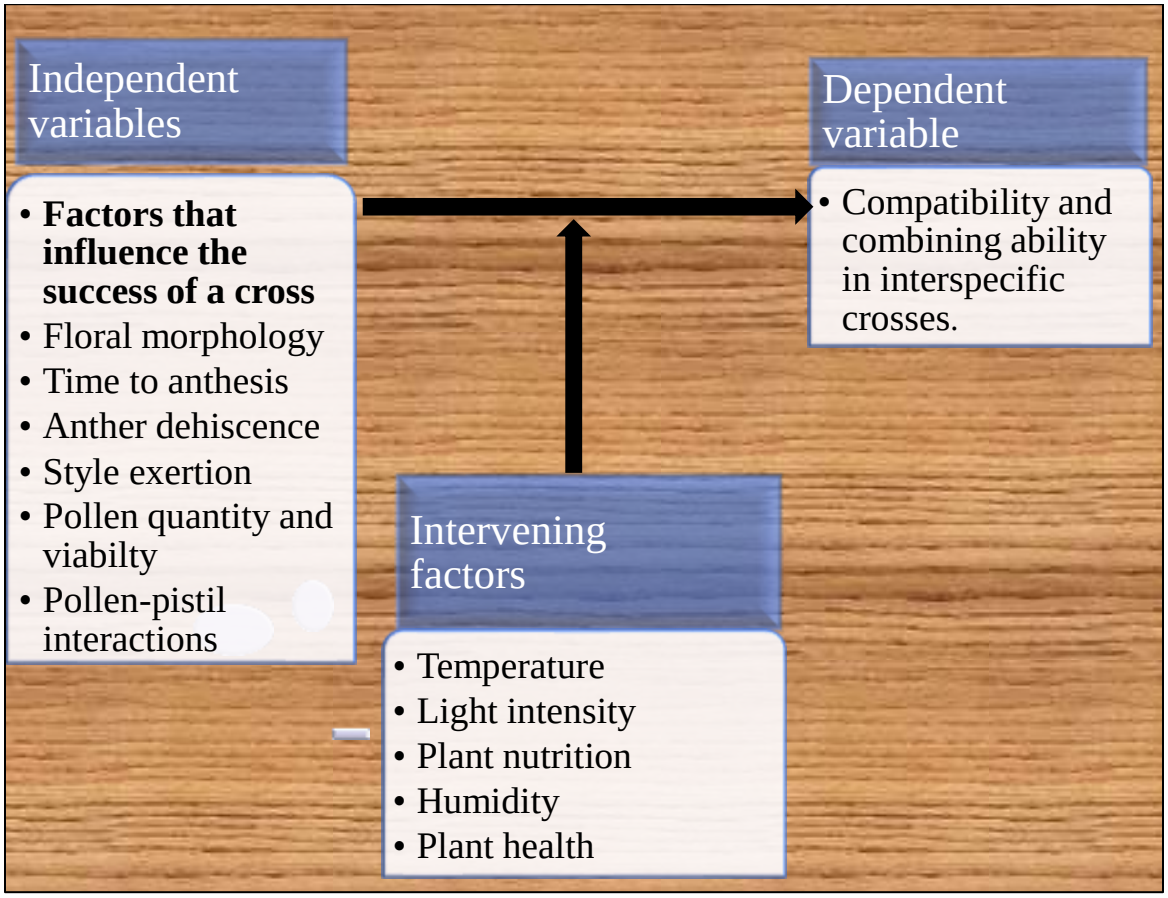


Figure 1: Conceptual Framework showing the factors which influence crossability.

CHARPTER TWO

LITERATURE REVIEW

2.1 The Origin, Botany and Biology of *Solanum aethiopicum* plant and floral morphology

Solanum aethiopicum (African eggplants) originated from tropical Africa and it was domesticated in the 20th century in Africa from its ancestor, *S. anguivi* (2n=24) (R. N. Lester, 1986; R. Lester & Niakan, 1986). *S. aethiopicum* is a diploid (2n = 2x = 24) belonging to *Solanaceae* family in the genus of *Solanum* (Sękara *et al.*, 2007; Acquadro *et al.*, 2017; Sseremba *et al.*, 2017). This crop has various types and forms which are having different morphological features with hundreds of indigenous varieties. The major cultivars of *Solanum aethiopicum* are; Gilo, Kumba, Shum and Aculeatum (R. N. Lester & Thitai, 1989; Sseremba *et al*., 2017). The leaves and fruits of *Solanum aethiopicum* are normally consumed. Fruits are utilized for Gilo, fruits and leaves are utilized Kumba, leaves are used for Shum and Aculetum for ornamental purposes (Adeniji *et al.*, 2013). Kumba is mainly grown in West Africa (Fock-Bastide *et al.*, 2003; Sseremba *et al.*, 2017). Gilo is grown worldwide and Shum group is mainly cultivated in Uganda (Adeniji *et al*., 2012; Nahamya *et al*., 2018).

Solanum aethiopicum is an annual (completes its lifecycle in one season) and perennial (completes its lifecycle in more than one season) herb/shrub which grows up to height of 2m and its branches and leaves have stellate hairs and prickles occasionally (Singh *et al*., 2018). Usually, the crop has bi-sexual flowers, a pedicel of 2-15mm long (Denkyirah, 2013; Sseremba *et al.*, 2017). In *Solanum aethiopicum*, number of flowers varies from 2-5 flowers per inflorescence. The crop is a hermaphrodite with flowers which are singular or clustered into two or more. Variations in the position and length of the style in relation to the stamen was done in *S. melongena* and *S.*

aethiopicum (intra-specific). The stigmas can be exserted (long-styled flowers), at the same level (intermediate styled flowers) or inserted (short- styled flowers) (Denkyirah, 2013; Buteme *et al*., 2021). The exserted and intermediated styled flowers are known to have well-formed nodules with capability to absorb pollen grains. *Solanum aethiopicum* that is solitary flowering has lower flower drop than the clustered flowering kind which can go up to 80% flower drop (Denkyirah., 2013). This may be attributed by low pollination rate, genetic make-up and trend of inheritance of inflorescence (Denkyirah., 2013). Therefore, the exserted-styled flower favor out-crossing compared to inserted-styled flowers which have reduced gynoecium, small stigma having unformed papillae and partial separation of anther spores (Denkyirah., 2013, Buteme *et al*., 2021).

Reports done on *S. lycopersicum* and *S. melongena* indicate that they are self-pollinated crops unlike *Solanum aethiopicum* which is partially self-incompatible and therefore cross pollination is recommended for successful fruits (Bedinger et al., 2011; Buteme et al., 2021). *Solanum aethiopicum* can cross-pollinate up to 20-30% (Adeniji *et al*., 2012; Denkyirah, 2013; Buteme *et al*., 2021). However, differences in floral morphology within *Solanum aethiopicum* were reported by Buteme *et al.*, (2021) and therefore this research seeks to evaluate floral morphology differences between *Solanum aethiopicum* and its relatives. The floral characteristics are important traits which indicate pollination mechanisms of the species and help to infer its various types of pollinators.

2.2 Socio-Economic importance of *Solanum aethiopicum*

S. aethiopicum is an essential vegetable in Africa and it plays a vital role as a traditional food. In Uganda, *Solanum aethiopicum* is grown both commercially and in subsistence majorly in peri-urban and urban places (Pincus, 2015; Nakyewa *et al.*, 2021). The crop provides significant amounts of minerals, primarily phosphorus, potassium, calcium, and magnesium, as well as dietary

fiber and vitamins (A, B1, and B6) (Abukutsa-Onyango, 2014; Nahamya *et al*., 2018). Additionally, it is widely used medicinally to treat conditions including glaucoma, convergence insufficiency, heart disease, arteriosclerosis, bronchitis, itch, bodily pains, and asthma (Igwe *et al.*, 2003; Okon *et al*., 2010; Han *et al*., 2021). *Solanum aethiopicum* has analgesic, anti-inflammatory, anti-hypertensive, and antibacterial properties that help treat and prevent various illnesses (Plazas *et al*., 2016).

Solanaceae family is widely distributed and produced in all regions in Uganda with 43.4% and production of *Solanum aethiopicum is higher* among the *Solanum* species with 12.4% (Sseremba *et al*., 2017). Due to increasing demand for *Solanum aethiopicum* in Uganda, *its* production has potential to significantly contribute to household nutrition and incomes. Reports show that *S. aethiopicum* Shum group earns about 4,000,000 people in Uganda who are involved directly through production and vending in peri- urban and urban areas (Nakyewa *et al*., 2021)**.**

2.3 Crossability and incompatibility mechanisms

2.3.1 Crossability

The crossability of *Solanum* species is determined by the occurrence of fruit set, seed set and germinability of F1 hybrids (Premabati Devi *et al*., 2015). The capability of two parents (between and within species) to cross-pollinate and produce fruits with viable seeds defines crossability (Acquaah, 2012.; Buteme *et al*., 2021). The success of crosses depends on whether the species are compatible in one direction (unilateral) or in both directions (bilateral) with wild species involved during crossing (Plazas *et al*., 2016; Maune *et al*., 2018; Buteme *et al*., 2021). For instance, crosses between *S. melongena* and its close relatives produced successful fruits, viable seeds and germination which also depended on the direction of crossing and cross combination (Afful *et al*.,

2018; Buteme *et al*., 2021). Incompatibility in *Solanum* species also depends on weather changes during flowering period, damaged pistil during emasculation and pollinating, variations in genetic makeup of the species, pollen failure to attach on the stigma, pollen tubes trapped in styles, and endosperm breakdown (Devi *et al.*, 2015; Afful *et al.*, 2018; Buteme *et al*., 2021).

Previously, intraspecific hybridization within the *Solanum aethiopicum* genotypes also showed that there was also success in fruits, seed set and germinability (Buteme *et al*., 2021). However, the crosses were compatible either unilaterally or bilaterally (Devi *et al.*, 2015; Maune *et al*., 2018; Buteme *et al*., 2021). Martins *et al*., 2015) and Jahkhaka, (2019) also reported that the rate of cross success between the complexes of *C. annuum* and *C. baccatum* was 2.2% to 14.6% without incompatibility barriers on selfed genotypes. 84% fruit, seed set and seed viability failures were observed when close species were used as female with *S. melongena*. Failure of the crosses is attributed to compatibility barriers (pre-zygotic and post zygotic reproductive barriers) like non receptive stigma, pollen viability, flowering time, style length, hybrid seed viability among others (Raimondi and Camadro, 2003;Hayes *et al*., 2005; Martins *et al*., 2015; Afful *et al*., 2018;Buteme *et al*., 2021). The potential crossing success for *Solanum aethiopicum* with other relatives has been underexplored as compared to other vegetables such as *S. lycopersicon, S. melongena, S. tuberosum* (Díez & Nuez, 2008; Hajjar & Hodgkin, 2007; Afful *et al*., 2018). Therefore, this study evaluated the crossability of *Solanum aethiopicum* (Shum and Gilo) with its close relatives (*S. anguivi*, *S. macrocarpon* and *S. incanum*).

2.3.2 Incompatibility Mechanisms

Incompatibility mechanisms in Solanaceae prevent inbreeding (self-pollination) and also out crossing (cross-pollination) (Eijlander *et al.,* 2000). Compatibility barriers have continued to hinder use of close relatives of *Solanum aethiopicum* in improving its cultivars with high yields,

tolerance and resistance to abiotic and biotic stress factors (Plazas *et al*., 2016; Afful *et al*., 2018; Buteme *et al*., 2021). Incompatibility mechanisms mostly hinder germplasm conservation and utilization (Maune *et al*., 2018). There are two main mechanisms of incompatibility; self-incompatibility mechanism (which prevents inbreeding through recognition and rejection of pollen within species) and, cross-incompatibility (which limits gene flow between different species) (Eijlander *et al*., 2000; Thomas & Franklin-Tong, 2004). The former promotes cross pollination and the latter limits gene flow which leads to speciation (Eijlander *et al.*, 2000; Maune *et al*., 2018).

Self-incompatibility (SI) is a widely spread genetic mechanism in angiosperms which prevents inbreeding through recognition and rejection of pollen in species which are close relatives (Maune *et al.*, 2018; Buteme *et al*., 2021). SI is controlled by a linked cluster of genes called "S-locus" and therefore the crops that have recognition alleles at the locus are not able to produce hybrids(Plazas *etal*., 2016; Maune *et al*., 2018). The SI is controlled by various S-allele /S-locus which exist in several species belonging to the Solanaceae family. The reactions at S-locus are in two types namely, Sporophytic Self-incompatibility (SSI) and Gametophytic Self-incompatibility (GSI). These incompatibilities can be determined on whether S-haplotype expressed by the pollen grain depending on the pollen donor species or by the species itself (Maune *et al*., 2018). In SSI, the pollen donor and pollen receiver may determine the compatibility of a cross-combination (Maune *et al.*, 2018). The pollen (gametophyte) reflects therefore, species of the pollen parent but not the actual species of the pollen itself (Eijlander *et al.*, 2000; Maune *et al*., 2018). There are majorly two categories of sporophytic SI which include heteromorphic and homomorphic incompatibility. In sporophytic heteromorphic self-incompatibility, there is an interaction between the incompatibility groups and floral morphology. Floral morphology has several parameters which include shape, size, color of petals and sepals, number of stamens, stamen length, and pistil length

and pollen quantity. The behavior of incompatibility depends on the floral appearance for example, studies have found out that inserted styled flowers can only pollinate plants with short anthers and flowers with exserted styles anthers can pollinate plants with long anthers (Eijlander *et al*., 2000; Plazas *et al.,* 2016).

Sporophytic Homomorphic self-incompatibility on the other hand is characterized by the fact that an association between pollen grains and stigmas is determined by dominance relationship between S-alleles of both parent (sporophytes), the pollen (gametophyte) carrying the information of pollen donor in the pollen coating (Eijlander *et al*., 2000). In gametophytic self-incompatibility (GSI), genotype of style and that of pollen its-self regulate whether the cross is incompatible or not. Therefore, the pollen (gametophyte) expresses the pollen's genotype. For instance, GSI occurs in potatoes where the S-locus is complex and contains a line of tightly linked genes-a single S-RNase and several (Entani *et al*., 2003). In GSI systems, occurrence of incompatibility is when pistils and pollen of a specific genotypic cross express the same S-haplotype and here, pollen tube growth is detained mostly in the upper part of the style (Bedinger *et al*., 2011; Maune *et al*., 2018). GSI system may be overcome through mutations in the SI genes, the action of genes like S-locus inhibitor, or by competitive association in diploid pollen grains having two or more different S-haplotypes, this clarifies the ability of polyploids with GSI to produce hybrids after selfing (de Nettancourt & de Nettancourt, 2001; Maune *et al.,* 2018).

Cross incompatibility (CI) mainly arises due to absence of genetic material in one of the species during hybridization(Jewell *et al*., 2020). This limits the transfer of genes which eventually leads to speciation (Cachi *et al*., 2014). Cross incompatibility can be expressed in one direction (unilateral) or in both directions (bilateral) (Hayes *et al.,* 2005; Bedinger *et al*., 2011). Unilateral Incompatibility (UI) is most commonly occurring during hybridization between self- compatible

and self-incompatible species whereby fertilization only occurs in one direction. This arises when a self-incompatible species is used as a pollinator (male), but not as pistillate parent. Therefore, it's compatible when pollen from SC species is used (SI x SC rule, SI x SC=F_1) but reciprocal crosses are incompatible (SC x SI= No F_1) for example the cytoplasmic male sterility in hybrids is derived from SC x SI crosses (Lewis & Crowe, 1958). The success and failure of interspecific hybridization in various species is explained by the SI x SC rule (Maune *et al*., 2018).

These reproductive incompatibility barriers may be pre-zygotic which reduces crossability by limiting gametes to combine to form a zygote and post-zygotic which mostly reduces the viability or reproductive potential of a hybrid (Bushell *et al*., 2013; Banaticla-Hilario *et al*., 2018). In *Solanum spp*, the pre-zygotic reproductive barriers are the ploidy levels, different gene pools, floral morphology, time to anthesis, time to adherence, stigma receptivity, pollen viability, stigma exertion and, flowering dates. The post-zygotic reproductive barriers include the poor viability of hybrid seeds, poor seed and fruit set, embryo death, hybrid sterility among others.

2.4 Pre-zygotic interspecific reproductive barriers

2.4.1 Pollen- pistil Interactions

Basing on the kind of interactions between pollen grain and pistil, the two main self-incompatibilities systems are; sporophytic and gametophytic. For sporophytic system, the self-incompatible reactions which depends on the expression of the parental sporophytic species in the pollen and in the pistil therefore the incompatibility interactions are controlled by the species of the donor and recipient. In the gametophytic system, the SI reaction depends on the appearance of the individual alleles existing in the pollen itself and in the pistil (Bedinger *et al*., 2011, 2017). During hybridization, fertilization depends on both physiological and physical relationship

between the pistils and origin/number of pollen grains germinating in the ovules (B. McClure *et al*., 2011; Pham *et al.,* 2016; Edmé & Mitchell, 2021). One of the key determinants of the success or failure of interspecific crosses in *Solanum* species is pollen-style interactions (Covey *et al.*, 2010; Gregory *et al.*, 2019; Martins *et al*., 2015). Failure of pollen tube growth has been documented in several species of the Solanaceae family, including wild potatoes, at various locations of the pistil (stigma; upper, middle, or bottom section of the style) (Hayes *et al*., 2005), wild tomatoes (Baek *et al*., 2015), and peppers (Onus & Pickersgill, 2004). Reports also show that there is a difference in self-pollen rejection (intraspecific spp) and interspecific pollen rejections in *Solanum peruvianum* (Chetelat *et al*., 2009). For instance, the pollen grains from *S. lycopersicon* are renounced in the upper section of the styles instead of stopping in the middle of the style as it is observed in SI species (Bedinger *et al*., 2011, 2017). Bedinger, (2011), also show that the top style of the pistils of *S. pennellii* SC accessions rejected tomato pollen tubes. These interactions have been widely studied in eggplants, tomatoes and potatoes while it remains limited in *S. aethiopicum*.

2.4.2 Gene pool and Endosperm Balance Numbers (EBN)

The closer the wild relative is during interspecific crossing, the easier it is to generate hybrids genetically which are fertile (Kaushik *et al*., 2016b). The presence of interspecific barriers shows how distant the species is genetically. *Solanum* species have wild relatives which belong to different gene pools (primary, secondary and tertiary) depending on whether they are more or less easily crossed (Bedinger *et al*., 2011). The primary gene pool (GP1) of *Solanum aethiopicum* is *Solanum* anguivi and hybrids are highly fertile (Kouassi *et al.*, 2019). The species that belong to the secondary Gene Pool (GP2) are *S. macrocarpon* and *S. incunam*, they produce partially fertile hybrids (Kouassi *et al*., 2019). The tertiary gene pool having S*olanum torvum* is highly

incompatible and most of the times sterile hybrids are produced (Kouassi *et al*., 2019). The *Solanum aethiopicum and* wild species belonging to different gene pools, respond differently to fruit set, seed set, hybrid seed germination and others (Syfert *et al*., 2016). There are more concentrated and intensive reproductive barriers as the genetic distance between species increases (Banaticla-Hilario *et al.*, 2013). Therefore, the genotypic differences among species are important factors to consider while obtaining interspecific hybrids in *Solanum species* (Kumchai *et al*., 2013; Premabati Devi *et al.*, 2015).

2.4.3 Ploidy levels and Endosperm

The level of successes in interspecific crosses between *S. melongena* and its relatives can depend on the species used in the crossing (Plazas *et al*., 2016). The variations in levels of ploidy among Solanaceae used in the crosses contribute either failure or success of the hybridization process (Lester, 1986; Fu & Yang, 2014). The development of hybrids is also determined by the ploidy levels of the parents/species used in crossing. After crossing of two species which are both diploids, the hybrids can be up to 100%. If there are differences in ploidy levels, the hybrids will be formed only after several crosses (Yang *et al*., 2014). Afful *et al* (2018), reported post-zygotic barriers for example development of abnormal/empty seeds, lack of vigor and sterility of hybrids in crosses made between *Solanum melongena* (2n=24) and *Solanum torvum* (2n=24,48).

2.4.4 Floral structure (Pollen size, style length, and stigma architecture)

The differences in floral structure have high effects on the rates of self-and cross pollination (Bedinger *et al*., 2011). In Solanaceae, the size of flowers, stigma exsertion are factors that tremendously influence the out-crossing level (Patricia *et al*., 2010). In eggplants, the solitary flowers are medium and long styled while cluster flowers widely have inserted styles (Laporta, 2005). The inserted and exserted flowers have very well-formed ovaries which are fertile while

the flowers with short styles are majorly sterile with either underdeveloped or rudimentary ovary (Hazra *et al*., 2007; Buteme *et al.*, 2021). Studies show that pollen size can inhibit germination and growth in the styles (Martins *et al*., 2015; Yermishin *et al.*, 2017). In some species of crops, there is a positive relationship between the pollen size and length of the style, which supports the theory that large pollen have more provisions and can navigate through longer styles to reach the ovaries (Aguilar *et al*., 2002; Laporta, 2005). The distance that pollen tubes travel through the stigma to get to the style's transmission tract is also associated with the size of the pollen grains (Quesada-Aguilar *et al*., 2008; Jia & Tan, 2012).

A lipid-rich exudate from Solanaceae wet stigmas contains cis-unsaturated triacyl glycerides that promote pollen hydration and germination (Fang *et al.*, 2013; Costa *et al*., 2014). Cross pollination is encouraged by the position of the stigma in reference to the tips of the anthers in long-styled flowers. The short-styled flowers have a small, reduced gynoecium that is frequently infertile, a small stigma with undeveloped papillae, a spatial separation of the anther pores, and a low sugar content that hinders pollen germination and causes pollen drop (Sękara & Bieniasz, 2012; Kumchai *et al*., 2013;). However, short-styled flowers are less exposed to the sun, wind, and rain and may stay receptive for a lengthy period of time (Enciso-Rodriguez *et al.*, 2019). The corolla lobe filaments on the stamens are short and thick, and they are inserted close to the base of the corolla tube. Racemose cymes (5–12) with a short or nonexistent peduncle and a short or excessively long rachis make up the lateral inflorescence (Chen *et al.*, 2018).

2.4.5 Stigma receptivity

The capability of a stigma to support viable and compatible pollen to germinate (Peña-Yam *et al*., 2019). It is one of the important stages in a maturing flowers which greatly influences pollination rate, successful pollination at different stages of a flower lifecycle, the likelihood of gametophytic

selection, the rate of competition caused by inappropriate pollen transmission, and the interference between male and female functions (Makwana & Akarsh, 2017). Studies show that stigmatic receptivity reduces intersexual interference and prevents intra floral selfing (Bawa *et al*., 2011). The facts about the stigma receptivity duration may be used for making a successful hybridization program (Makwana & Akarsh, 2017).

Different studies have evaluated stigma receptivity in different times of the day using hydrogen peroxide (peroxide water) (Sheffield *et al*., 2005; Makwana & Akarsh, 2017; Peña-Yam *et al*., 2019). Receptive stigmas are described using a high enzymatic activity and presence of various enzymes is found in concurrence with this developmental stage of a flower (Monika and Akash, 2017). Several methods used in evaluating stigma receptivity are in-vitro which bases on identifying the enzymatic activity (Sheffield *et al*., 2005; Makwana & Akarsh, 2017; Peña-Yam *et al*., 2019). Stigma receptivity studies were done in various crops like *Solanum lycopersicum, Capsicum chinense and Solanum melongena.* Studies show that the stigma becomes receptive on the day of anthesis and remains sensitive for two days (Sheffield *et al*., 2005; Makwana & Akarsh, 2017; Peña-Yam *et al*., 2019). In eggplants, the stigma appears shiny and sticky when fully receptive and the stigma is very receptive normally on the day of flower opening. Receptivity also decreases with age of a flower, on the 5^{th} day, flowering is negligible therefore the stigma also gradually turns brown (Vara Prasad *et al.*, 2002; Das *et al.*, 2017). Das *et al*., (2017) reported that the stigma was highly receptive at 8:00am and just after anthesis and declined gradually yet receptivity was at maximum on the day of anthesis. Reports also indicate that there was no fruit set when flowers were pollinated 3 days before and 1 day after anthesis (Das *et al*., 2017); although He *et al*., (2017) showed that stigmas were receptive up to 9 days after anthesis. However, stigma receptivity remained positive from one day before anthesis until one day after anthesis indicating

that it increased during anthesis and diminished after (Aleemullah *et al*., 2000; Laura *et al*., 2019). Monika and Akash, (2017) also reported that *S. lycopersicum* stigmas became receptive one day before opening of the flower and remained receptive up to the withering stage in *S. lycopersicum*. However, the stigma receptivity was reported fully after the flower bud had opened (Monika and Akash, 2017). It is therefore commended to carry out hybridization (crossing) one day before anthesis (at floral bud) because the anthers are fully closed in most crops (Laura *et al*., 2019). Information on stigma receptivity has been reported in only a few species of *Solanum* (*Solanum melongena* and *Solanum lycopersicon*) (Monika and Akarsh, 2017; Peña-Yam *et al.*, 2019; Yi *et al.*, 2006). Although stigma receptivity has been reported in different Solanum species, information on the receptivity of stigmas in *Solanum aethiopicum* remains limited (Aleemullah *et al*., 2000; Monika and Akash, 2017; Laura *et al*., 2019). Therefore, this study evaluated stigma receptivity of *Solanum aethiopicum* and wild relatives.

2.4.6 Anthesis and anthesis time

Anthesis refers to the opening a flower bud and time of the flower bud opening (Zhao *et al*., 2011). Investigation on quantity of flowers at anthesis during different times of the day provides guidance on the best period when hybridization/hand pollination can take place (Neeraja Puthiamadom, 2017). The appearance of a visually recognizable flower bud is considered to be an indication for onset of flowering initiation in *Solanum species* (Neeraja Puthiamadom, 2017). Neeraja, (2017), reported that initial flowering time in cultivated *Solanum melongena* takes 8 weeks after planting and then flowers throughout the growing season. Flowering opening started at 7:00am to 8:00am and then closed at 2:00pm in most of the flowers observed (Prasad and Prakash, 1968). Reports show that anthesis begins from 7:30am to 11:30 am, with a peak period of anthesis being 8:30 and 10:30am (Rashid and Singh, 2000). In eggplants, anthesis is between 6:45 to 9:45 am (Hazra *et*

al., 2003). The flowers then start to close from 2pm and are completely closed at a night and the cycle continues for about 5 days and the corolla gradually turns brown and withers (Chattopadhyay *et al.*, 2009). However, flower opening is influenced by the flowering date, temperatures and relative humidity of the environment (Neejara, 2017). In other *Solanum species,* the stigma becomes receptive on the day of anthesis and remains sensitive for two days. Pollen grains become fertile one day before anthesis with maximum fertility on the day of anthesis(Douglas & Freyre, 2010) and the pollen is released from the anthers 1-4 hours after anthesis depending on the cultivar used during crossing (Aleemullah *et al*., 2000). Reports also show that flowers open at different times in a day in different genotypes of *Solanum aethiopicum* Shum and there is a gradual decrease in the number of flowers that open from 8am to 4pm (Buteme *et al*., 2021). Most flowers at anthesis are observed at 8am and very less at 4pm with in *Solanum aethiopicum* (Shum) (Buteme *et al*., 2021). This study evaluated the number of flowers at anthesis at varying periods in *Solanum aethiopicum* and its relatives in order to assess the period of the day that would be ideal for crossing.

2.4.7 Anther dehiscence

Anther indehiscence is a process in which the anthers do not open to release pollen grains hence interfering with pollination in crops (Matsui *et al*., 2000; Wang *et al*., 2021). It is a vital process whereby by mature pollen grains are released from the locules of the anther to enable pollination of the stigma (Sanders *et al*, 2005). Anther dehiscence is an event that determines the success of pollination and it greatly depends on the interaction between the crop and the environment (Aleemullah *et al*., 2000). The understanding of pollen release provides an effective mechanism for the control of male sterility and for hybrid generation (Wilson *et al*., 2011; Peña-Yam *et al.*, 2019). Male sterility is associated not only with lack of viable pollen but also with the failure of

pollen release(Wilson *et al*., 2011). Anther dehiscence is a highly regulated process which appears to be very sensitive to abiotic stress as reported (Aleemullah *et al*., 2000; Wilson *et al*., 2011).

The conditions in the environment especially high temperature, have a dramatic effect on anther dehiscence and pollen viability (Sakata *et al*., 2008; Zinn *et al*., 2010). Heat stress was shown to result in protein expression changes, particularly associated with dehiscence in *Capsicum annuum* (Peña-Yam *et al*., 2019). High temperature is also linked to the reduction in the swelling of pollen which provides the force for anther opening (Matsui *et al.*, 2000). Dehiscence of anthers occurs longitudinally and pollen is seen one hour after flower opening in *Capsicum annuum* (Aleemullah *et al*., 2000; Peña-Yam *et al*., 2019). (Khanduri *et al*., 2013) revealed that in *Cornus capitata*, the opening of the anther also occurs from the top to the bottom. According to Peña-Yam *et al*., (2019), emasculation and pollination are done in the stage of flower bud before the anthers open to release pollen to ensure that anthers are closed to reduce the risks of self-pollination. In *S. Lycopersicum*, the anther wall ruptures while the flower still in bud form and anther rapture/ pollen exposure is a direct result of anthesis (Bonner & Dickinson, 1989). Although morphological changes in anthers during dehiscence has been thoroughly described in crops such as *Solanum melongena, S. Lycopersicum, Capsicum annuum,* anther dehiscence in *Solanum aethiopicum* remains relatively unknown. This study therefore, evaluated the time to anther dehiscence in *Solanum aethiopicum* and its relatives in order to assess the stage of the flower that would be appropriate for emasculating and crossing.

2.4.8 Pollen viability

Determination of pollen viability is important in the hybridization process (Ginoya *et al.*, 2022). In artificial pollination, particularly when several species or genera are involved, determining pollen viability is extremely important; and it a reliable method (P, 2018). The pollen quality is determined basing on the viability and vigor of pollen grains (Sulusoglu & Cavusoglu, 2014). The rate at which the pollen grains germinate and growth of pollen tube is known as pollen vigor (Sulusoglu & Cavusoglu, 2014). Pollen is considered viable if it is capable of growing on the stigma of a gynoecium of an associated plant species. Assessing the ability of pollen to germinate is a very important process of artificial hybridization (França *et al*., 2009). Upon germination, the pollen develops a tube which penetrates into the style and enters the ovule through the micropyle to release two nuclei (Abdullateef, Zakaria, Hasali, & Osman, 2012). According to Peña-Yam (*et al*., 2019b) the capability of a pollen tube to emerge from the pollen coat and pollen diameter are possibly indicators of pollen viability. (Singh *et al*., 2018) also pointed out that the performance of pollen is variable depending on the genetic combination of the pollen. Pollen quality can be affected by several internal and external factors such as the stage of flower development, high temperatures (above 40^0C), low temperatures (below 15^0C), nutrition of the plant, light intensity, agricultural pesticides among others (França *et al*., 2009).

The viability of pollen grain (degree of male fertility) may be assessed by different techniques (Dafni & Maués, 1998; Buteme *et al*., 2021). The methods used are in-vitro and in-vivo pollen germination tests, staining methods and finally the analysis of seed set (França *et al*., 2009; Buteme *et al.*, 2021). The choice of technique depends on the crop or species (Dafni & Maués, 1998; Dafni & Firmage, 2000). Reports show that, pollen grains are fertile one day before anthesis and the maximum fertility is on the day of anthesis in *Solanum species* (Plazas *et al.*, 2016) . On the other

hand, viability of pollen in some hybrids was low or even inviable (Premabati Devi *et al*., 2015; Thorat *et al*., 2020). No or reduced pollen viability is due to the cytoplasmic-genic interactions and several meiotic abnormalities like univalent, lagging chromosomes and arrested meiosis (Edlund *et al.*, 2004).

4.2.8.1 Pollen tube growth in-vitro

In vitro pollen germination is an accurate test for pollen viability. Additionally, it answers many fundamental problems about sexual reproduction and is especially helpful in widespread hybridization (P, 2018)**.** With this method, a culturing medium is made (100g/l of potassium Nitrate, 200g/l of magnesium sulphate, 100g/l of boric acid, 300g/l of Calcium nitrate and 20g/l of sucrose granules (França *et al.*, 2009). Pollen is dusted on drop of the nutrient medium, which is then incubated for 24 hours at 27^0C (França *et al.*, 2009; Ginoya *et al*., 2022). Pollen of flowering plants requires a carbon source, boron, and other nutrients in induction of germination (Das *et al*., 2017). This strategy provides a stimulation of conditions on the stigma (moisture) and in pollen.

Pollen germination using in vitro and in vivo in *S. melongena* and *S. Lycopersicon* showed that pollen grains germinate with in the 24 hours within nutrient medium (França et al., 2009; Ginoya et al., 2022). The pollen grains are seen under light microscope, where those that develop tubes that at least half of the diameter of the pollen grains are considered viable (Einhardt *et al*., 2006; França et al., 2009). Pollen viability is then determined as a percentage by dividing number of germinated pollens by the total number of pollen grains multiplied by 100. Genotypes with above 30% viability of pollen are regarded as viable and can germinate on the stigma of *S. melongena* which is a close relative to *S. aethiopicum* (Rodriguez-riano & Dafni, 2000; Geetha *et al.*, 2013). In eggplant genotypes, pollen viability ranged between 49% to 79% after 8 hours of incubation (Tatis *et al*., 2012). Das *et al.*, (2017) found average viability of 51.74% to 76.59% in *S. melongena*

after 24 hours of incubation. On other hand, Valadares *et al*., (2019) and Ginoya et al., (2022) obtained a pollen viability ranging from 20.7% to 69.9% after incubating pollen for 24 hours. Buteme *et al.,* (2021), also obtained pollen viability between 56.4% to 66.54% in *S. aethiopicum* genotypes. This study applied the hanging drop method for examining the viability of pollen, growing pollen in nutrient medium for 24 hours to acquire maximum germination of *S. aethiopicum* and its relatives.

4.2.8.1 Pollen tube growth in-vivo

In in-vivo pollen tube growth, the pollen tube enters the pistil, grows between the walls of the stigma cells, and then goes through an extracellular matrix within the style's transmission tissue to the ovary (Baek *et al.*, 2016; Buteme *et al.*, 2021). During in-vivo pollen growth analysis, staining using callose material with anline is used and later observed using the florescent microscope (Martinez-Gomez *et al*., 2000; Buteme *et al.*, 2021). Modification of the protocols is recommended since observations may be hindered by large amounts in the style shown as yellow or brown inflorescence, large amounts of callose having vascular strands or inadequate callose within the tubes and on the stigma.

Several studies have used this method in studying incompatibility within and between species of Solanaceae. Incompatibility in inter and intra hybridization of *S. melongena, S. lycopersicon*, chilense with its relatives using in-vivo pollen growth has been widely reported (Zhao *et al*., 2011; Premabati Devi *et al.*, 2015; Baek *et al.*, 2016). Incompatibility in one direction (unilateral incompatibility) is related to Self-incompatibility; where self-pollen and foreign pollen is rejected in the style of the recipient. Pollen tube growth stops at the upper, middle, bottom of the pistil or even in the ovary of the female parent (Bedinger *et al*., 2011; Buteme *et al.*, 2021). Conclusions have been made that unilateral incompatibility follows SCxSI rule; where viable pollen from SI

species can germinate on SC species while SC viable pollen grains do not germinate SI species and are inhibited in the stigma, style or even ovary (McClure *et al*., 2000; Baek *et al*., 2015; Buteme *et al*., 2021). In several *Solanum* species, the failure of pollen to germinate in the pistils to the ovary is widely evaluated using in-vivo method where anline stain is applied and observed in florescent light. This study sought to use the in-vivo method for examining pollen growth in the pistils, growing pollen of *S. aethiopicum* and its relatives.

2.5 Post-zygotic interspecific reproductive barriers

The post-zygotic reproductive barriers inhibit proper formation of the embryo, resulting in to embryo abortion and poor/no seed development (Afful *et al.*, 2018; Buteme *et al*., 2021). The development of seeds begins after fertilization. Fertilization happens when the female and male gametes are mature enough. When pollen grains land on the stigma, the pollen tube grows down to the style, through the micropyle and embryo sac (He *et al*., 2017). Several barriers occur after fertilization during interspecific hybridization of *S. melongena*, *S. lycopersicon* (Kondo *et al.*, 2002; Afful *et al.*, 2018). These include seed failure, no F_1 hybrid germination and sterile hybrids (Afful *et al*., 2018; Buteme *et al*., 2021).

2.5.1 Fruit and seed set

Low fruit set is obtained in various interspecific cross between cultivated and wild species (Afful *et al*., 2018). This is attributed to environmental conditions which occur during the period of flowering, emasculation carried out on not fully matured flower bud, low nutrition and protective function of the perianths, pistil damage when emasculating or even degeneration of ovules, genetic origin of parental lines (Sękara *et al.*, 2007; Kumchai *et al*., 2013; Afful *et al*., 2018).

Studies have reported varying fruit sets in different *Solanum* species and genotypes. Plazas *et al*., (2016) recorded the fruit set successes ranging from 3 to 7% in *C. chinese* and Manzur *et al*., (2015) also revealed fruit set between 1.3 to 6% in *C. annuum*. A range of 1.3 to 6% of fruit set successes was observed in interspecific crosses between *S. melongena* and its relatives (Afful *et al.,* 2018). In intra-specific crosses with *S. aethiopicum,* fruit set success ranged from 3 to 93% (Buteme *et al*., 2021). Variations in fruit set successes noted by various researchers may be related to the use of various accessions of wild species and variations in the environment in which hybridization studies were conducted (Afful *et al*., 2018).

Furthermore, these wild species and domesticated species come from various gene pools, which means that they could respond to fruit set in various ways (Syfert *et al.*, 2016). Failure of a fruit to set a seed (seedless fruit) is referred to as parthenocarpy (Dhatt & Kaur, 2016). Parthenocarpy could be a result of allelic incompatibility during fertilization and the growth of normal fruit. Several studies have reported parthenocarpic fruits in crosses of *S. melongena, S. tuberosum* and *Capsicum* species with their relatives (Kouassi *et al*., 2014; Plazas *et al*., 2014; Premabati Devi *et al*., 2015; Ye & Fan, 2021).

Endosperm breakdown, which is commonly recognized as the cause of seed failure in both interploidy crossings within a species and interspecific crosses, can also be used to explain the success or failure of crosses between related plant species (Asatryan & Tel-Zur, 2014; Yermishin *et al*., 2017). Although the fruit and seed sets are obtained and discussed in other *Solanum* species (Kouassi *et al*., 2014; Plazas *et al*., 2014; Premabati Devi *et al*., 2015; Afful *et al*., 2018; Ye & Fan, 2021), little is known about seed and fruit sets in interspecific crosses of *S. aethiopicum*. Therefore, this study evaluated the fruit and seed set successes/failures obtained between *S. aethiopicum* and its relatives.

2.5.2 Poor germination of hybrid seeds

Seed viability is a phenomenon which has been widely investigated (Asatryan & Tel-Zur, 2014; Afful *et al*., 2018; Mondo *et al*., 2020; Buteme *et al*., 2021). The variations in genetic background of the parental lines lead to failure or poor seed germination in some hybrids (Kumchai *et al.*, 2013). Studies showed that environmental conditions also influence the viability of F_1 hybrid seeds (Bhatla &Lal, 2018). Prolonged drought and low soil moisture reduces the size of the seed specifically during flowering and seed fill (Munim Farooq, 2012). Occurrence of drought before and during flowering affects seed formation and size of the seed.

The performance of seeds to regenerate when planted is affected by seed viability and vigor. Failure of seed to germinate in interspecific crosses may be attributed to hybrid lethality, which is genetically controlled and occurs at seedling stage in interspecific hybrids (Premabati Devi *et al*., 2015; Afful *et al.*, 2018). This leads to zygote abortion after fertilization and death of the cell in the tissues of seedlings after germination (Mino *et al*., 2002). The incompatibility of the chromosomes and genes of the two parental species as they are combined in the hybrid nuclei, the discordant interaction of the chromosomes or genes of one species with the cytoplasm of the other, and the endosperm's inhibition of the formation of the hybrid embryo are all factors that contribute to seed viability (Abdullateef, *et al*., 2012).

Several studies have reported failure of hybrids to germinate in *S. melongena* (Afful *et al*., 2018). In *S. aethiopicum*, failure of fruit was observed in only one's intra-specific cross (Buteme *et al.*, 2021). Therefore, this study established the seed viability of interspecific crosses between *S. aethiopicum* and its relatives (*S. anguivi, S. incanum* and *S. macrocarpon*).

2.6 Combining ability of yield and yield related traits

Akinyode *et al*., (2018) reported a high phenotypic and genotypic coefficient of variation for yield and yield related traits (plant height, number of leaves, leaf area, fruit length, number of seeds per fruit, fruit weight and flower length). Very high broad sense heritability estimate was reported for fruit length, fruit weight and days to flowering in study by Kumar *et al* (2012). He also identified good combiners among the parental lines used and some hybrids that out-performed the parental lines.

Combining ability shows its potential to cross with each other (Olfati *et al*., 2012; Liu *et al*., 2021). Combining ability analysis aids in identifying the appropriate breeding method as well as the parents to use for hybridization (Gissa *et al.*, 2007; Ansari, 2014). Its concept was developed by Sprague (1936) (Olfati *et al*., 2012). The average performance of a parental line in a series of hybrids is determined by general combing ability (GCA). Based on the average performance of the lines involved, specific combining ability (SCA) calculates the deviation of certain expected combinations (Acquaah, 2012; Olfati *et al*., 2012). When the GCA is low (either positive or negative), it means that the average of a parent crossed with the other parent does not deviate significantly from the general mean of crosses (Olfati *et al*., 2012; Acquaah, 2012). If the GCA is high, it shows that the mean of the parental lines is superior or inferior to the grand mean, which shows additive gene action, narrow sense heritability and homozygosity, less environmental effect and large adaptability (Acquaah, 2012). This was in relation with genetic gain, breeding value (high achievement in selection) and is heritable and fixable (Acquaah, 2012). Certain hybrid combinations present better or poor performance of the yield and yield related traits than expected depending on the mean performance of the lines (Maune *et al*., 2018); showing non-additive gene

effect (dominant effects, dominant x dominant or epistatic effects), broad sense heritability, heterozygosity and hybrid vigor/heterosis (Kumar and Arumugam, 2013).

Studies on combining ability for several crop features was done in several species including Solanaceae but particularly *S. lycopersicum, S. melongena* (Fasahat, 2016). Fasahat, (2016) found out that superiority of GCA is more relevant than that of SCA. Amin, (2012) also revealed that none of parental lines expressed desirable GCA effects for all traits (nature of inheritance for days to first fruit set, days to first picking, plant height, number of primary branches per plant, fruit size, flesh thickness, number of fruits per plant, weight of fruits, fruit yield per plant and number of locules); in individual as well as in pooled over environments for a 10 parental diallel cross of *S. lycopersicum* L. Both additive and non-additive gene effects are involved in inheritance of major traits such as locule number, pericarp thickness and total solids studied in crosses of *S. lycopersicum* (Singh and Asati, 2011). These findings were similar to those by (Saleem *et al*., 2009) who studied genetic analysis to identify suitable parents for hybrid seed production in *S. lycopersicum.* In intraspecifc and interspecific hybridization study between *S. melongena* and *S. macrocarpon,* the estimates high SCA effects showed in superior specific combinations for plant height, total fruit yield, number of branches per plant and fruit number per plant (Hamada et al., 2016).

In drought study on *S. aethiopicum,* SCA effects for crosses within *S. aethiopicum* species were found more significant compared to GCA effects for leaf area, number of leaves per plant, fresh leaf yield and leaf mass area (Sseremba, 2019). In tomatoes, the studies showed that additive gene action (with highly significant SCA effects) influenced the inheritance of fruit weight, fruit height, fruit diameter, and the number of fruits per cluster (Izzo *et al*.,2021). Similarly, (Liu *et al*., (2021) reported that the additive genetic effect is dominant in trait inheritance in tomatoes.

The effect of a gene is additive when each additional gene enhances the appearance of the trait by equal increments (Acquaah, 2012). For example, the effect of aabb=0, Aabb=1, AABb=3 and AABB=4. In the case of a single locus (A, a), the heterozygote would be exactly intermediate between the parents. That is, the performance of an allele is the same, irrespective of other alleles at the same locus. This means that the phenotype reflects the genotype in additive action, assuming the absence of environment effect.

Even if, these studies were widely done in *S. melongena* and *S. lycoperscon*, combining ability was not yet determined in *Solanum aethiopicum* with its close relatives. In the study, the researcher studied the combining ability of *S. aethiopicum* and its relatives for yield related traits. This study therefore assessed the combining ability and heritability (in both the broad and narrow senses) of interspecific crosses between *S. aethiopicum* and its relatives (*S. anguivi*, *S. incanum* and *S. macrocarpon*).

CHAPTER THREE

METHODS AND MATERIALS

3.1 Objective 1: Identifying the major pre-zygotic reproductive barriers in *Solanum aethiopicum* and its relatives

3.1.1 Study site and Planting materials

Six genotypes were used for the study which included *Solanum aethiopicum* (Gilo and Shum), *Solanum anguivi, Solanum macrocarpon* and *Solanum incanum* which were obtained from the Faculty of Agricultural Sciences (FAS) seed bank at Uganda Christian University. The list and features of genotypes are described in Table 1.

Table 1: Description of genotypes used in the study

Species	**Accession No.**	**Description and characterization**	**Source**
S. aethiopicum Shum (2n=24)	N11	Large leaf size, green stem and glabrous, white flowers and simple raceme inflorescence, green fruits and red when ripe. 	Sseremba *et al*., 2018

S. aethiopicum Shum **(**2n=24)	N4	Small leaf size, Purple and glabrous stem, white flowers and raceme inflorescence, light green fruits when raw and orange when ripe. 	Sseremba *et al*., 2018
S. aethiopicum G*ilo* **(**2n=24)	G4	Large leaf size, light green and glabrous stem, white flowers and simple raceme inflorescence, whitish fruits when raw and orange when ripe 	Sseremba *et al*., 2018
S. macrocarpon ***(***2n=24,36)	E12	Large leaves, green and glabrous stem, purplish corolla, racemose inflorescence, stripped dark green fruits when raw, 	(Bukenya and Carasco, 1995)

		yellowish to brownish fruits when ripe	
S. anguivi (2n=24)	A1	Small leaves,  pale purple and glabrous stem, white purple and raceme-like cyme inflorescence, green fruits with stripes when raw and red when ripe	Sseremba *et al*., 2017
S. incanum (2n= 48)	In1	Medium sized leaves, many hairs and spines on leaves and  stem, violet-colored petals, light green stripped fruits when raw, and Golden yellow berries when ripe.	(Knapp et al., 2013)

3.1.2 Study site

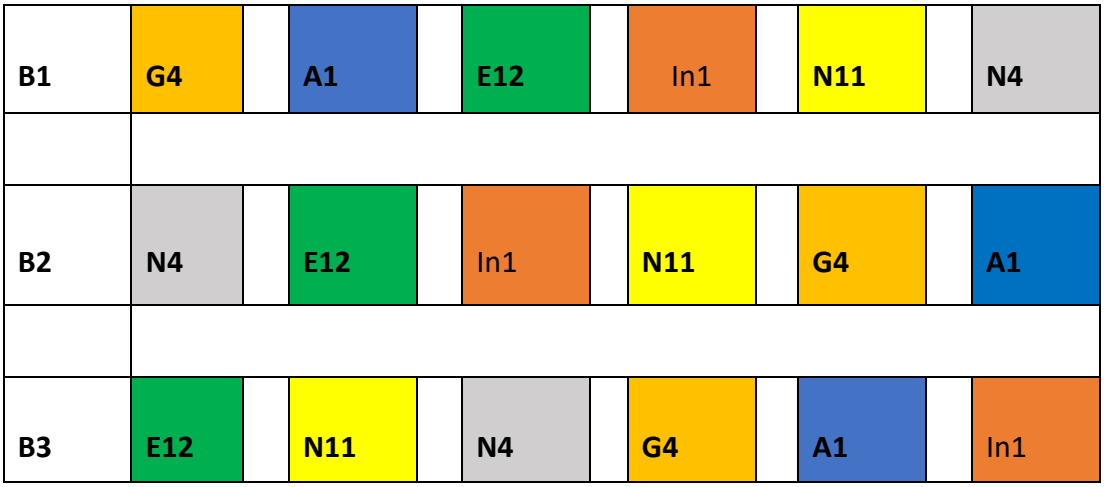
The study was carried out at Faculty of Agricultural Sciences (FAS) in Uganda Christian University (UCU)-Mukono Campus between September, 2020 to February, 2022. Mukono District is located in Central Uganda approximately 22 kilometers East of Kampala and at an elevation of 1158 to 1219 meters above sea level. Mukono receives a range between 1100 to

1400mm of rainfall annually with temperatures ranging from 21 to 29°C, and is located at 00°20'N 32°45'E.

3.1.3 Experimental design

The experiment was done on the Randomized Complete Block Design (RCBD) having three replications/blocks. The six genotypes were planted in 18 plots, each genotype having three plots. Plots of 4mx4m were used each having 36 plants totaling to 108 plants per genotype. The spacing of 60cm x30cm was used between lines and plants.

Experimental layout



3.1.4 Agronomic and management practices

Before planting, the seeds were immersed in gibberellic acid for 24 hours to break the seed dormancy (Mihaiela *et al.*, 2020; Tang *et al*., 2019). Seeds were directly sown in seedling trays with sterile black soil and organic manure at a ratio of 1:3 (Buteme *et al*., 2021). After 4 weeks, seedlings were transplanted to the field for evaluation and others into pots in the screenhouse which were female parents. Fertilizers were applied 7 days after transplanting with NPK (17: 17:17) at 10 g per plant (312 kg/ha) and urea at 10g per plant was applied at the start of flowering in order

to boost vegetative growth and flowering of plants (Buteme *et al*., 2021). Watering was done once a day. At flowering stage though, irrigation was done two times a day (morning and evening). Hand weeding was carried out two to three times a month depending on weed incidence and as need be.

3.1.5 Data collection

Data collection was conducted at flowering stage.

3.1.5.1 Floral structure

Data collection was conducted at 7 weeks after planting (for *S. aethiopicum*) and 9 to 8 weeks after planting for wild relatives. Randomly selected 10 plants per plot were tagged for data collection on the following floral morphological traits.

No	Floral traits	Scales and Description
1.	**Days of flowering**	Six genotypes were observed from time of transplanting to know when the plants started flowering. The days to 50% flowering and 100% flowering of the genotypes was determined.
2.	**Inflorescence number for each plant**	The total amount of inflorescence per plant was collected at 50% flowering.
3.	**Type of inflorescence**	The structure of inflorescence was recorded for the 10 flowers per plant. The scale used was 3- raceme 4-cyme
4.	**Number of flowers per inflorescence**	Number of flowers per inflorescence was recorded at 50% flowering.

5.	**Number of flowers for each plant**	This was recorded for each of the 10 plants
6.	**Flower breadth**	A meter rule was used to determine the flower breadth in centimeters (cm) for 10 flowers per plant for each of 10plants per plot.
7.	**Petal length**	Using meter rule, the length of petal was measured (cm) for 10 flowers per plant for each of 10plants per plot.
8.	**Corolla color**	Petal colour for 10 flowers per plant was assessed using a scale from a *Solanum aethiopicum* descriptor (Buteme *et al*., 2021). The scale was greenish white, white, pale violet; light violet and purplish white.
9.	**Sepal colour**	Colour of the sepals per 10 flowers for each of 10 plants per plot was assessed using a scale from a *Solanum aethiopicum* descriptor (Nahamya *et al.*, 2018). The scale was white, light purple, dark purple, light green, green 6- green purple.
10.	**Stamen length**	This was determined using a meter rule and stamen length for 10flowers in centimeters (cm) per plant for each of 10 plants per plot.
11.	**Pistil length**	Length of the pistil for 10 flowers per plant from 10 plants per plot were determined using a meter rule in cm
12.	**Style Length**	The meter rule was used to measure the length of the style for 10 flowers per plant from 10 plants per plot

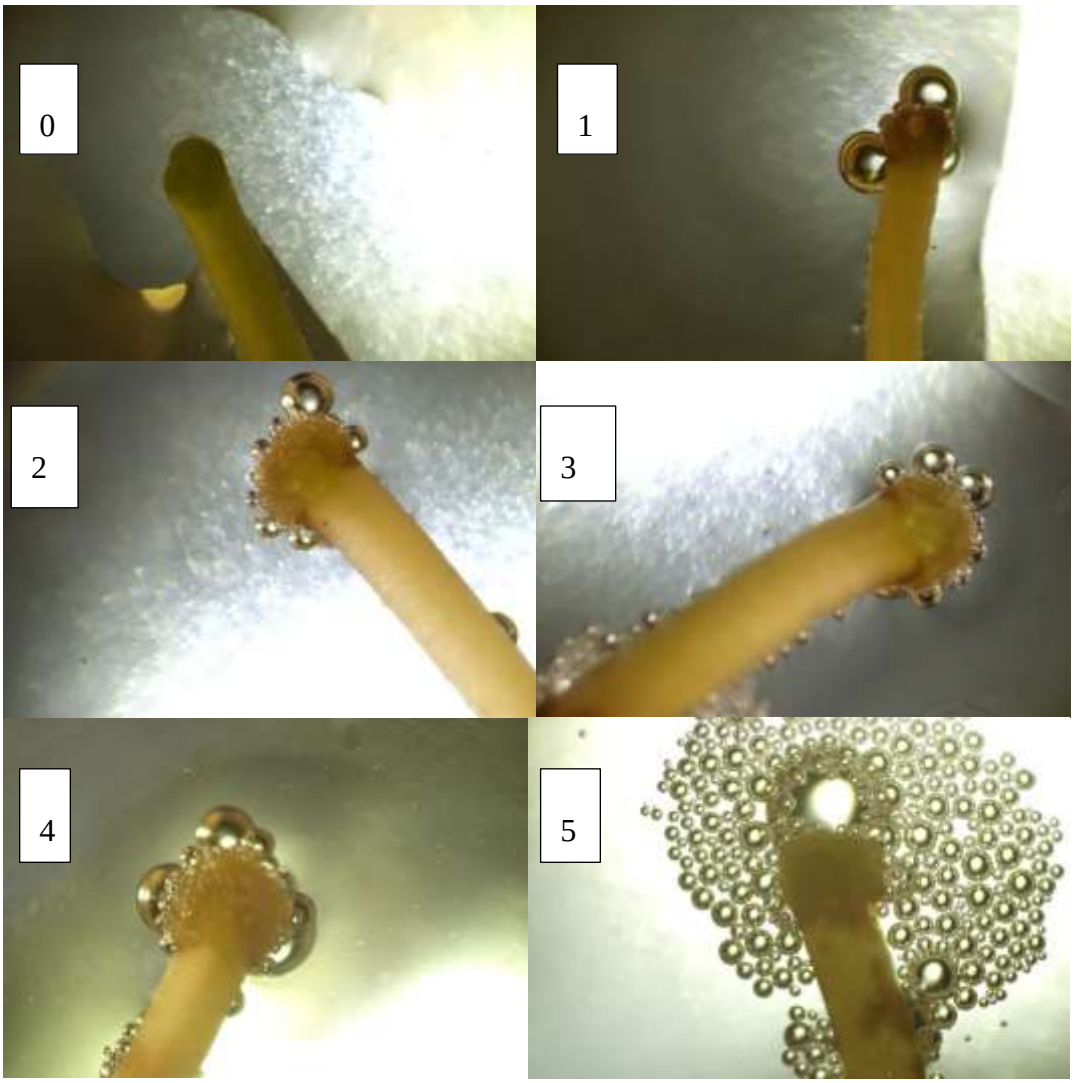
13.	**Number of anthers**	The anther number per 10 flowers for each plant per 10 plants were counted and recorded.
14	**Pistil position with regard to the anthers**	The pistil/stigma exsertion was determined per 10 flowers for each of the 10 plants. The pistil position was evaluated with regard to the anthers so that the probability of cross-pollination to occur was determined in every genotype. The scale of 1 to 3 was used; 1 (inserted pistil), 2 (pistil at the same level as the anthers), 3 (pistil exserted) (Peña-Yam et al., 2019b).
15.	**Anthesis**	A total of 360 flowers were evaluated. Sixty flowers per genotype whereby 2 flowers per 10 selected plants per plot were collected. Flowers in anthesis (bloom stage) were recorded per plant. The opened flowers were counted, recorded and removed from the plant to avoid recounting them. The flowers were counted daily in 4 hourly intervals every day for 10 days; 8:00am, 12:00pm and 4:00pm (Peña-Yam *et al*., 2019; Buteme *et al.*, 2021) . Anthesis was assessed in order to determine the period of the day when the highest number of flowers were registered and when to collect many male flowers in the day for pollination. It aided in knowing which time of the day the highest amount of pollen could be extracted during crossing.
17.	**Anther dehiscence**	2 flowers from 10 plants for each plot (60 flowers for each genotype and total of 360 flowers) were evaluated per each flower stage. Therefore 60 selected flowers from 30 plants at

bud stage (day before anthesis) and 60 flowers at bloom stage (day of anthesis) and at withering stage in 3 time tables of 8:00am, 10:00am and 12:00pm for each genotype were evaluated (Peña-Yam *et al*., 2019). This was used to determine whether the anthers were dehiscent or not. Anther dehiscence was evaluated 3 weeks after start of flowering, when the flowers per plant remained constant. The initial assessment was based on the observation of whitish powder that resembled pollens with the naked eyes. Using a hand lens, the discharged pollen grains were observed. (Aleemullah *et al.*, 2000; Thorat *et al*., 2020).

3.1.5.2 Stigma receptivity

Stigma receptivity was assessed using a drop of hydrogen peroxide (H_2O_2) at 6 %. Two flowers from 10 selected plants per plot were picked and 60 flowers were evaluated per genotype. A total of 360 flowers for every flowering stage (bud stage, full bloom and withering stage) were evaluated. The observations were recorded at 8 am, 12 pm and 4 pm. Stigma sensitivity was assessed using the methodology by (Dafni and Maués, 1998). The stigmas from flowers were put on a cavity slide and hydrogen peroxide (6%) was then dropped on the stigma. It was observed under a light microscope at 10 X and bubbling from the stigma was considered as mark of receptivity of the stigma. The estimated rate of production of bubbles from the stigma surface in which hydrogen peroxide was applied was associated with level of stigma receptivity (Sheffield *et al*., 2005; Valdiani *et al*., 2012; Makwana and Akarsh, 2017). A modified grading scale based on Gupta *et al*., (2015) of 0, 1, 2, 3, 4 and 5 was used in ranking stigma receptivity, 0 = No bubble

production, 3= less bubble production while 5= Very rapid bubble production (shown in figure 2). Receptivity was scored based on the extent of bubbling. This method tests the level of oxygen generation (Makwana and Akarsh, 2017). Stigma receptivity at every stage of a flower (bud, bloom and withering) and at hourly intervals was recorded.



Receptivity of the stigma of Solanum aethiopicum and Wild relatives evaluated with 3% Hydrogen Peroxide. (0) Non reactivity of stigma (No bubbles produced), (1) Very Less reactivity, (2) Less Reactivity, (3) Moderate reactivity, (4) High reactivity, (5) Very High reactivity.

Figure 2: Grading scale used to determine the receptivity of the stigmas of the genotypes under study

3.1.5.3 Pollen production (Quantity)

Two flowers per plant from 10 selected plants per plot were taken from 7:00-8:30am (Buteme *et al*., 2021). A total of 60 flowers for each genotype were assessed. 360 flowers were evaluated altogether. Five anthers from every flower were crushed gently and put in the test tube and then added 2ml of distilled water to make a suspension. Then the pollen quantity was counted using a haemocytometer (Neubauer Improved Cell Counting Chamber Hemocytometer (3x3 mm)). A cover slip was first placed over the counting surface before loading the pollen suspension and then filled haemocytometer chambers with drops of the suspension using a micro-pipette (10-100µl). The sample was allowed to settle for 2-3 minutes and then viewed under a fluorescence microscope (Model BS.1153-PLI~110-240/50-60Hz) at 4x and 10x magnification. To get the total number of pollen grains for each flower, numbers of pollen in the 10 main squares of the haemocytometer were counted and recorded. Summation of pollen grains in 2mls of distilled water was calculated as following a method by Provost and Wallert, (2015).

3.1.5.3 Pollen viability

The two randomly selected flowers in anthesis from 10 plants per plot (60 flowers per genotype) were used. Three methods were used to determine pollen viability (Buteme *et al*. 2021).

Staining method using 2% acetocarmine.

Pollen viability was evaluated following the method described by Alexander, (1969). Pollen was stained using the acetocarmine 2%. Two to four drops of acetocarmine were put on pollen shed on a glass slide, mixed gently and cover slipped. Stained pollen grains were observed under fluorescence microscopy with a camera to make ensure that pollen grains had absorbed the stain (Gao *et al*., 2015, Martins *et al*., 2015). Pollen grains which were stained red were considered

viable and those unstained was be considered as sterile. To determine viability of pollen, the number of viable grains was divided by the total amount of pollen seen, multiplied by 100.

In-vitro Pollen tube growth

Pollen viability was evaluated for capability of the pollen to germinate on the in-vitro media. Flowers at dehiscence stage were harvested from the 6 genotypes. Two flowers from 10 plants per plot in the field were collected totaling up to 60 flowers per genotype (Buteme *et al*. 2021). The pollen was dusted in 35-mm petri-dishes containing the culture medium. The growth medium contained 10% sucrose, 100mg Boric acid (H_3BO_3), 300ppm of calcium Nitrate ($Ca(NO_3)_2$, 200mg magnesium sulfate and 100mg potassium nitrate as reported by (Abdul-Baki, 2019; P, 2018b). The pollen grains were dusted uniformly onto a slide containing the medium. The pollen grains were incubated for 24 hours incubation at 22^0C in darkness to determine the percentage of the germinated pollens (Abdul-Baki, 2019; Buteme *et al*., 2021). The germinated pollen from five randomly selected locations on the slide were counted and recorded. Only pollen grains having pollen tubes with length exceeding pollen grain diameter were considered to have germinated (Buteme *et al*., 2021).

***In-vivo* pollen tube growth**

In vivo pollen tube growth was done for cross combinations that were not successful (Buteme *et al*., 2021). To measure the pollen growth, 2 pollinated pistils from 10 plants per plot were collected after 24, 48 and 72hrs per combination after crossing. The pistils were stored in a solution containing ethanol and acetic acid at a ratio of 3:1. They were rinsed with distilled water and then moved to 8N Sodium hydroxide overnight at room temperature under darkness to soften the pistil. After, pistils were rinsed then stained in 0.1% w/v aniline blue fluorochrome dissolved in 0.1N $K_2PO_4$for 8hrs, in darkness at -4°C (freezer was required) as reported by (Covey *et al*., 2010;

Buteme *et al.,* 2021). Three drops of 50% glycerine were put on pistils on the glass slide and then observed and measured using fluorescence microscope (Dafni *et al*., 2005; Abdelgadir *et al*., 2012). Successful pollen tube growth was determined when pollen grain tubes were observed in the ovary and around the ovules (Martin, 1959). Photographs of pollen tube growth were taken using a fluorescent microscope equipped having sCMEX-6 USB3.0 with a blue, Ultra-violet, green filters at magnification of 10 and 4 (Buteme *et al*., 2021). Pollen tubes which did not reach the ovules were considered incompatible and pollen tubes which reached the ovules were considered compatible.

3.1.5.4 Data analysis

Data collected was entered, summarized and cleaned in Microsoft Excel and then was exported to GenStat Version 14 and R-statistics software version 4.2.2 for analyzing. For Floral qualitative morphological characteristics like color of the flower and style exertion, percentages were determined. The means and standard deviation were determined for days to first flowering, stamen length, petal length, relative style length, flowers per plant, pistil length, anther number per flower, anthesis, pollen viability, stigma receptivity and anther dehiscence were generated. Data transformation was done were necessary before a post hoc tests using logbase 10/ exponential/ power transformations for pollen viability. Two way and three way-Analysis of Variance was done for each quantitative variable which was followed with a separation of means using Bonferroni multiple comparison test at $P \geq 0.05$.

3.2 Objective 2; Determining the level of success between *Solanum aethiopicum* and its close relatives

3.2.1 Materials and methods

3.2.1.1 Germplasm and study site

The study was conducted in screen house at the FAS in UCU-Mukono between September, 2020 and January, 2022. A total of six genotypes (domesticated and wild genotypes) were evaluated.

3.2.1.2 Experimental design

Seeds were directly planted in seedling trays having sterile black soil plus manure at ratio of 1:3 and then transplanted in pots after 4 weeks. 16 plants (one plant per pot) for each genotype were planted in individual plastic pots in a Randomized Complete Block Design (RCBD). A total of 48 pots/ plants per genotypes were grown in 3 replicates.

3.2.1.3 Field management

Fertilizers were applied 7 days after transplanting using NPK (17: 17:17) at the rate of 4 g/plant (312 kg/ha) and urea was applied at the rate of 4 g/per at the start of flowering in order to boost and flowering of plants. Watering was done daily and at flowering stage, irrigation was done two times a day (morning and evening). Hand weeding was done if there were emergency of weeds.

3.2.1.4 Mating design and hybridization (mating design).

A full diallel mating design by Griffings, (1956) was used during cross-pollination. The genotypes crossed had equal chances of being both female and male parents (Christie and Shattuck, 2010; Buteme *et al.*, 2021). Six genotypes were mated as both female and male parents resulting in 30 direct and reciprocal cross-combinations and 6 self-combinations. For each Cross combination, crosses were done depending on the availability of flowers (Afful *et al*., 2018; Buteme *et al*., 2021).

3.2.1.5 Hand pollination

At 50% flowering, flower buds were emasculated, bagged and tagged. Emasculated flowers were then pollinated in the morning (6 to 9 am) by dusting pollen from the selected male plant (donor) onto the stigma of the emasculated female flower (recipient) (Afful *et al.*, 2018). Each pollinated flower was tagged and labeled with the cross details (cross combination, date and time of pollination and flower number). The flowers which were pollinated were covered with organza netted bags to prevent any additional pollen grains from other sources from getting into contact with the stigma. The bags were then removed 2 days after pollination to avoid molding of the pollinated flower. Successful fertilization was considered when the pollinated flower remained intact (not aborted) having a well-developed fruit and viable seeds (Martínez-García *et al*., 2014; Buteme *et al*., 2021). When fruits of the crosses were fully ripe, they were harvested and then seed were extracted. The fruits with very small or no seeds were considered seedless (Buteme *et al.*, 2021). After the seeds were dried and stored for raising the F_1 generation.

3.2.1.6 Data collection

The crosses made were counted and noted. Successfully formed fruits from crosses made per combination were counted and recorded. Fruits with seeds were counted overall and the number of seeds per fruit were recorded. Finally, the hybrid viability was evaluated (Afful *et al*., 2018). Integral reproductive success index (IRSI) was explored between different sets of self, intraspecific and interspecific crosses. Each at a point pertaining to fruit and seed characteristics, as well as the final germination percentage all populations, was labelled and pooled according to pollination treatment (self- and cross-pollination) for inclusion in the analysis (Gallego-Fernández & García-Franco, 2021). In order to represent the total reproductive success in a single value for the crosses and selfed, the integral reproductive success index (IRSI) was constructed and obtained as follows:

$IRSI = fruit\ set\ x\ relative\ seed\ set\ x\ final\ germination$; where a value of 1 indicates very high success in all reproductive phases (fruit set, Seed set and F_1 germination percentage) (Gallego-Fernández & García-Franco, 2021).

3.2.1.7 Data analysis.

Percentage of fruit set was determined by dividing the number of developing fruits by total number of fertilized flower buds. Percentage of germinated seeds was calculated as number of parent and F_1 seeds which germinated divided by total number of planted seeds. The clean data was exported to GenStat version 14 for analysis and later means of fruit set, seed set and germination ability of F_1 hybrids with the parents were obtained. One way Analysis of variance ($P \leq 0.05$) was obtained to assess the difference between the parents as recipients and donors (each cross-combination) and the followed by Bonferroni multiple comparison test.

3.3 Objective 3; Combining ability of yield related traits in F_1 hybrids of *Solanum aethiopicum* and its close relatives.

3.3 Materials and methods

3.3.1.1 Germplasm and study site

The study was conducted at the Faculty of Agricultural Sciences at Uganda Christian University between September, 2021 and May, 2022. A total of six parental lines (domesticated and wild genotypes) and 15 successful F_1 hybrids got from crossing were evaluated. The F_1 hybrids are listed as below;

Table 2: List of the F_1 hybrids utilized in ability combining analyses

No	F_1 hybrid	Female	Male	No	F1 hybrid	Female	Male
1	A1xN11	A1	N11	9	N4xN11	N4	N11
2	A1xN4	A1	N4	10	N4xG4	N4	G4
3	N11xA1	N11	A1	11	N4xIn1	N4	In1
4	N11xE12	N11	E12	12	G4xA1	G4	A1
5	N11xN4	N11	N4	13	G4xN11	G4	N11
6	N11xG4	N11	G4	14	G4xE12	G4	E12
7	N11xIn1	N11	In1	15	G4xIn1	G4	In1
8	N4XA1	N4	A1				

3.3.1.2 Experimental design

The parental lines and F_1 population seeds were planted in trays and then transplanted to the field after 4 weeks. F_1 hybrids with their parents were evaluated in a Randomized Complete Block Design (RCBD) having 3 Replications. The plots of 4mx4m were made and seedlings were transplanted at a spacing of 60 × 30 cm^2 and every plot had 30 plants. In total, 90 plants were planted per parental line and F_I hybrids. Fertilizers were applied once a week after transplanting using NPK at a ratio of 17: 17:17 at the rate of 10 grams per plant (312 kilograms per hectare) while urea at the rate of 10 grams per plant when flowering began. Watering was periodically done during dry times since the experiment was in an open field. Hand weeding was carried out two to three times a month depending on weed incidence and as need be.

3.3.4 Data collection

3.3.4.1 Vegetative traits

Successful crosses were further examined for combining ability (Cazzola *et al.*, 2020). A total of 15 successful cross-combinations and 6 selfs obtained after crossing were used in this study. Data was collected on germination and seedling data 4 weeks after planting, vegetative data at 4, 6 to 8 weeks after transplanting. At seedling stage, the data collected was germination percentage, cotyledon leaf length and width ratio, vigor and length of the seedling. The following quantitative

traits were evaluated at vegetative stage, weight of fresh leaf, plant height, branches per plant, leaves per plant, leaf area, stem girth, leaf lobe base length, number of leaf lobes, dry leaf weight, weight of plants without leaves, weight of 10 plants per plot and data on harvest index.

3.3.3.2 Fruiting stage

Data was collected at fruiting stage, on the number of fruits per plant, fruit pedicel length, presence of prickles on pedicel and calyx, the weight of fruits, the number of seeds per fruit, harvest index (HI). HI was determined as leaf yield (weight of fresh leaves only) divided by total biomass (weight of plants) expressed as a percentage.

3.3.4 Data analysis

R-statistics version 4.2.2 was used to determine the combining ability (General Combining Ability and Specific Combining Ability). The multivariate linear mixed model general form $y = X\beta + Zu + \varepsilon$ available in the R-sommer package was used (Covarrubias-Pazaran, 2016). This was employed in determining the General Combining Ability (GCA) and Specific Combining Ability (SCA) variance components in the incomplete diallel design with missing values (15 of the 30 cross-combinations where successful after crossing making it incomplete crosses) (Buteme *et al*., 2021). Whereby y = assessed phenotypes, X= incidence matrix for fixed effects such as grand mean (μ), Z = the incidence matrix for random effects such as GCA and SCA, β = the vector for BLUEs, u = vector for BLUPs and ε = the residuals /environmental variance (*VE*) (Bu *et al*., 2015; Covarrubias-Pazaran, 2016). The BLUEs is referred to as best linear unbiased estimates of fixed effects and BLUPs are the best linear unbiased predictions of random effects. The phenotypic data from plants was predicted as; $y = X\beta + ZuGCAmales + ZuGCAfemales + ZuSCA + \varepsilon$. whereby *y* was the observed phenotype (leaves per plant, branches per plant, leaf area, fruit size, plant height, Harvest index and others), *X* was the matrix for fixed effects *(μ,* blocks*)* and *Z* was the incidence matrix for random effects (GCA males, GCA females and SCA). Using a 5% error

margin, the significant values of the GCA and SCA effects were determined; later the variance component estimates were done as conducted by Falconer and Mackay (1996), Kang (2002), and as summarized by Sseremba *et al*. (2018). To decide on the significance of combining ability effects, critical values of z-ratio; 1.64, 2.33 and 3.09 (Bu *et al*., 2015; Covarrubias-Pazaran, 2016), set at 0.05, 0.01 and 0.001, respectively to determine probability of variations by chance (α). General and specific combing abilities variance estimates were produced and used in determining the additive (V_A) and dominance genetic variance (V_D) respectively as shown below; $VA = 4 * VGCA$ and $VD = 4 * VSCA$ where $VGCA = VGCAmales + VGCAfemales$ respectively. Genotypic variance (V_G) was determined as $VG = VA + VD$. The measured or phenotypic variance (V_P) was calculated as: $VP = VA + VD + VE/n$; on the hypothesis of negligible epistatic effects (Falconer and Mackay, 1996; Kang, 2002), so that estimation of heritability of traits studied; n=3 which was the number of blocks (3).

General combining ability of parents and crosses were calculated as follows (Sseremba *et al*., 2018);

i. $GCA_{males} = Mean\ peformance\ of\ progeny\ of\ a\ given\ male - Mean\ of\ progeny\ from\ all\ males$
ii. $GCA_{females} = Mean\ peformance\ of\ progeny\ of\ a\ given\ female - Mean\ of\ progeny\ from\ all\ females$
iii. $SCA\ effect = Observed\ mean\ of\ a\ cross - Expected\ mean\ of\ the\ cross$
iv. $Expected\ mean\ of\ a\ cross = overall\ mean\ (\mu) + GCA\ for\ a\ given\ male + GCA\ for\ a\ given\ female.$

The broad- (H^2) and narrow-sense (h^2) heritability was calculated: $H^2 = \frac{V_G}{V_P}$ and $h^2 = \frac{V_A}{V_P}$.

Whereby V_G, V_A and V_P refers to genetic variance, additive genetic variance and phenotypic variance, respectively.

CHAPTER FOUR

RESULTS

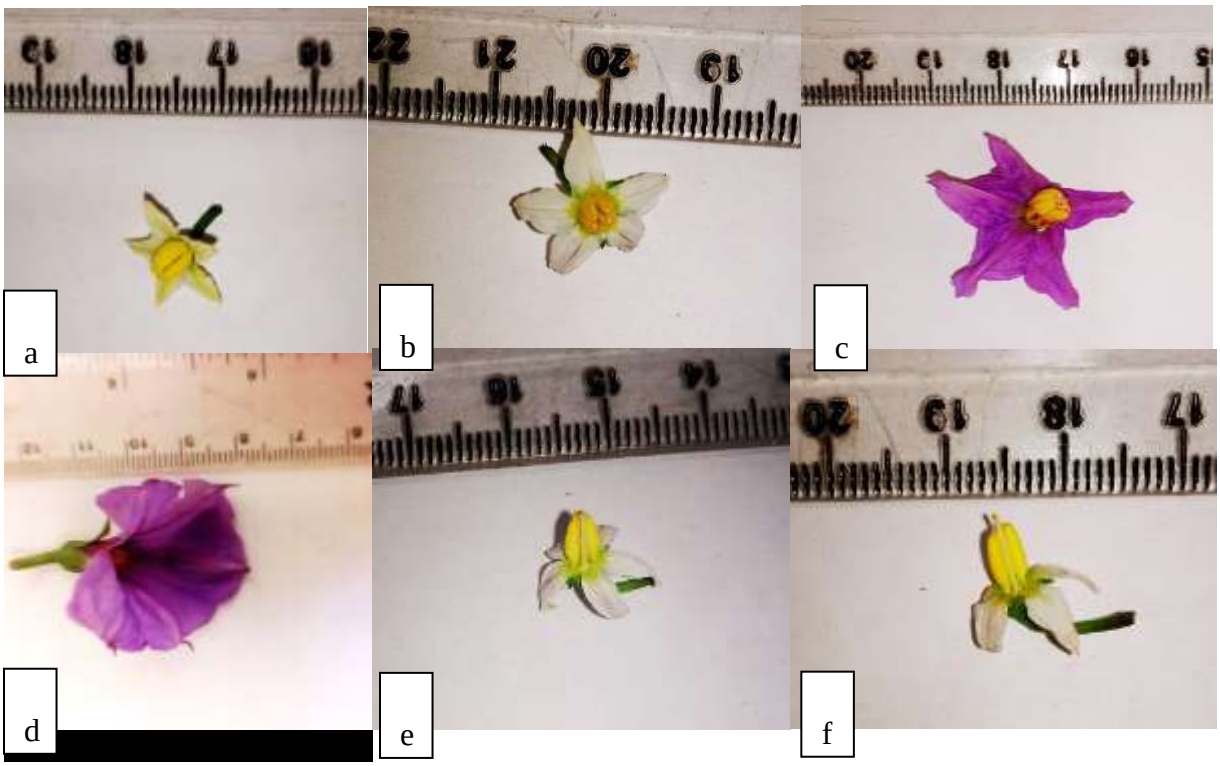
4.1 Objective 2: Pre-zygotic reproductive barriers in crosses of *Solanum aethiopicum* and its relatives

4.1.1 Qualitative floral traits of *Solanum aethiopicum* and wild relatives

The study found out that there are variations among species for qualitative floral traits (Table 2). G4 and A1 had white petals, N11 had white yellow petals, N4 had white purple petal, N12 had pale violet petals and In1 had light violet petals. The exertions of the style were differing between and within the genotypes were varying. All the genotypes had many flowers with intermediate styles and for each genotype; they had varied number of exerted and inserted styles. E12 had the highest percentage of the inserted styles with 31%, while N4, A1, G4 and N11 had the lowest percentage of inserted styles (2%, 2%, 3% and 6%). Majority of the genotypes were having intermediate styles, N4, G4, N11 and In1 had high percentages with 64%, 61%, 58 and 51% respectively. Only A1 had high percentage of intermediate styles with 61%.

Table 3: The qualitative floral traits for *S. aethiopicum* genotypes and its wild relatives

Genotype	Corolla Color (n=300)	Style Exsertion (n=300)	Sepal color
A1	White (100%)	Inserted (2%), Intermediate (2%) Exserted (96)	Green purple (100%)
N11	White Yellow (100%)	Inserted (6%), Intermediate (64%), Exserted (30%)	Green (100%)
E12	Pale violet (100%)	Inserted (64%), Intermediate (31%), Exserted (8%)	Green purple (100%)
N4	White purple (100%)	Inserted (2%), Intermediate (57%), Exserted (41%)	Green (100%)
G4	White (100%)	Inserted (3%), Intermediate (61%), Exserted (36)	Light green (100%)
In1	Light Violet (100%)	Inserted (18%), Intermediate (51%), Exserted (31)	Green purple (100%)



Corolla color and style exertion (a- white yellow flower, b-white purple flower, c and d-purple flowers, e-intermediate style, f-exserted style, g- inserted style).

Figure 3: Variations in corolla color and style exsertion among genotypes

4.1.2 Quantitative floral traits of *Solanum aethiopicum* and wild relatives

There was significant difference (P<.001) among the genotypes for quantitative floral traits (Table 3). N4 had the shortest days to first flowering and G4 had the shortest time to 50% flowering. In1 had the longest days to first, 50% flowering and 100% flowering while N11 and E12 had the widest flowers and longest petals and sepals while N11 had the smallest flower width, petal and sepal length. E12 had the longest stamen while A1 and N11 had the shortest stamens (Table 3). For, style length, E12 had the longest style and N11 had the shortest style length, E12 had the longest pistil length and N11 had the shortest pistil length (Table 6 and figure 3). Among the genotypes, N11 had the highest flower number; E12 had had the lowest flower numbers per plant.

Table 4: Quantitative floral traits of *S. aethiopicum* and its relatives

Genotype	DFL	DFFL	DOF	STL	FB	PEL	RSL	NOFP	PL	NAF
A1	73±10[a]	82±9[a]	96±10[a]	0.54±0.11[a]	1.61 ±0.4[a]	0.85±0.27[a]	0.53±0.12[b]	129±59.5[c]	0.74±0.17[b]	5±0.13[a]
N11	68±9[a]	73±4[a]	88±6[a]	0.54±0.09[a]	1.58 ±0.21[a]	0.78±0.14[a]	0.47±0.12[a]	139±41.3[d]	0.66±0.16[b]	5.2±0.4[b]
E12	73±8[a]	81±4[a]	91±6[a]	0.9±0.26[d]	4.73 ±1.25[d]	2.44±0.61[d]	0.77±0.28[d]	23±8.59[a]	1.18±0.34[e]	6±0.86[c]
N4	66±8[a]	72±6[a]	79±6[a]	0.55±0.11[a]	1.65±0.36[a]	0.8±0.18[a]	0.53±0.12[b]	127±55.1[c]	0.74±0.15[b]	5.1±0.4[ab]
G4	67±4[a]	72±6[a]	80±6[a]	0.67±0.15[b]	1.89±0.33[b]	0.93±0.21[b]	0.58±0.09[bc]	27±7.77[a]	0.88±0.12[d]	6±0.73d
In1	108±12[b]	122±12[b]	138±12[b]	0.8±0.14[c]	3.08±0.52[c]	1.45±0.31[c]	0.59±0.42[c]	42±20.17[b]	0.81±0.49[c]	5±0.17[a]
l. s. d	8.56	9.25	11.63	0.024	0.08	0.052	0.036	6.209	0.043	0.085
p-Value	<.001	<.001	<.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a],[b],[c],[ab, bc] in table 2 above show the statistical significance of differences between means of genotypes for the quantitative characteristics. DFL- Days to first flowering, DFFL- Days to 50% flowering, DOF- Days to 100% flowering, STL- Stamen length, PEL-Petal length RSL-Relative style length, NOFP-Number of flowers per plant, PL-Pistil Length, NAF- Number of anthers

4.1.3 Anthesis of flowers of *S aethiopicum* and its relatives

There was a significant difference observed for number of open flowers among the genotypes, time and days of anthesis (Figure 4 and Table 5). A significant interaction ($p < 0.001$) was observed between genotype and time, genotype and day, time and day, genotype, time and day. The largest number of open flowers was recorded at 8:00am for all genotypes and the lowest number of flowers at anthesis was observed at 4pm for all genotypes (Figure 4). The highest number of open flowers over 10 days was observed in N11 (13 flowers) whereas the lowest number of open flowers was recorded in E12 (3 flowers). N11 had largest number of open flowers was observed at 8:00am and 12pm with 22 and 8 flowers respectively and E12 had the lowest number of open flowers at 8:00am, 12:00pm and 4:00pm with 7, 2 and 1 flowers respectively.

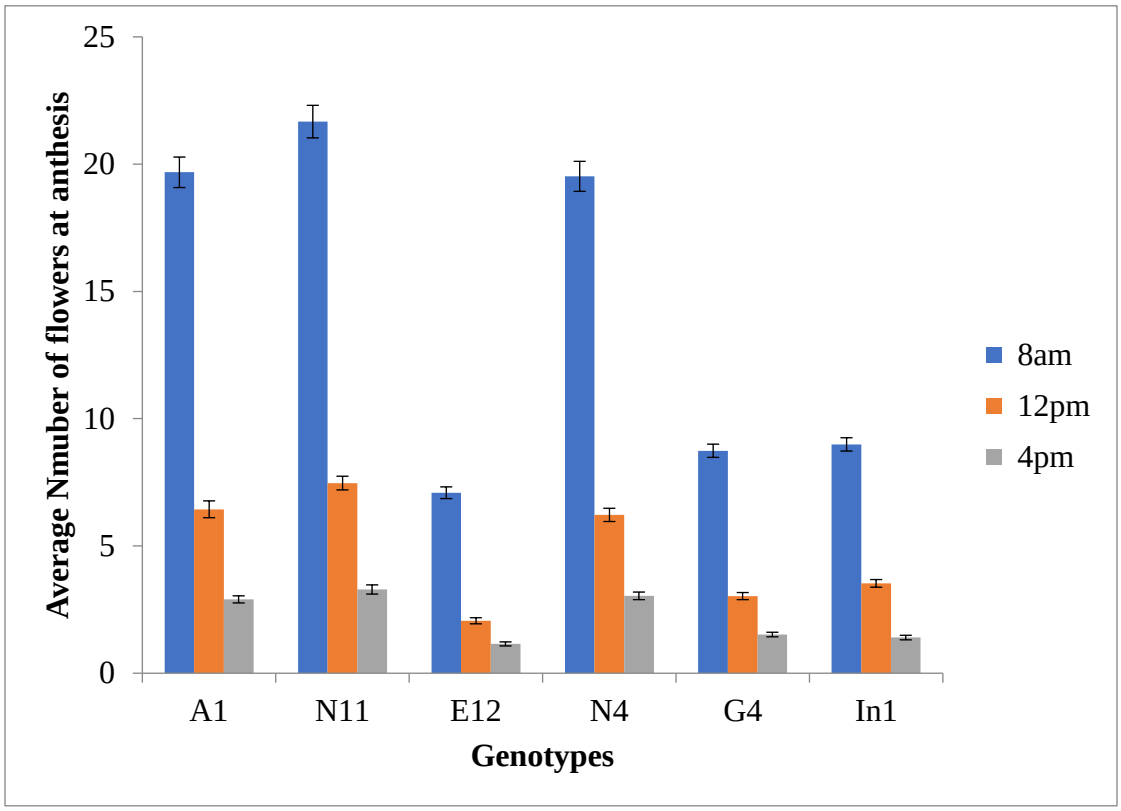


Figure 4: Number of flowers at anthesis per genotype at 8:00am, 12:00pm and 4:00pm

Table 5: Analysis of variance of flowers at anthesis at different times of the day by genotypes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	5	47932.97	9586.59	389.71	<.001
Time	2	145314.5	72657.24	2953.61	<.001
Day	9	8518.89	946.54	38.48	<.001
Genotype*Time	10	27171.85	2717.18	110.46	<.001
Genotype*Day	45	4609.49	102.43	4.16	<.001
Time*Day	18	5566.98	309.28	12.57	<.001
Genotype*Time*Day	90	4780.3	53.11	2.16	<.001
Residuals	5218	128359.8	24.6		

4.1.4 Anther dehiscence

There was no difference in anther dehiscence among genotypes (Table 6 and 7). However, there was a significant difference in anther dehiscence at bud, bloom and withering stages (P<.001). There was no interaction in anther dehiscence between genotypes and floral stages (Table 7).

Table 6: Mean anther dehiscence at the different flower development stages

Genotype	Bud	Bloom	Withering
A1	0.00^{a}	1.00^{a}	1.00^{a}
N11	0.00^{a}	0.98^{a}	1.00^{a}
E12	0.00^{a}	1.00^{a}	1.00^{a}
N4	0.00^{a}	0.98^{a}	1.00^{a}
G4	0.00^{a}	1.00^{a}	1.00^{a}
In1	0.00^{a}	1.00^{a}	1.00^{a}

Tabl**e 7;** ANOVA table showing the differences in anther dehiscence among genotypes and flower stages

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	5	0.019753	0.003951	1.62	0.152
Stage	2	357.3531	178.6765	73186.32	<.001
Genotype x Stage	10	0.039506	0.003951	1.62	0.096
Residual	1602	3.911111	0.002441		

4.1.5 Stigma receptivity

All stigmas in all genotypes were receptive at all stages (Table 8 and 9). More receptive stigmas were recorded at bloom stage, relatively low at withering stage and very low at bud stage. There was a significant difference (P<.001) in stigma receptivity among genotypes, stages and time at (Table 9). There was an interaction between genotypes and stages (P<.001), stages and time (P<.001), among genotypes, stages and time (P<.003) however no interaction was found between genotypes and time (P<.214) 001 (Table 6). *S. aethiopicum* Shum (N11 and N4) had the highest mean stigma receptivity of 3.31 and 2.88±1.32 respectively (Table 8) while In1 had the least stigma receptivity of 2.31(Table 8). At bud stage, N11 showed high receptivity of the stigmas at 8:00am, 12:00pm and 4:00pm with 3.78, 3.27 and 2.85 respectively. Very low stigma receptivity was observed in In1 at 8:00am, 12:00pm and 4:00pm with 2.17, 1.85 and 1.85 respectively (Table 8). At bloom stage, N11 showed high receptivity of the stigmas at 8:00am and 4:00pm with 4.22 and 3.37 respectively and A1 had highest stigma receptivity at 12:00pm with 3.37. Low stigma receptivity was observed in In1 at 8:00am, 12:00pm and 4:00pm with 3.27, 2.62 and 2.35respectively (Table 8). At withering stage, N11 showed high receptivity of the stigmas at 8:00am, 12:00pm and 4:00pm with 3.18, 3.07 and 3.02 respectively. In1 had the lowest stigma receptivity at 8:00am, 12:00pm and 4:00pm with 2.25, 2.42 and 1.83 respectively (Table 8).

Table 8: Mean, standard deviation, significance differences and l.s.d stigma receptivity of *Solanum aethiopicum* and wild relatives for every stage and time

Genotype	Overall	Bud stage			Bloom stage			Withering stage		
		8:00am	12:00pm	4:00pm	8:00am	12:00pm	4:00pm	8:00am	12:00pm	4:00pm
A1	2.78±1.40^{b}	2.5±1.42^{ab}	1.83±1.33^{a}	1.78±1.29^{a}	3.9±1.19^{b}	3.37±1.18^{b}	3.2±1.04^{b}	3.08±1.14b^{c}	2.95±1.14^{ab}	2.4±1.39^{abc}
N11	3.31± 1.09^{c}	3.78±1.19^{c}	3.27±1.33^{b}	2.85±1.42^{b}	4.22±0.9^{b}	3.05±1.20^{ab}	3.37±1.06^{b}	3.18±1.27^{c}	3.07±1.23^{b}	3.02±1.3^{c}
E12	2.5±1.41^{a}	2.7±1.27^{ab}	1.98±1.08^{a}	1.77±1.33^{a}	3.28±1.37^{a}	2.62±1.46^{a}	3.07±1.33^{b}	2.8±1.34^{abc}	2.33±1.3^{a}	1.92±1.44^{a}
N4	2.88±1.32^{b}	3±1.33^{b}	1.92±1.21^{a}	1.65±1.15^{a}	4.28±0.76^{b}	3.22±0.96^{ab}	3.5±1.07^{b}	2.88±1.15^{abc}	2.68±1.17^{ab}	2.78±1.17^{bc}
G4	2.84±1.22^{b}	3.07±1.15^{b}	2.7±0.89^{b}	2.02±0.93^{a}	4.15±0.88^{b}	3.28±0.96^{b}	3.12±1.06^{b}	2.48±1.41^{ab}	2.42±1.06^{a}	2.32±1.13^{ab}
In1	2.31± 1.1^{a}	2.17±1.01^{a}	1.85±1.2^{a}	1.97±1.28^{a}	3.27±1.3^{a}	2.62±1.14^{a}	2.35±1.16^{a}	2.25±1.3a	2.47±1.2^{ab}	1.83±1.11^{a}
p- value	<0.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.002	<.001
l.s.d	0.157	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.5

^{a}, ^{b}, ^{c}, ^{ab}, $^{bc, cd, abc}$, ^{d}, ^{e} in table 5 above show the statistical significance of differences between means of genotypes in stigma receptivity. Means of genotypes with same letters are not significantly different for stigma receptivity.

Table 9: ANOVA table showing the differences in stigma receptivity among genotypes, floral development stages and time

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	5	323.22	64.644	45.15	<.001
Stages	2	528.675	264.337	184.61	<.001
Time	2	268.708	134.354	93.83	<.001
Genotype*Stages	10	129.718	12.972	9.06	<.001
Genotype*Time	10	18.873	1.887	1.32	0.214
Stages*Time	4	53.683	13.421	9.37	<.001
Genotype*Stages*Time	20	59.691	2.985	2.08	0.003
Residual	3184	4559.057	1.432		
Total	3239	5943.852			

4.1.6 Pollen Quantity

There was a significant difference ($p<0.047$) in pollen quantity between genotypes (Table 10). The results showed that E12 had the highest average number of pollen grains at 218,267 (Min 34,000 and Max 752,000) and lowest average number for A1 at 141,333 (Min 26,000 and Max 450,000).

Table 10: Mean, standard deviation, significance differences and l.s.d for number of pollen grains per flower in *Solanum aethiopicum* and wild relatives

Genotypes	Pollen quantity	Min	Max
A1	141,333±73,993[a]	26,000	450,000
N11	195,467± 119,635[a]	26,000	516,000
E12	218,267±158,913[a]	34,000	752,000
N4	211,033±130,070[a]	36,000	544,000
G4	208,100±153,781[a]	42,000	648,000
In1	190,767± 190,651[a]	28,000	1,086,000
l.s.d	51289.4		
p-values	0.047		

[e] *in table 5 above show the statistical significance of differences between means of genotypes in pollen quantity*

4.1.7 Pollen Viability

Generally, there was a significant difference ($p<0.001$) in percentages of pollen viability amongst genotypes when both in-vitro and staining methods were used (Table 11). Results showed a high

pollen viability (87.55%) in N11 with in-vitro pollen tube growth method and a relatively low viability (80.24%) in A1. Further, a highest pollen viability revealed; E12 (93.34%) and N11 (93.01%) and lowest viability of 69.42% in G4 when Acetocarmine stain was used.

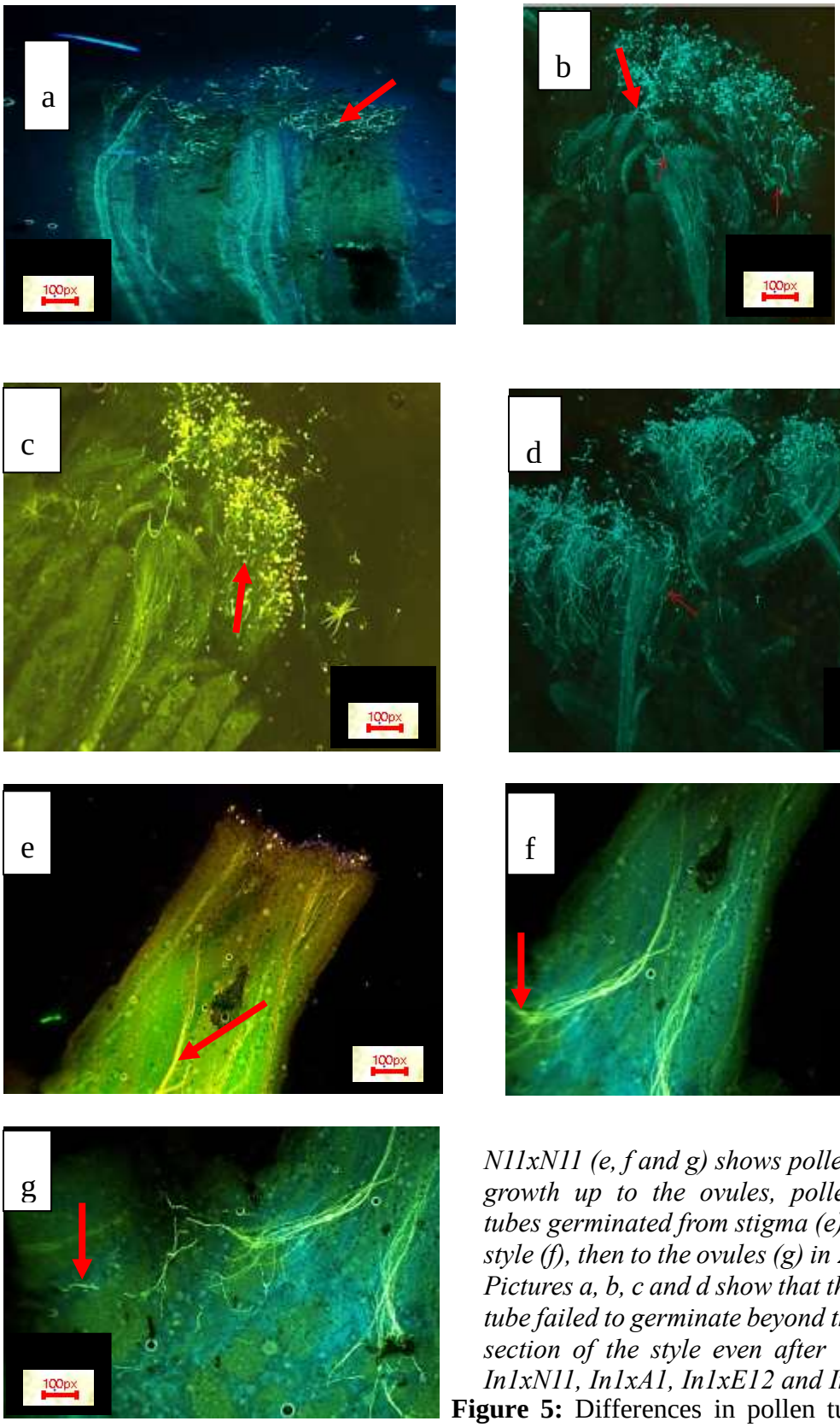
Table 11: Mean, standard deviation, significance differences and l.s.d stigma receptivity of *Solanum aethiopicum* and wild relatives in pollen Viability (%) per flower using In-vitro-pollen tube growth and 2% Acetocarmine staining

Genotypes	In-vitro pollen tube growth	Min	Max	2% acetocarmine stain	Min	Max
A1	80.24±7.75^{a}	55.17	98.31	86.89± 11.19^{b}	57.20	100.00
N11	87.55±7.45^{c}	72.09	100.00	93.01±9.90^{b}	45.24	100.00
E12	85.45±13.97^{abc}	32.50	100.00	93.34± 5.58^{b}	78.89	99.77
N4	86.94±6.15^{c}	70.00	100.00	89.79±11.73^{b}	40.45	99.63
G4	80.77±13.23^{ab}	39.47	100.00	69.42± 21.84^{a}	9.82	98.09
In1	86.36±13.22^{bc}	37.88	100.00	91.64±9.65^{b}	58.58	100.00
l.s.d	3.874			4.547		
p-values	<0.001			<0.001		

^{a}, ^{b}, ^{c}, ^{ab}, $^{bc, abc}$ in table 5 above show the statistical significance of differences between means of genotypes in pollen viability

4.1.8 In vivo pollen tube growth

In vivo pollen tube germination using fluorescent microscope examination showed variations in level of success in pollen tube growth between the selfed and crosses (Figure 5). For all the four crosses that failed to set fruits after crossing (In1xN11, In1xN4, In1xE12 and In1xA1), stigmas had germinated pollen grains every time of examination. There was pollen tube growth observed in In1xN11, In1xN4, In1xE12 and In1xA1 from the stigma however the pollen tubes failed to grow beyond the upper and middle part of the style even after 72 hours after pollination. The post-zygotic barriers could have led to failure of fruit set in the crosses. In the selfed genotype (N11xN11) which was used as a control, there was compatibility detected; the pollen tubes germinated from the stigma to the ovules within 24hrs after pollination.



N11xN11 (e, f and g) shows pollen grains growth up to the ovules, pollen grain tubes germinated from stigma (e) into the style (f), then to the ovules (g) in 24hours. Pictures a, b, c and d show that the pollen tube failed to germinate beyond the upper section of the style even after 72hrs in In1xN11, In1xA1, In1xE12 and In1xE4.

Figure 5: Differences in pollen tube germination and growth for the failed crosses and controls which were selfs of *S. aethiopicum*

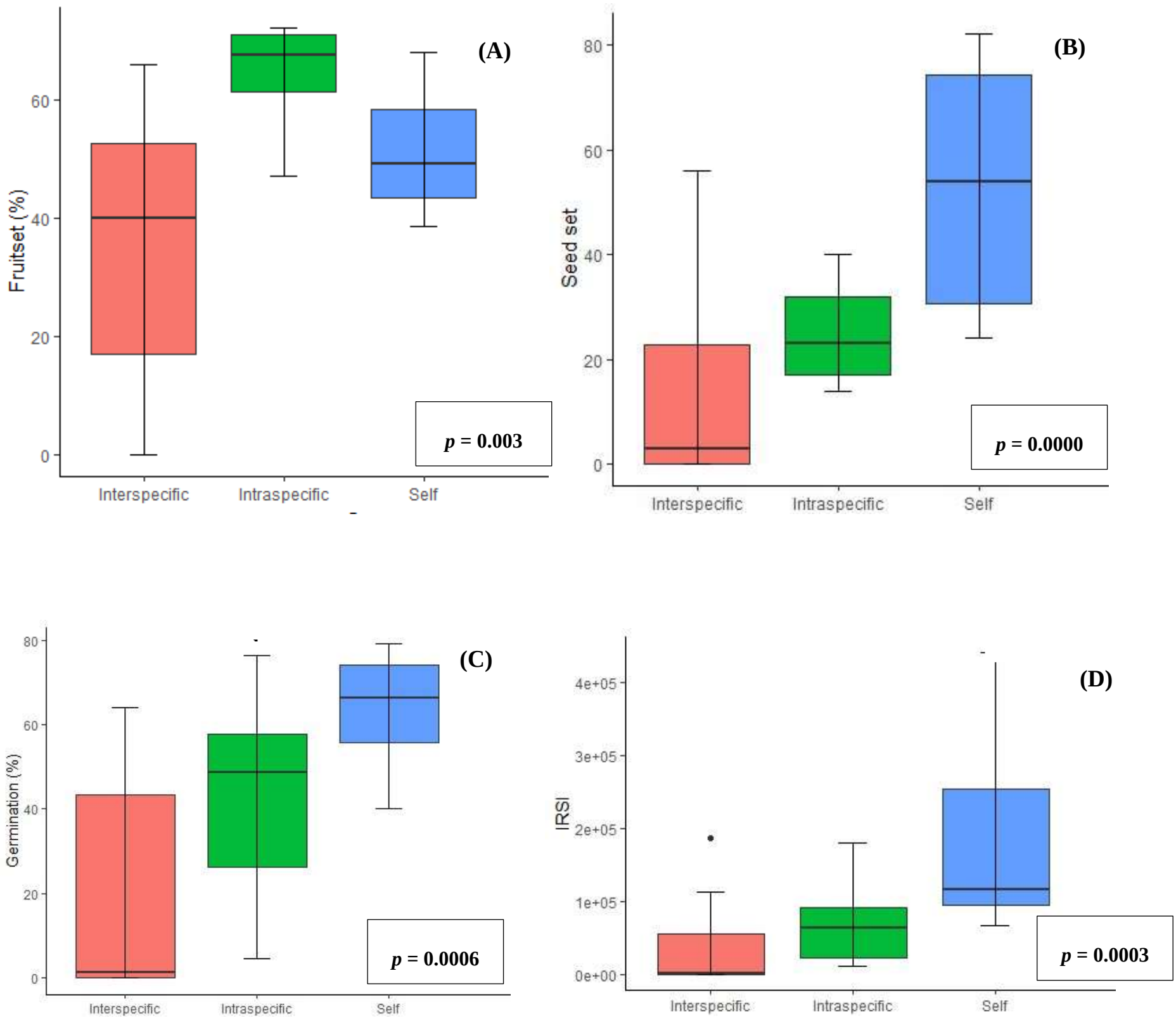
4.2 Objective 2: Determining the level of crossing success between *Solanum aethiopicum* and its close relatives

4.2.1 Crossability of *S. aethiopicum* genotypes with wild species

A total of 6828 crosses were made in 36 cross-combinations (Table 12). All six selfs (N11xN11, N4xN4, E12xE12, A1xA1, G4xG4 and In1xIn1) showed successful fruit set, seed set and germinability. The highest (67.93%) and lowest (38.54%) number of fruit set were observed in N11 and In1 respectively. Among the crosses, 4 out of the 30 cross-combinations done produced no fruit (In1XA1), In1X N11, In1XE12, and In1XN4) (Table 12). Out of 26 crosses with successful fruit set, five did not produce seed (A1XG4, E12XA1, E12XN11, E12XN4, and E12XG4). Highest fruit set was obtained in N4XN11, N4XG4, N11XN4, N11XG4, and N11XE12 with percentages of 71.97%, 71.39, 69.15%, 66%, 65.89% respectively. Fruit set was lowest in In1XG4, E12XA1 and E12XN4 with 8.6%, 9.76 and 25.5% fruit set respectively. The means of seed set per fruit for crosses varied with the highest seed set (56 seeds) and (45seeds) in N11XA1 and G4XA1 respectively. The lowest seed set was obtained in A1XE12 (2seeds) and A1XIn1 (4seeds). The seeds from successful crosses were planted and assessed for germination viability. Assessment of germination viability of seeds from crosses revealed the highest seed germination at 76.33% and 60.67% in N11XN4 and N11XA1 respectively, and no germination at all in 5 cross combinations (A1XE12, E12XIn1, N4XE12, AXIn1, G4XN4). All selfs showed successful fruit set, seed set and seed germinability (Table 12).

Table 12: Fruit set (%), number of seeds per fruit and germination (%) of F_1 seeds in selfs and crosses of *S. aethiopicum* and wild relatives.

Cross-Combination	Number of Selfs/ Cross-pollinations	Fruit set (%)	Seed per fruit	Germination (%)
A1	133	46.3±29.81[a]	24±13[a]	59.67±5.07[c]
N11	211	67.93±15.6[a]	82±51[b]	79±18.02[c]
E12	126	51.94±22.59[bc]	77±67[b]	74.67±2.57[b]
N4	238	60.39±24.32[a]	27±26[a]	72.67±5.07[d]
G4	171	42.54±21.50[a]	66±52[a]	40±4.39[bc]
In1	90	38.54±26.14[b]	42±21[b]	54.33±14.36
A1xN11	160	52.71±23.41[a]	19±4.42[a]	44±22.39[bc]
A1xE12	292	42.19±27.02[a]	2±1.4[a]	0±0[a]
A1xN4	68	53.54±23.02[a]	29±18.27[a]	43±28.77[b]
A1xG4	118	37.8±23.02[a]	0±0[a]	N/A
A1xIn1	240	32.44±24.06[a]	4±4.12[a]	0±0[a]
N11xA1	277	55±18.83[a]	56±34.23[ab]	60.67±12.04[b]
N11xE12	512	65.89±26.48[a]	36±15.97[ab]	39.33±12.16[a]
N11xN4	236	69.15±15.16[a]	34±30.40[a]	76.33±5.4[c]
N11xG4	231	66±21.78[a]	20±10.43[a]	60±10.41[b]
N11xIn1	510	60.65±18.26[a]	29±21.49[a]	64±18.72[b]
E12xA1	101	9.76±11.15[a]	0±0[a]	N/A
E12xN11	114	19.45±28.30[a]	0±0[a]	N/A
E12xN4	77	25.5±15.59[ab]	0±0[a]	N/A
E12xG4	102	29.39±16.42[abc]	0±0[a]	N/A
E12xIn1	146	52.56±17.75[c]	2± 1.6[a]	0±0[a]
N4xA1	283	51.57±23.91[b]	22±21[a]	57±12.85[c]
N4xN11	289	71.97±19.41[b]	26±25[a]	51±9.24[c]
N4xE12	414	19.98±19.12[b]	9±4.12[a]	0±0[a]
N4xG4	143	71.39±15.25[b]	14±14[a]	46.33±8.48[c]
N4xIn1	206	57.37±20.86[b]	6±6[a]	24±14.55[b]
G4xA1	157	44.16±20.57[a]	45±44[a]	48±7.24[d]
G4xN11	132	46.98±27.5[a]	16±16[a]	19.67±8.52[b]
G4xE12	189	52.08±28.99[a]	20±20[a]	33.67±23.08[c]
G4xN4	124	59.69±11.91[a]	40±39[a]	0±0[a]
G4xIn1	166	44.21±21.84[a]	25±24[a]	46.33±12.9[d]
In1xA1	132	0±0[a]	N/A	N/A
In1xN11	69	0±0[a]	N/A	N/A
In1xE12	197	0±0[a]	N/A	N/A
In1xN4	87	0±0[a]	N/A	N/A
In1xG4	87	8.6±10.24[a]	0±0[a]	N/A
Total	5859			
p-value		<0.001	<0.001	<0.001
Lsd			26.01	6.23



The characteristics of fruit set (A), seed set (B), Germination (C) and the integral reproductive success index (IRSI) (D) for the groups of selfs, intraspecific and interspecific crosses of S. aethiopicum and its relatives, obtained by self (Self) and cross pollination (Cross). The result of the analysis of variance is shown in each graph. The different letters above the boxes indicate significant differences (multiple comparisons, p≤0.001), Boxes show the average (x), median (-) and quartiles. ^{a}, ^{b}, ^{ab}, *in Fig 3 above show the statistical significance of differences between means of set of crosses and selfs in Fruit set, Seed set,* F_1 *germination percentage and IRSI.*

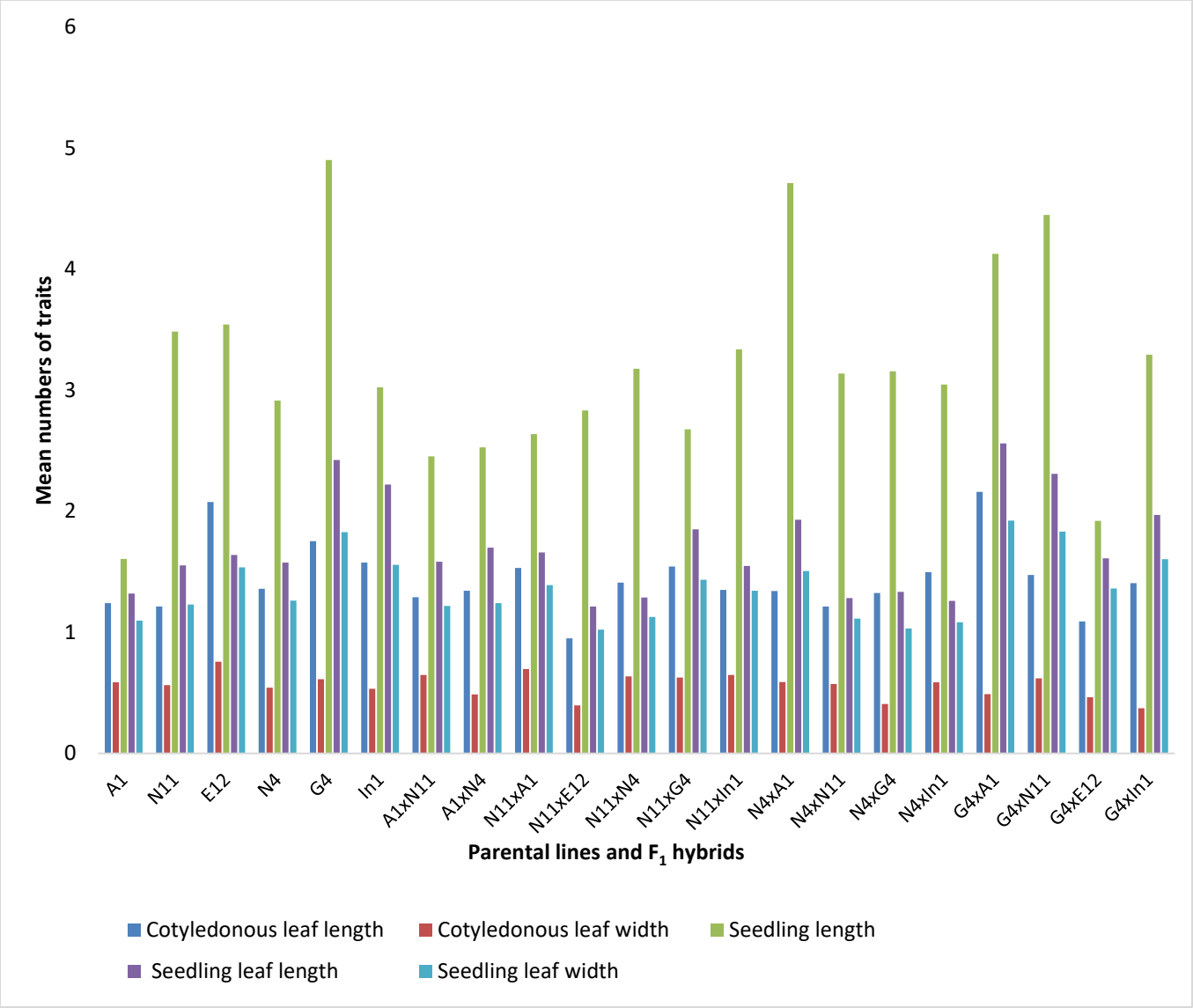
Figure 6: Comparisons between the groups of selfs, intraspecific and interspecific crosses of *S. aethiopicum* and its relatives for fruit set, seed set, Germination and the integral reproductive success index.

4.3 Objective 3: Combining ability of yield related traits in F1 hybrids of *Solanum aethiopicum* and its close relatives.

4.3.1 Performance of the parental genotypes and their hybrids for yield related traits

4.3.1.1 Performance of the parental genotypes and their hybrids at Seedling stage

For parents, E12 had the longest Cotyledonous leaf with length of 2.08cm and N11 had the shortest Cotyledonous leaf with the length of 1.21cm (Figure 6). N12 had the broadest Cotyledonous leaf with average width of 0.76cm and In1 had the smallest Cotyledonous leaf with the width of 0.53cm. G4 was the tallest with 4.9 cm and G4xE12 was the shortest with 1.61cm. Findings also revealed that G4 had the longest and widest seedling leaf with length of 2.42cm and width of 1.83cm. A1 had the shortest and smallest seedling leaf with length of 1.32cm and width of 1.1cm. For F_1 hybrids, G4xA1 had the highest longest Cotyledonous leaf with length of 2.16cm and N11xE12 had the shortest Cotyledonous leaf with the length of 0.95cm (figure 6). N11xA1 had the broadest Cotyledonous leaf with average width of 0.7cm and N11xE12 had the smallest Cotyledonous leaf with the width of 0.4cm. In regards with seedling height, N4xA1 was the tallest with 4.71cm and G4xE12 was the shortest with 1.92cm. Findings also revealed that G4xA1 had the longest and widest seedling leaf with length of 2.56cm and width of 1.92cm. N11xE12 had the shortest and smallest seedling leaf with length of 1.21cm and width of 1.02cm. At seedling stage, all traits (CLL, CLW, SL, SLL and SLW) were significantly different ($p<.001$) amongst all genotypes and F_1 hybrids.



*Significant different at **P<.001** among hybrids and parents*

Figure 7: Performance of parental genotypes and their hybrids for yield related traits at seedling stage

4.3.1.2 Performance of the parental genotypes and their hybrids at vegetative stage

Generally, there was significant difference (P<0.001) between the performance of quantitative traits (Leaf area, plant height, stem girth, plant width, number of leaves and number of branches) between parents and F_1 hybrids at 4WAT (Table 13 and 14). There was no significant difference between in quantitative traits between seasons at P (0.828).

At four weeks after transplanting, the study revealed that there was a significant difference for all the measured traits for the parents and F_1 hybrids at Four weeks after transplanting P (0.001) (Table 14). N4 was the tallest with plant length of 30.13cm and A1 was the shortest with height of 15.55cm among the parents. The hybrids, N4xA1, A1xN4 and G4xN11 had the tallest plants with height of 49.38cm, 36.74cm and 34.86cm respectively and N4xIn1 and N11xIn1 had the shortest plants (19.24cm and 18.88cm) respectively.

The parental lines N4 had the highest branch number (9) and In1 had the lowest number of branches (2). For the hybrids, N4xA1 and G4xN11 had the highest number of branches with (10) and (9) respectively. Among parents, for stem girth G4 had the widest stem (0.78cm) and In1 had small stem girth (0.42cm). Among the hybrids, N11xE12 and G4xN11 had the widest stem 0.95 and 0.81 respectively while N11xIn1 had the smallest stem girth of 0.52cm. Number of leaves per plant were high in N4 (35) and lowest in In1 with 12 leaves. For the hybrids, N4xA1 and G4xN11 had the highest number of leaves with (48) and (39) respectively and N11xIn1 had the lowest number of leaves (18). For leaf area, G4 had the largest leaf with area of 285.02cm^2 and N4 had the smallest leaf with area of 139.09cm^2. Among the F_1 hybrids, G4xA1 and N11x E12 had the largest leaves with average leaf area of 346.35cm2 and 326.10cm2 respectively. N4xIn1 and N11xIn1 had the smallest leaves with average leaf area of 190.92cm^2 and 160.39cm^2 respectively. At Six weeks after transplanting, there was significant difference ($P<0.001$ and $P<0.004$) between the performance of plant height, stem girth, plant width, number of leaves, number of branches and between parents and F_1 hybrids (Table 15). There was no significant difference between in quantitative traits between seasons at P (0.369). There was a significant difference between genotypes (Parents and offspring's) in all traits (plant height, stem girth, number of leaves per plant, number of branches and leaf area) at $P<.001$ (Table 16).

For the parents, N4 and G4 had the tallest plants (41.78 and 40.2cm) respectively and E12 had the shortest plants with 24.73cm. Amongst the hybrids, N4xG4 and N4xA1 had the tallest plants with height of 79.38cm and 70.71cm respectively and N11xIn1 had the shortest plants (31.08cm) respectively.

For the parental lines, N4 had the highest number of branches (12) and In1 had the lowest number of branches (7). Among hybrids, N4xA1, N4xN11 and N4xG4 had the highest number of branches with (14), (13) and (13) respectively.

Among the parental lines, stem girth ranged from 0.52 to 0.96cm with G4 having the widest stem (0.96cm) and In1 had small stem girth (0.52cm). Among the hybrids, N11xE12 and G4xN11 had the widest stem 0.95 and 0.81 respectively while N11xIn1 had small stem girth with 0.52cm.

For the parental lines, average number of leaves per plant ranged from 23 to 47 with N4 (35) having the highest number of leaves (47) and In1 having the lowest number of leaves (23). Among hybrids, highest numbers of leaves per plant were obtained in N4xG4 (101) and G4xN11 (68) and lowest number of leaves were obtained in G4xA1 (33leaves) and N11xIn1 (37 leaves).

For leaf area, G4 had the largest leaf with area of 345.23cm^2 and N4 had the smallest leaf with area of 179.53cm^2. Among F_1 hybrids, N11xA1, N11x E12 and G4xA1 had the largest leaves with average leaf area of 413.41cm^2, 404.37cm^2 and 377.02cm^2 respectively while N11xIn1 and A1xN4 had the smallest leaves with average leaf area of 224.54cm^2 and 242.38cm^2 respectively.

Table 13: Overall performance of quantitative traits at Six weeks after transplanting for parents Vs F_1 hybrids

Variables (4WAT)	Parents		F1 hybrids		
	Mean	Range of variation	Mean	Range of variation	Prob.t
Plant Height (cm)	20.88	(9.1-38.1)	28.58	(10.9-68.7)	<0.001
Plant width (cm)	35.58	(4.6-90.5)	42.53	(6.9-71.2)	<0.001
Number of Leaves	21	(4-53)	30	(6-95)	<0.001
No of branches	6	(0-14)	7	(0-14)	<0.001
Leaf area (cm^2)	200.8	(29.28-596.2)	256.1	(21.2-624.8)	<0.001
Stem girth(cm)	0.61	(0.1-1.1)	0.74	(0.1-1.9)	<0.001

Table 14: Average performance of parental genotypes and their hybrids for yield related traits at 4WAT

	PH	NOB	SG	NLP	LA
Parents					
A1	15.55±4.66^{a}	6±4^{cdefg}	0.65±0.26^{b}	14±7^{a}	163.51±51.70^{ab}
N11	19.61±7.88^{a}	6±4^{bcde}	0.67±0.30^{bc}	21±11^{bc}	234.90±134.08^{cde}
E12	16.46±5.42^{a}	5±2^{bc}	0.66±0.15^{bc}	18±8^{ab}	204.60±81.29^{bcd}
N4	30.13±5.40^{def}	9±3^{jkl}	0.53±0.17^{a}	35±11^{6ef}	139.09±55.46^{a}
G4	25.38±5.90^{bc}	7±1^{defgh}	0.78±0.19^{cd}	26±8^{cd}	285.02±73.16^{efg}
In1	18.70±3.97^{a}	2±1^{a}	0.42±0.09^{a}	12±4^{a}	191.56±57.74^{bc}
F_1 hybrids					
A1xN11	24.51±11.69^{bc}	5±2^{bcd}	0.63±0.25^{b}	24±17^{bc}	195.59±93.48^{bc}
A1xN4	36.74±14.36^{h}	8±2^{jkl}	0.69±0.21^{bcd}	32±16^{def}	240.10±85.46^{cde}
N11xA1	23.81±8.75^{b}	8±2^{ghij}	0.71±0.32^{bcd}	32±13^{def}	309.24±120.74^{gh}
N11xE12	33.58±11.29^{fgh}	8±2^{ijk}	0.95±0.18^{e}	35±18^{ef}	326.10±110.73^{gh}
N11xN4	24.36±7.05^{bc}	7±2^{ghi}	0.69±0.15^{bcd}	32±12^{def}	218.59±80.92^{cd}
N11xG4	28.39±6.14^{cde}	7±1^{efgh}	0.80±0.22^{d}	28±10^{cde}	297.87±74.38^{fgh}
N11xIn1	18.88±5.66^{a}	5±3^{b}	0.52±0.21^{a}	18±12^{ab}	160.39±95.77^{ab}
N4xA1	49.38±12.53^{i}	10±2^{l}	0.74±0.19^{bcd}	48±14^{g}	250.76±76.94^{def}
N4xN11	24.05±8.22b^{c}	6 ±2^{defg}	0.71±0.18^{bcd}	27±11^{cd}	232.74±74.00^{cde}
N4xG4	32.28±6.29^{efg}	8±1^{hij}	0.76±0.16^{bcd}	35±12^{ef}	282.46±66.48^{efg}
N4xIn1	19.24±5.48^{a}	7±1^{fghi}	0.78±0.18^{cd}	23±10^{bc}	190.92±73.48^{bc}
G4xA1	27.94±8.82^{bcd}	6±1^{cdef}	0.73±0.23^{bcd}	24±13^{bc}	346.35±151.64^{h}
G4xN11	34.86±7.88^{gh}	9±2^{kl}	0.81±0.19^{d}	39±22^{f}	273.67±89.83^{efg}

G4xE12	23.97±5.45^{bc}	6±1^{bcdef}	0.78±0.13^{cd}	23±6^{bc}	234.64±61.63^{cde}
G4xIn1	25.56±8.54^{bc}	7±2^{efghi}	0.72±0.29^{bcd}	29±15^{cde}	277.43±134.48^{efg}
P-value	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
l.s.d	**3.824**	**1.2065**	**0.10691**	**6.437**	**45.76**

a, b, c, ab, abc, bc, cd, cde, cdef, *d*, def, defg, *e*, ef, efg, efgh, f, fgh, fghi, g, gh, ghi, ghij, h, ijk, I, jkl *in table 14 above show the statistical significance of differences between means of parents and F_1 hybrids for vegetative traits at 4WAT. Means of genotypes with same letters are not significantly different for the vegetative traits.*

PH-Plant height, NOB-Number of branches, NLP- Number of leaves per plant, SG- Stem Girth, L A- Leaf area

Table 15: Overall Performance of quantitative traits at Six weeks after transplanting for parents Vs F_1 hybrids.

Variables (6WAT)	Parents		F1 hybrids		
	Mean	**Range of variation**	**Mean**	**Range of variation**	**Prob.t**
Plant Height (cm)	34.34	(13-58.6)	48.45	(8.2-425)	<.001
Plant width (cm)	46.62	(13.4-70.1)	51.44	(5-90.3)	<.001
Number of Leaves	34	(8-94)	53	(4-173)	<.001
No of branches	10	(2-18)	11	(3-32)	<.001
Leaf area (cm^2)	278.6	(28.38-629.8)	308.2	(54.4-873.1)	0.004
Stem girth(cm)	0.716	(0.2-1.2)	0.852	(0.2-1.8)	<.001

Table 16: Average performance of parental genotypes and their hybrids for yield related traits at Six weeks after transplanting

	PH	NOB	SG	NLP	LA
Parents					
A1	29.27±7.16^{ab}	10±3^{cde}	0.68±0.2^{abc}	26±16^{ab}	267.78±126.98^{abcde}
N11	36.63±9.25^{abcd}	9±2^{bcd}	0.77±0.25^{bcdef}	34±12^{abcd}	287.04±125.55^{bcdef}
E12	24.73±5.51^{a}	10±2^{cde}	0.78±0.18^{bcdefg}	34±12^{abcd}	280.32±79^{bcdef}
N4	41.78±8.67^{abcdef}	12±2^{efgh}	0.62±0.13^{ab}	47±22^{cdefg}	179.53±54^{a}
G4	40.2±8.51^{abcdef}	9±2^{abcd}	0.96±0.12^{ghi}	42±11^{bcdef}	345.23±91.23^{defg}
In1	34.41±8.21^{abc}	7±2^{a}	0.52±0.11^{a}	23±7^{a}	322.72±102^{cdefg}
Hybrids					
A1xN11	38.76±12.99^{abcde}	11±3^{defgh}	0.75±0.25^{bcde}	41±28^{abcdef}	278.21±120.55^{bcde}
A1xN4	55.35±16.1^{efg}	11±3^{cdefg}	0.82±0.2^{cdefgh}	59±32^{fgh}	242.38±65.79^{abc}
N11xA1	48.74±11.63^{cdef}	11±3^{cdefg}	0.98±0.24^{hi}	50±20^{cdefgh}	413.41±165.84^{g}
N11xE12	52.33±17.75^{def}	12±5^{efgh}	1.04±0.15^{i}	42±18^{abcdef}	404.37±93.98^{g}
N11xN4	39.66±6.89^{abcdef}	10±3^{cdef}	0.79±0.19^{bcdefgh}	53±20^{defgh}	251.43±91.36^{abcd}
N11xG4	48.55±12.11^{cdef}	10±2^{cde}	0.9±0.23^{defghi}	45±24^{bcdef}	364.49±78.38^{efg}
N11xIn1	31.08±6.95^{abc}	11±3^{cdefg}	0.63±0.23^{ab}	37±26^{abcde}	224.54±126^{ab}
N4xA1	70.71±15.98^{gh}	14±3^{h}	0.83±0.16^{cdefgh}	67±26^{gh}	265.12±87.35^{abcd}
N4xN11	44.28±12.59^{bcdef}	13±2^{fgh}	0.85±0.26^{cdefgh}	51±15^{cdefgh}	286.93±65.25^{bcdef}
N4xG4	79.38±33.2^{h}	13±7^{gh}	0.94±0.18^{fghi}	101±35^{i}	322.06±60.35^{cdefg}
N4xIn1	42.31±13.1^{abcdef}	10±2^{cde}	0.92±0.14^{defghi}	56±22^{efgh}	283.91±79.34^{bcdef}
G4xA1	37.23±7.89^{abcd}	9±2^{abc}	0.74±0.27^{bcd}	33±21^{abc}	377.02±160.02^{fg}
G4xN11	57.15±15.94^{fg}	11±2^{cdefg}	0.86±0.25^{cdefghi}	68±19^{h}	288.79±66.81^{bcdef}
G4xE12	44.59±8.48^{bcdef}	7±2^{ab}	0.93±0.1^{efghi}	48±11^{cdefgh}	295.11±59.68^{bcdef}
G4xIn1	45.36±9.56^{bcdef}	10±2^{cdef}	0.86±0.27^{cdefghi}	58±39^{efgh}	327.49±121.41^{cdefg}
P-value	<.001	<.001	<.001	<.001	<.001
l.s.d	9.473	1.358	0.102	10.556	51.62

a, b, c, ab, abc, abcd, abcdef, bc, bcdef, bcdefgh, cd, cde, cdef, cdefg, cdefghi, d, def, defg, defgh, e, ef, efg, efgh, f, fg, fgh, fghi, g, gh, ghi, ghij, h, hi, ijk, I, jkl in table 14 above show the statistical significance of differences between means of parents and F_1 hybrids for vegetative traits at 6WAT. Means of genotypes with same letters are not significantly different for the vegetative traits.

PH-Plant height, NOB-Number of branches, NLP- Number of leaves per plant, SG- Stem Girth, L A- Leaf area

4.2.1.3 Performance of the parental genotypes and their hybrids at Fruit Stage

At fruit stage, there was significant difference (P<0.001) between the performance of number of Fruits per inflorescence, number of Fruits per Plant, Fruit length, Fruit breadth, Fruit weight, Number of seeds per fruit (Table 17). There was no significant difference between in quantitative traits between seasons at P (0.369). There was a significant difference (P<.001) between genotypes (Parents and offspring's) in all traits (plant height, stem girth, number of leaves per plant, number of branches and leaf area) (Table 17).

There was a significant difference (P<.001) between genotypes (Parents and offspring's) in all traits (plant height, stem girth, number of leaves per plant, number of branches and leaf area).

For the parental lines, N11 had the highest number of fruits per inflorescence (11) and G4 had the lowest number of branches (3). Among hybrids, N11xA1 and N11xE12 had the highest number of fruits per inflorescence (13 and 11 respectively). G4xA1, G4xIn1 and N4xA1, had the lowest of number fruits per inflorescence with 5, 5 and 6 respectively.

Number of Fruits per Plant was highest for N11 (153) and lowest for E12 (22). Among hybrids, N11xA1 and N4xG4 had the highest number fruits per plant with 253 and 272 respectively and G4xA1 had the lowest number of fruits per plant with 56.

Among the parental lines, fruit length ranged from 9.87 to 50.79cm with G4 having the longest fruit (50.79cm) and A1 had shortest fruit (9.87cm). Among the hybrids, G4xA1, G4xIn1 and N11XG4 had the longest fruit with length of 30.71, 24.72, and 24.17cm respectively. N4xIn1, N4xN11 and N11xN4 had the shortest fruits with length of 12.07, 12.1 and 12.22cm respectively.

Among the parental lines, fruit breadth ranged from 11.11 to 43.85cm with E12 having the widest fruit (43.85cm) and A1 had smallest fruit (11.11cm). Among the hybrids, G4xE12, N11XG4 and

G4xIn1 had the widest fruit with width of 25.32, 25.29, and 23.41cm respectively. N4xA1, G4xN11 and A1xN4 had the smallest fruits with width of 15.78, 16.36and 16.39cm respectively.

For the parental lines, E12 had the highest fruit weight (39.26g) and A1 had the lowest number fruit weight (0.91g). Among hybrids, N11xG4 and G4xE12 had the highest fruit weight (8.66 and 8.18g respectively) and N4xIn1 and N4xA1 had the lowest of number fruits per inflorescence with 2.07 and 2.13g respectively.

Number of seeds per fruit was highest for E12 (937) and lowest for A1 (56). Among hybrids, N11x1n1, N4xIn1 and N4xN11 had the highest number seeds per plant with 85, 85 and 83 seeds respectively and G4xE12 and N11xE12 had the lowest number of seeds per fruit with 7 and 10 seeds respectively.

Weight of 100 seeds was highest for E12 (0.55) and lowest for A1 (0.19). For the F_1 hybrids, G4x1n1, G4xE12 and G4xN11 had the highest weight for 100 seeds with 0.45, 0.42 and 0.42g respectively and N4xA1 and N11xIn1 had the lowest weight for 100 seeds 0.22 and 0.23g respectively.

Table 17: Average performance of parental genotypes and their hybrids for yield related traits at fruit stage

	NFTI	NFPP	FL	FTB	FW	SPF	Weight of 100 seeds
Parents							
A1	10±3^{efgh}	131±57^{cde}	9.87±2.88^{a}	11.11±1.62^{a}	0.91±0.4^{a}	56±21^{bc}	0.19±0.04^{a}
N11	11±3^{fgh}	153±53^{efg}	16.09±2.46^{f}	22.37±2.78^{ef}	5.17±1.43^{e}	152±69^{h}	0.23±0.02^{abc}
E12	5±3^{abc}	22±9^{a}	30.71±9.05^{j}	43.85±2.78^{j}	39.26±25.56^{i}	937±303^{j}	0.55±0.34^{g}
N4	6±2^{abcd}	128±55^{cde}	12.69±5.49^{bc}	15.92±1.65^{b}	2.02±0.47^{b}	104±28^{g}	0.22±0.04^{abc}
G4	3±1^{a}	26±8^{a}	50.79±10.4^{k}	38.91±8.43^{i}	38.98±16.23^{i}	207±86^{i}	0.25±0.06^{bcd}
In1	9±3d^{efg}	42±20^{ab}	14.55±8.66^{e}	23.36±4.32^{fgh}	12.67±5.22^{h}	71±32^{cdef}	0.5±0.04^{f}
F1 hybrids							
A1xN11	8±2^{bcdefg}	124±49^{cd}	19.05±6.96^{g}	19±3.2^{cd}	3.96±1.92^{d}	55±28^{bc}	0.31±0.06^{e}
A1xN4	7±4^{bcde}	181±78.8^{hi}	13.67±2.45^{cde}	16.39±9.52^{b}	2.34±0.73^{bc}	62±26^{f}	0.24±0.04^{bc}
N11xA1	13±3^{hi}	253±148^{j}	12.79±1.97^{bcd}	16.97±2.01^{bc}	2.49±0.87^{bc}	41±27^{b}	0.24±0.05^{bc}
N11xE12	11±3^{gh}	144±70^{def}	13.95±1.74^{de}	20.27±2.7^{d}	3.22±0.85^{cd}	10±4^{a}	0.32±0.07^{e}
N11xN4	8±2^{bcdefg}	130±56^{cde}	12.22±1.33^{b}	17.28±1.81^{bc}	2.37±0.59^{bc}	80±24^{f}	0.26±0.05^{bcd}
N11xG4	9±3^{defg}	154±83^{efg}	24.17±3.06^{i}	25.29±14.97^{gh}	8.66±2.05^{g}	80±29^{f}	0.29±0.03^{de}
N11xIn1	9±2^{defg}	176±116^{ghi}	12.87±2.16^{bcd}	16.57±2.59^{b}	2.56±0.94^{bc}	85±34^{f}	0.23±0.04^{abc}
N4xA1	6±2^{abcd}	131±78^{cde}	13.26±1.88^{bcd}	15.78±7.14^{b}	2.13±0.65^{b}	72±25^{def}	0.22±0.04^{ab}
N4xN11	10±4^{efgh}	160±122^{fgh}	12.1±1.18^{b}	16.42±3.08^{b}	2.41±0.4^{bc}	83±22^{f}	0.26±0.03^{bcd}
N4xG4	16±6^{i}	272±138^{j}	19.68±2.35^{gh}	19.39±2.06^{d}	3.88±1.32^{d}	62±22^{cde}	0.34±0.03^{e}
N4xIn1	7±2^{bcdef}	195±66^{i}	12.07±1.39^{b}	17.11±1.44^{bc}	2.07±0.84^{b}	85±19^{f}	0.25±0.02^{bcd}
G4xA1	5±2^{ab}	56±27^{b}	30.71±7.28^{j}	20.62±3.04^{de}	6.54±2.46^{f}	40±21^{b}	0.26±0.04^{cd}
G4xN11	6±2^{abcd}	121±66^{cd}	12.75±1.94^{bcd}	16.36±1.47^{b}	2.28±0.54^{bc}	75±22^{ef}	0.42±0.05^{f}
G4xE12	9±2^{cdefg}	151±43^{efg}	20.38±2.09^{h}	25.32±2.72^{h}	8.18±2.13^{g}	7±4^{a}	0.42±0.11^{f}
G4xIn1	5±2^{ab}	112±54^{c}	24.72±6.56^{i}	23.41±2.58^{fg}	5.85±2.3^{ef}	57±24^{cd}	0.45±0.41^{f}
p-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001

a, b, c, ab, abc, abcd, abcdef, *bc,* bcdef, bcdefgh, *cd,* cde, cdef, cdefg, cdefghi, *d,* def, defg, defgh, *e,* ef, efg, efgh, f, fg, fgh, fghi, g, gh, ghi, ghij, h, hi, j, i *in table 14 above show the statistical significance of differences between means of parents and F_1 hybrids for vegetative traits at 6WAT.*

NFTI- Number of Fruits per inflorescence, NFPP- Number of Fruits per Plant, FL= Fruit length, FTB- Fruit breadth, FW- Fruit weight, SPF- Number of seeds per fruit

4.3.2 Combining ability analysis for yield and yield related traits in the F_1 hybrids of *S. aethiopicum* and its relatives.

4.3.2.1 Determining heritability for vegetative traits

Broad sense heritability (H^2) value for each of the variables (plant height, number of branches, plant width, leaves per plant, leaf area and stem girth) was greater than 80% (as shown in Table 18). At four weeks after transplanting, plant height and leaves per plant showed highest broad sense heritability at 96 percent, while stem girth had the lowest proportion at 83%. number of branches exhibited the highest narrow sense heritability (52%) while plant height and stem girth had zero narrow sense inheritance. Number of branches had the highest broad sense heritability at 6WAT, with a 92 percent, and plant breadth had the lowest percentage (80%), whereas number of branches had the highest narrow sense heredity at 55%, and stem girth had the lowest percentage (0%).

4.3.2.2 Determining heritability for fruit traits

For each of the variables (Number of fruits per plant, fruit length, fruit breadth, fruit weight, seed number per fruit and weight of 100 seeds) had broad sense heritability (H^2) value above 80 percent (Table 19). Fruit weight, fruit breadth and weight of 100 seeds showed highest broad sense heritability value (100%) followed by fruit length (99%), while the number of fruits per inflorescence had the lowest percentage at 35%. Number of seeds per fruit exhibited the highest narrow sense heritability (78%), while number of fruits per inflorescence lowest narrow sense heritability (20%).

4.3.2.3 Identifying appropriate traits for selection of parents and hybrids with good combining ability

For vegetative traits, analysis of variance for combining ability revealed that SCA effects were significant ($p<0.01$) and had the largest mean squares for plant height, plant width, leaves per plant, leaf area and stem girth compared to GCA effects for same traits at both 4WAT and 6WAT (Table 20 and Table 21). SCA were highly significant ($p<0.01$) for plant height, number of leaves per plant and leaf area at 4WAT. On the other hand, whereas SCA effects for number of branches per plant were significant, they were lower than GCA effects at 4WAT and 6WAT. For all leaf yield traits, the general combining abilities were not significant.

For fruit traits, Analysis of variance for combining ability revealed that SCA effects were significant for number of fruits per plant, fruit length, fruit breadth, fruit weight, seed number per fruit and weight of 100 seeds besides for number of fruits per inflorescence (Table 22). For all leaf yield traits, the general combining abilities were not significant. Specific combining ability were highly significant ($p<0.01$) for fruit length while specific combining ability were non-significant for number of fruits per in florescence. Whereas SCA effects for number of fruits per plant, fruit breadth, fruit weight, seed number per fruit and weight of 100 seeds were significant, they had lower mean squares than GCA female effects.

Table 18: Broad and narrow sense heritability estimates for leaf yield traits at 4WAT and 6WAT

Traits	Additive Variance		Dominance Variance		Environmental Variance		Phenotypic variance		H^2		h^2	
	4WAT	**6WAT**	**4WAT**	**6WAT**	**4WAT**	**6WAT**	**4WAT**	**6WAT**	**4WAT**	**6WAT**	**4WAT**	**6WAT**
PH	0	83.16	242.72	556.6	10.4	69.8	253.1	709.6	0.96	0.90	0.00	0.12
NOB	9.28	9.4	7.56	6.32	1.1	1.4	17.9	17.2	0.94	0.92	0.52	0.55
PW	4.72	42.16	122.08	63	19.8	23.4	146.6	128.5	0.87	0.82	0.03	0.33
LPP	38.6	315.2	249	878.8	30.7	86.7	318.3	1280.7	0.90	0.93	0.12	0.25
LA	4156.8	5302.8	8249.6	7652.4	1652.3	2071.7	14058.7	15026.9	0.88	0.86	0.30	0.35
SG	0	0	0.04	0.04	0.008	0.008	0.0	0.0	0.83	0.83	0.00	0.00

4WAT- Four Weeks after Transplanting, 6WAT- Four Weeks after Transplanting, H^2- Broad sense heritability, h^2- Narrow sense heritability

Table 19: Broad and narrow sense heritability estimates for fruit yield traits

Traits	Additive Variance	Dominance Variance	Environmental Variance	Phenotypic variance	H^2	h^2
Number of fruits per inflorescence	9.8	7.628	32.0	49.4	0.35	0.20
Number of fruits per plant	10016	8964	1513.8	20493.8	0.93	0.49
Fruit length	225.52	179.64	3.3	408.5	0.99	0.55
Fruit breadth	356.2	70.72	1.6	428.5	1.00	0.63
Fruit weight	557.8	218.8	2.2	778.8	1.00	0.72
Number of seed per fruit	503751.2	8392.8	576.3	512720.3	1.00	0.78
Weight of 100 seeds	0.04	0.04	0.004	0.1	0.95	0.48

Table 20: Analysis of variance for general and specific combining ability for leaf traits of *S. aethiopicum* and its relatives at 4WAT

Source of variation	Plant height	Number of plant branches per plant	Plant width	Number of leaves per plant	leaf Area	Stem girth
GCA*Females*	0	2.22	0	9.47	621.8	0
GCA*Males*	0	0.1	1.18	0.18	417.4	0
SCA	60.68**	1.89**	30.52*	62.25*	2062.4**	0.01*
Residual	52.04***	5.48***	98.86***	153.4***	8261.6***	0.04***

**, ** and ***significance at 0.05, 0.01 and 0.001, respectively*

Table 21: Analysis of variance for general and specific combining ability for leaf traits of *S. aethiopicum* and its relatives at 6WAT

Source of variation	Plant height	Number of plant branches per plant	Plant width	Number of leaves per plant	leaf Area	Stem girth
GCA*Females*	20.79	2.35	0	78.8	293.2	0
GCA*Males*	0	0	10.54	0	1032.5	0
SCA	139.15*	1.58**	15.75*	219.7*	1913.1**	0.01**
Residual	349.03***	7.18***	116.82***	433.5***	10358.7***	0.04***

*, ** and ***significance at 0.05, 0.01 and 0.001, respectively

Table 22: Analysis of variance for general and specific combining ability for fruit traits of *S. aethiopicum* and its relatives

Source of variation	Number of fruits per inflorescence	Number of fruits per plant	Fruit length	Fruit breadth	Fruit weight	Number of seed per fruit	Weight of 100 seeds
GCA *Females*	2.45	2504	32.4	78.99	128.43	125624.5	0.01
GCA *Males*	0	0	23.98	10.06	11.02	313.3	0
SCA	1.907	2241**	44.91**	17.68*	54.7*	2098.2**	0.01*
Residual	159.93***	7569***	16.50***	8.01***	11***	2881.3***	0.02***

**, ** and ***significance at 0.05, 0.01 and 0.001, respectively*

4.3.3.4 Identifying parents with good GCA and hybrids with good SCA in crosses between S aethiopicum and close relatives

4.3.3.4.1 Identifying parents with good GCA for vegetative traits (at Four weeks after transplanting and Six weeks after transplanting)

For general combining ability effects (as shown in table 23), regarding the leaf yield traits, the GCA effects were both positive and negative for female and male parents (table 23). Among the females, N4 exhibited the highest positive GCA effects at 4WAT and 6WAT with 6.94 and 13.85 respectively for plant height, while In1 had the lowest negative GCA effects at 4WAT and 6WAT (-5.40 and -5.99 respectively) (Table 24). N4 exhibited the highest GCA effect for females at both 4WAT and 6WAT and E12 showed the lowest negative GCA effects. For, plant width, N4 showed largest positive GCA effects at 4WAT and 6WAT (4.04 and 4.27) whereas E12 exhibited the lowest negative GCA effect at 4WAT (-2.51) and A1 had the lowest negative GCA effect at 6WAT (-0.29). In terms of leaf area, G4 displayed the highest GCA effects at both the 4WAT and the 6WAT (59.18 and 30.56, respectively), while N4 displayed the lowest negative GCA effects at both the 4WAT and the 6WAT (-10.65 and 33.58 respectively). N4 showed the greatest GCA effects for the number of leaves per plant at both 4WAT and 6WAT (9.69 and 19.52, respectively), whereas A1 showed the least negative GCA at both the 4WAT and the 6WAT (-0.59 and -0.14 respectively). In terms of stem girth, N11 displayed the highest GCA effect at 4WAT and 6WAT (0.07 and 0.09, respectively), while In1 displayed a negative GCA effect at 4WAT (-0.24) and A1 had the lowest negative GCA effects at 6WAT (-0.01).

Among the males, N4 showed the largest positive general combining ability effect for males at 4WAT (4.03) and G4 showed that G4 had the highest positive GCA effect at 6WAT (9.23) for plant height, whereas N11 exhibited the lowest negative GCA at 4WAT (-0.72) and 6WAT (-0.55). N4 exhibited the highest positive GCA effect at 4WAT and 6WAT with 6.94 and 13.85 respectively for number of branches, while N11 displayed a negative GCA effect at 4WAT (-0.09)

and In1 had the lowest negative GCA effect at 6WAT (-0.11). For, plant width, G4 showed largest positive GCA for male at 4WAT and 6WAT (4.91 and 6.79 respectively), whereas N11 exhibited the lowest negative GCA effects at 4WAT and 6WAT (-0.99 and -1.61 respectively). For, leaf area, G4 showed largest positive GCA effect for male at 4WAT and 6WAT (47.9 and 46.12 respectively), whereas N11 exhibited the lowest negative GCA effect at 4WAT and 6WAT (-7.36 and -15.14 respectively). N4 showed the greatest GCA effect for the number of leaves per plant at both the 4WAT and the 6WAT (5.49 and 5.13 respectively), whereas E12 showed the least negative GCA effects at both the 4WAT (-2.18) and In1 showed the least negative GCA effect at both the 6WAT (-3.85). In terms of stem girth, E12 displayed the greatest GCA effect at 4WAT (0.09) and G4 had the highest GCA effects at 6WAT (0.11), whereas N4 displayed the lowest GCA effect at 4WAT (-0.07) and A1 displayed the highest GCA at 6WAT (-0.01) (Table 23).

4.3.3.4.2 Identifying parents with good GCA for fruit traits

General combining ability effects for female parents for fruit yield traits were positive and negative (Table 24). Among the females, N4 showed the largest positive GCA effect for number of fruits per inflorescence (69.03) whereas E12 exhibited the lowest negative GCA (-86.07) (Table 24). N11 displayed the highest positive general combining ability effect (2.39) for the number of fruits per plant, whereas E12 displayed the lowest negative GCA (-2.94). For, fruit length, E12 showed largest positive GCA (12.03), while A1 exhibited the lowest negative GCA (-4.68) as a female for same trait. E12 showed the greatest positive GCA for fruit breadth (20.83), whereas E12 showed the least negative GCA effect (-3.77). For fruit weight, E12 showed largest positive GCA (27.68) as a female and A1 showed the lowest negative GCA (-7.18). For number of seeds per fruit, E12 showed largest positive general combining ability effect (722.37) and G4 showed lowest negative

GCA (-154.44). E12 displayed the highest positive GCA (0.19) for the weight of 100 seeds per fruit, whereas A1 and N4 displayed the lowest negative GCA (-0.11) for the same attribute.

General combining ability effects for male parents for fruit yield traits were positive and negative (Table 25). Among the male, G4 showed the largest positive GCA effect for number of fruits per inflorescence (14.4) whereas E12 exhibited the lowest negative GCA (-29.56). G4 displayed the highest positive general combining ability effect (0.9) for the number of fruits per plant, whereas N4 displayed the lowest negative GCA (-1.39). For, fruit length, G4 showed largest positive GCA (9.93), while A1 exhibited the lowest negative GCA (-4.75) as a female for same trait. G4 showed the greatest positive GCA for fruit breadth (5.43), whereas A1 showed the least negative GCA effect (-8.32). For fruit weight, G4 showed largest positive GCA (-7.12) as a female and A1 showed the lowest negative GCA (-2.47). G4 displayed the highest positive general combining ability effect (21.36) for the number of seeds per fruit, and E12 displayed the lowest negative GCA (-25). For the weight of 100 seeds per fruit, E12 showed largest positive GCA (0.1) as a female and A1 showed the lowest negative (-0.07) for the same trait (as shown in Table 24).

4.3.3.4.3 Identifying hybrids with good Specific Combining Ability for vegetative traits (at Four weeks after transplanting and Six weeks after transplanting)

Table 25 shows specific combining ability for the leaf yields at 4WAT and 6WAT. The crosses showed negative SCA and positive SCA for plant height, number of branches per plant, plant width, leaf area, stem girth and number of leaves per plant. Cross N4xA1 had the highest positive (best) specific combining ability for plant height at 4WAT and 6WAT (10.63 and 10.76 respectively), while N4xA1 had the lowest specific combining ability at 4WAT (-10.74) and N4xA1 had the lowest specific combining ability at 6WAT (-13.53). For number of branches per plant, G4xN11 had the best SCA effect followed by G4xIn1 at 4WAT and 6WAT, whereas G4xA1 had the worst SCA effect at 4WAT and G4xE12 had the worst SCA effect at 6WAT. N11XE12

had the best specific combining effect for plant width at 4WAT and N4XIn1 had the best specific combining effect at 6WAT, whereas N11XIn1 exhibited worst SCA for plant width at 4WAT AND 6WAT. A1XN4 and N11XA1 had the best specific combining effects for leaf area at 4WAT (52.52) and 6WAT (56.14), respectively, while G4XE12 and N11XIn1 had the worst specific combining abilities for leaf area at 4WAT (-95.04) AND 6WAT (-90.7), respectively. With regard to the number of leaves per plant, N4XA1 and N4XG4 had the best specific combining effects at 4WAT (6.04) and 6WAT (23.06), respectively, whereas G4XE12 and G4XA1 had the worst specific combining abilities at 4WAT (-9.83) AND 6WAT (-18.11), respectively. For stem girth at 4WAT and 6WAT, N4XIn1 exhibited the best specific combining effects, whereas N11XIn1 had the worst specific combining abilities (Table 25).

4.3.3.4.4 Identifying hybrids with good Specific Combining Ability for fruit traits

Cross G4xE12 had the best (positive) specific combining ability effect for number of fruits per inflorescence followed by N4xG4 while N4xA1 had the lowest negative (worst) effect (Table 26). The best (positive) specific combining ability effect for number of fruits per plant was exhibited in N4xG4, followed by G4xE12. No cross showed a negative specific combining ability effect for number of fruits per plant. For fruit length, A1xN11 had the best (positive) specific combining ability effect A1xN4 while G4xN11 exhibited the worst (negative) effect. In terms of fruit breadth, A1xN11 had the best specific combining ability effect and G4xN11 was the only cross which displayed a negative SCA effect. For fruit weight, A1xN11 expressed the best (positive) SCA effect followed by N4xA1, whereas N4xN11 was the only cross which showed poor (negative) SCA effect. For both the number of seeds per fruit and weight of 100 seeds per fruit, all crosses showed good specific combining ability effects. However, N4xA1 exhibited the best SCA effect for number of

seeds per fruit followed by and N4xG4 showed the best SCA effect for weight of 100 seeds per fruit (Table 26).

Table 23: Estimates of general combining ability effects for parental genotypes for Yield related traits (4WAT and 6WAT)

Genotype	Role played	Plant Height		Number of Branches		Plant width		Leaf area		Stem Girth		Leaves per Plant	
		4WAT	6WAT	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT
A1	Female	1.66	0.72	0.92	0.91	-3.02	-0.24	-26.11	-33.96	0.01	-0.01	-0.59	-0.14
N11	Female	0.74	2.43	0.85	0.62	3.65	0.56	33.10	27.46	0.07	0.09	4.14	1.12
E12	Female	-7.64	-15.67	-0.94	-0.03	-2.51	-2.9	-21.24	-16.44	0.00	0.02	-5.80	-8.4
N4	Female	6.94	13.85	2.05	2.46	1.28	0.98	-10.65	-33.58	0.04	0.06	9.69	19.52
G4	Female	3.70	4.67	1.19	-0.64	4.04	4.27	59.18	30.56	0.11	0.1	4.65	7.57
In1	Female	-5.40	-5.99	-4.08	-3.33	-3.44	-2.66	-34.28	25.96	-0.24	-0.24	-12.10	-19.67
A1	Male	2.57	1.73	0.62	0.4	2.37	1.26	25.88	30.2	0.00	-0.01	1.94	-3.85
N11	Male	-0.72	-0.55	-0.09	0.57	-0.99	-1.61	-7.36	-15.4	0.00	-0.01	0.31	0.40
E12	Male	-1.78	-4.39	-0.43	-0.88	0.82	0.53	14.49	27.12	0.09	0.1	-2.18	-7.16
N4	Male	4.03	0.85	1.35	0.86	-2.81	-5.3	-42.32	-76.19	-0.07	-0.08	5.49	5.13
G4	Male	1.94	9.23	0.32	0.15	4.91	6.79	47.90	46.12	0.08	0.11	1.85	11
In1	Male	-6.04	-6.88	-1.77	-1.1	-4.29	-1.66	-38.60	-11.84	-0.11	-0.1	-7.41	-5.53

4WAT- Four weeks after transplanting, 6WAT- Six weeks after transplanting

Table 24: Estimates of general combining ability effects for parental genotypes for Yield related traits at fruit

Genotype	Role played	Number of Fruits per inflorescence	Number of Fruits per Plant	Fruit length	Fruit breadth	Fruit weight	Number of seeds per fruit	100 seeds weight (gm)
A1	Female	37.49	0.54	-4.68	-8.32	-9.18	-150.83	-0.11
N11	Female	60.65	2.39	-3.53	-4.07	-7.5	-140.57	-0.1
E12	Female	-86.07	-2.94	12.03	20.83	27.68	722.37	0.19
N4	Female	69.03	1.14	-5.32	-6.97	-9.18	-132.11	-0.11
G4	Female	-14.73	-2.38	5.82	-1.16	-2.93	-154.44	0
In1	Female	-66.37	1.24	-4.32	-0.30	1.1	-144.42	0.14
A1	Male	6.40	0.26	-0.94	-3.77	-2.47	-23.64	-0.07
N11	Male	3.39	0.59	-2.61	-1.25	-2.03	14.86	0.01
E12	Male	-29.56	0.25	0.06	3.96	1.74	-25	0.1
N4	Male	10.39	-1.39	-4.75	-3.43	-3.25	12.37	-0.06
G4	Male	14.40	0.90	9.93	5.43	7.12	21.36	-0.01
In1	Male	-5.02	-0.61	-1.7	-0.93	-1.11	0.05	0.03

Table 25: Specific combining ability for yield related traits in *Solanum aethiopicum* for vegetative stage (Four and six weeks after transplanting)

F_1 hybrid	Plant height		Number of branches per plant		Plant width		Leaf area		Stem girth		Number of leaves per plant	
	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT	4WAT	6WAT
A1xN11	-5.01	-5.77	-2.62	-0.68	0.87	2.99	-26.96	27.95	-0.11	-0.04	-5.50	-6.62
A1xN4	2.77	9.43	-0.73	-1.06	1.97	4.14	52.52	52.92	0.02	0.1	-2.97	6.62
N11xA1	-8.08	0.22	-1.12	-0.48	-2.67	4.82	-5.76	56.14	-0.09	0.09	-3.95	5.63
N11xE12	6.04	9.93	0.66	1.53	2.68	3.71	22.50	50.17	0.05	0.05	2.97	0.44
N11xN4	-8.99	-7.97	-2.29	-1.51	-3.03	-0.19	-28.21	0.55	-0.05	-0.03	-7.43	-0.51
N11xG4	-2.87	-7.47	-1.48	-1.37	-1.50	-2.83	-39.14	-8.7	-0.08	-0.11	-7.89	-14.74
N11xIn1	-4.39	-8.82	-1.65	0.94	-9.76	-7.3	-90.13	-90.7	-0.20	-0.17	-8.27	-6.15
N4xA1	10.63	10.76	-0.15	0.45	1.99	-2.69	-20.49	-31.11	-0.04	-0.03	6.04	3.67
N4xN11	-10.74	-13.38	-2.81	-0.96	-3.00	-2.62	-5.27	36.29	-0.07	-0.01	-13.37	-16.61
N4xG4	-5.18	11.93	-1.78	0.33	-2.55	3.47	-10.80	9.91	-0.10	-0.04	-6.75	23.06
N4xIn1	-10.23	-9.02	-0.42	-2.01	-5.48	5.27	-15.85	29.72	0.11	0.15	-9.24	-5.31
G4xA1	-6.91	-13.53	-2.89	-1.56	-0.47	-2.09	5.28	16.65	-0.12	-0.16	-12.19	-18.11
G4xN11	3.30	8.67	1.02	0.77	-3.03	-1.11	-34.16	-25.98	-0.04	-0.04	4.07	12.57
G4xE12	-6.53	-0.05	-2.21	-2.21	-9.42	-3.21	-95.04	-63.19	-0.16	-0.08	-9.83	-0.12
G4xIn1	-0.67	3.22	0.44	1.59	2.58	0.63	0.84	12.16	-0.02	0.05	1.52	8.84

Table 26: Specific combining ability for yield related traits in *Solanum aethiopicum* for Fruit stage

F1 hybrids	Number of Fruits per inflorescence	Number of Fruits per Plant	Fruit length	Fruit breadth	Fruit weight	Number of seeds per fruit	100 seeds weight (gm)
A1xN11	-74.10	6.62	9.38	9.74	11.24	129.82	0.12
A1xN4	-24.10	7.84	6.13	8.80	10.83	159.11	0.11
N11xA1	29.00	10.33	0.30	5.98	8.54	144.16	0.11
N11xE12	-44.14	8.87	0.46	1.54	5.04	114.50	0.01
N11xN4	-98.79	6.69	3.53	5.94	9.19	146.86	0.12
N11xG4	-78.54	5.55	0.80	3.87	5.11	138.44	0.10
N11xIn1	-37.11	7.13	1.14	2.74	7.24	164.23	0.00
N4xA1	-102.15	4.28	2.59	7.32	9.85	167.23	0.09
N4xN11	-70.08	8.41	3.06	5.85	-1.52	139.45	0.06
N4xG4	30.95	13.50	-1.90	2.09	2.01	112.33	0.16
N4xIn1	-25.90	7.05	2.13	6.17	8.43	156.20	0.03
G4xA1	-93.09	6.87	8.87	6.72	8.01	157.50	0.04
G4xN11	-24.55	7.93	-7.43	-0.06	3.31	153.39	0.11
G4xE12	37.97	10.84	-2.46	3.69	5.43	125.10	0.02
G4xIn1	-25.60	7.63	3.64	5.12	5.96	151.10	0.13

CHAPTER FIVE

5.0 DISCUSSION

Traditional planting breeding methods are the most widely used approaches to genetic improvement of crops including Solanaceae to generate hybrids. In Uganda, commercial crops varieties have largely been developed using the conventional approaches, though with difficulty attributed to reproductive barrier. There has been limited research to promote understanding of the incompatibility barriers especially the interspecific reproductive barriers that hinder introgressive hybridization of *S. aethiopicum* and its relatives both in the wild and domestic interface. Therefore, this study examined the interspecific reproductive barriers and combining ability between *S. aethiopicum* and its relatives.

The first objective sought to identify pre-zygotic reproductive barriers in *S. aethiopicum* and its relatives. The major pre- zygotic barriers looked at included floral morphological features and phenology. Flowering plants have features which aid pollination (self- and cross- pollination). Results showed that the relative position of stigma and anthers has an important effect on success of female reproduction (Larrinaga *et al.*, 2009) in terms of pistil/style exertion. Only A1, which is *Solanum anguivi* presented high number exserted styles and intermediated styles present in S. *aethiopicum*. The study concurs with Buteme *et al*., (2021), who found out that *S. aethiopicum* had heterostyly (with stigmas at same level with anthers) with 77-96% of flowers. Studies indicate that long and medium- styled flowers fruit under normal field conditions and fruit set is high in long-styled flowers with 70-80% (Prasad and Prakash 1968; Siddique and Husain 1974; Kowalska 2003, 2006). With these results, there is a possibility to confirm that a high level of autogamy

exists in the *S. aethiopicum* genotypes and have low levels of allogamy. Only *S. macrocarpon* had short-styled flowers at 64%, where anthers are above the stigma. In such flowers, pollen does not reach the stigma easily and flowers commonly abort (Nothmann *et al*., 1983; Kowalska 2006). In this study, *S. macrocarpon* had the least fruit set in crosses which were parthenocarpic. Very low or no fruit set (0-16.7%) is observed in short-styled flowers as reported by (Kowalska, 2006). The variations in fruit set in species is because long- styles have stigma with well-developed nodules and well-permeable tissues which are rich in polysaccharides, proteins and other nutrients which support pollen tube growth(Kowalska, 2003, 2008).

S. aethiopicum showed the shortest time to first flowering of the studied species. Differences in flowering days between the species do not support cross-pollination between *S. aethiopicum* and *S. incanum* which takes long to flower. The quantitative trait loci (QTLs) are predominately responsible for variations in flowering times in *Solanum* Species (Jiménez-Gómez et al., 2007). Flowering time is also controlled by environmental factors like photoperiod and temperatures (Pierre *et al.,* 2008). With these results, it appears that interspecific hybridization is limited by staggered flowering times. Therefore, staggered planting in hybridization of *S. aethiopicum* and *S. incanum* could promote crossing.

Regarding the anthesis, the highest numbers of flowers were recorded at 08:00am in all genotypes with most of the flowers in anthesis found in *S. aethiopicum* and least in *S. macrocarpon*. The findings are in correspondence with Buteme *et al*. (2021) who analyzed crossability and compatibility within *S. aethiopicum,* and reported that highest number of flowers (11-28 flowers) in anthesis were recorded at 08:00am. Other similar study on the floral biology of *Capsicum*

chinense, reported a greater number of flowers in anthesis were recorded at 08:00am Peña-Yam *et al*. (2019a) while studies on cross compatibilities in *S. melongena* showed that full bloom occurred in the morning hours between 0645h and 0945h (Sękara & Bieniasz, 2012; Das *et al.*, 2017). This empirical evidence strongly suggests that more flowers at anthesis in *S. aethiopicum, S. anguivi, S. macrocarpon and S. incanum* occurs in the morning than evenings. This could mean that the appropriate time of the day the largest amount of pollen is collected from donors is at 8:00am. This knowledge is important in development of protocol for research in interspecific hybridization of *S. aethiopicum*.

The study demonstrated a variation in anther dehiscence in the different floral stages (bud, bloom and withering), with all genotypes registering anther dehiscence at bloom stage. With these results, it implies emasculation of flowers and hand pollination can be carried out at bud stage of a flower given that anthers are still closed (100%) in *S. aethiopicum* and its relatives. This could reduce risks of self-pollination during hybridization. In a similar study by Pena-Yam *et al*., (2019) which analyzed floral biology of *Capsicum chinense,* it observed no evidence of anther dehiscence at bud stage of the flower. Comparable results were reported in *Capsicum species* were dehiscence of anthers occurred one hour after flower opening (Crispim *et al*., 2017; Peña-Yam *et al*., 2019). The study however, did not establish the exact time when anthers opened to release pollen grains.

Generally moderate stigma receptivity was observed at bud stage, increased during at bloom stage and diminished at withering stages, with more receptivity recorded at 8:00am for all the studied species. *S. aethiopicum* showed the highest stigma receptivity among the species studied with receptive stigma even at withering stages. This implies that crossing in *S. aethiopicum* can be done

one day before anthesis (flower bud stage) in order to avoid contamination of the stigmas with pollen. Comparative research conducted on stigma receptivity in tomatoes revealed that stigmas were most receptive at bloom stage (Makwana and Akarsh, 2017). Aleemullah *et al*., (2000). Pena-Yam *et al*., (2019) determined in *C. annum* and *C. chinense* that pistils were receptive from one day before anthesis to one day after anthesis however increased receptivity was registered at anthesis. Previously, studies in ornamental chilli also showed that flowers are receptive from bud stage but more receptive at bloom stage (Crispim *et al*., 2017). (Karapanos *et al*., 2008) reported that stigmas of *C. annuum* maintain its receptivity for three days. The significant difference in stigma receptivity amongst the species and floral stages is due to the variation's stigmatic papillae elongation and increased amounts of stigmatic exudates that inundated papillae in the species (Ganders, 1979).

Pollen viability was generally greater than 80% for all species, with significant differences ($P<0.01$) among them. Maximum viable pollen was observed in *S. aethiopicum* (N11 and N4) in in-vitro pollen growth with 2% acetocarmine staining (87.4% and 93.34% respectively). This is in contrast to examined compatibility barriers in intraspecific crosses of *S. aethiopicum* by Buteme *et al*. 2021 where pollen viability varied from 56.4% to 66.54%. The variations in pollen viability be could be due to time of pollen collection and pollen density on the culturing media (Dafni and Firmage, 2000). Viability of pollen in *S. melongena* (Das *et al.*, 2017; Jayaprakash *et al*., 2018; Buteme *et al.*, 2021)at more than 50% is regarded as good viability. Consequently, failure of pollen tube growth in crosses made was not due to pollen viability but other forms incompatibility.

Therefore, for the first hypothesis stated that anthesis is the major contributor to incompatibility in *S. aethiopicum* and its relatives. The findings showed that anthesis, flowering time, anther dehiscence and stigma receptivity which were all significantly different among the species and flower stage are the major contributors to cross-incompatibility between *S. aethiopicum* and its relatives. Hence, the hypothesis is rejected. For instance, *S. aethiopicum* majorly has medium-styled species*; S. anguivi* has the highest proportion of exserted styles; and has a greater number of flowers with short styles. *S. aethiopicum* showed a high stigma receptivity while *S. incanum* had low receptivity. *S. aethiopicum* Shum had the shortest time to flowering whereas *S. incanum* had the longest time to flowering.

The second objective thought to elucidate crossing success between *Solanum aethiopicum* and its relatives. No fruits were produced in the crosses where *S. incanum* was the female parent. Further studies on the pollen tube growth for the four incompatible combinations in this study showed that they stopped in the middle and base of the style implying pollen rejection. All selfs used as controls showed that pollen tubes reached the ovules in 24 hours after pollination. The incompatibility observed between *S. aethiopicum* and *S. incanum* could be explained by female parent functioning which may be attributed to the presence of the S-locus which constitute a major limitation of pollen-tube growth (Eijlander *et al*., 2000; Bedinger *et al.*, 2011; Zhao *et al*., 2011). The findings are in contrary to Plazas et al., (2016) who had successful crosses between *S. melongena* (male parent) and *S. incanum* as a female parent. Although *S. melongena* is a closely related domesticated species of *S. aethiopicum*, *S. incanum* is compatible with *S. melongena* since *S. incanum* is considered to be a member of the primary gene pool with *S. melongena*. This study did not explore the presence of S-genes in *S. incanum* style nor were molecular approaches used

in explaining causes of incompatibility. For instance, testing the presence and absence of HT-proteins (HT-A and HT-B) (Covey et al., 2010), S-RNase binding proteins (Hua and Kao, 2006; O'Brien et al., 2004; Bedinger et al., 2017), Na- transmitting tract specific (NaTTS) in the style (Lush & Clarke, 1997), S-locus F-box proteins (Bedinger et al., 2011b; Li & Chetelat, 2015), and SCF ubiquitin ligase components(Li & Chetelat, 2015) that influence incompatibility. Further studies could be done using molecular approaches in explaining causes of incompatibility.

Pathenocarpic fruits were produced in selected crosses between *S. macrocarpon* and *S. anguivii*, (E12xA1), *S. macrocarpon* and *S. aethiopicum* (SE12xN4, E12xN11, E12xG4), *S. incanum* and *S. aethiopicum* Gilo (In1xG4) and *S. anguivi* and *S. aethiopicum* Gilo (A1xG4). The fruits showed abnormal seeds with varying sizes, devoid of mesocarp while others were empty with degenerated seeds(S. Jansky, 2006; S. H. Jansky *et al.*, 2014). Pathenocarpic fruits are due to degeneration of the endosperm and later resulting into embryo death (Rodrangboon *et al.,* 2002). It might be linked with allelic incompatibility at fertilization and normal fruit formation (Behera and Singh, 2002). To note is the fact that *S. aethiopicum* Gilo (G4) as a donor produced parthenocarpic fruits. In African eggplants, particularly *S. aethiopicum* Gilo, where seed is a limiting factor during consumption, parthenocarpy could be used to improve yield, quality, and processing qualities (Dhatt and Kaur, 2016).

F_1 hybrid seed from the interspecific crosses *S. anguivi* and *S. macrocarpon* (A1xE12), *S. macrocarpon* and *S. incanum* (E12xIn1), *S. aethiopicum* Shum and *S. macrocarpon* (N4xE12), *S. anguivi and S. incanum* (AxIn1), and the intraspecific cross *S. aethiopicum* Gilo (G4xN4) failed to germinate. Failure of seed to germinate in interspecific crosses may be attributed to hybrid

lethality, which is genetically controlled and occurs at seedling stage in interspecific hybrids (Mino *et al*., 2002). This leads to zygote abortion after fertilization and death of the cell in the tissues of seedlings after germination (Mino *et al*., 2002). The Interspecific crosses which had the lowest IRSI suggests that the crosses can be a subject to different selections of reproductive factors compared to the selfed. The greater integral reproductive success of the selfed crosses can be an important factor in their establishment success. Therefore, IRSI could support in a clearer understanding of reproductive success in conditions where each parameter (fruit, seed set and F_1 germination) may vary.

Therefore, second hypothesis stated that *S. aethiopicum* and its relatives will show crossing failure in 84% of the crosses as in S. *melongena*. The findings showed that 50% of the crosses between *S. aethiopicum* and its relatives showed incompatibility (fruit set, seed set and seed germination failures). With these findings, the hypothesis is rejected.

The third objective sought to determine the combining ability of yield related traits in F_1 hybrids of *Solanum aethiopicum* and its close relatives. Specific combining ability effects were more significant than general combining effects which is in correspondence with Sseremba *et al*., (2019) and Buteme *et al*., (2021) findings in *S. aethiopicum* Shum. These results imply that the inheritance of the traits understudied can be explained by significant non-additive gene action (dominant, additive*dominant, and dominant*dominant effects). Traits controlled by non-additive gene action are needed to determine the most superior hybrids to select during hybridization. Among the parents, N4 was a good combiner for plant height and number of leaves when used as a female. Among the relatives *S. anguivi* was a good combiner for plant height, number of leaves and number

of fruits per plant. *S. anguivi* has high numbers of leaves and fruits which make it a best donor while improving *S. aethiopicum Gilo*.

Broad sense heritability values were high than narrow sense heritability values showing that most variation observed is genetically determined and that selection among hybrids will be efficient in interspecific breeding of *S. aethiopicum* (Lawand *et al*., 2022; Namugga *et al*., 2018; Sseremba *et al.*, 2019). This was supported by the fact that the narrow sense heritability, which takes in account additive variance, had lower values for all yield related traits examined. From this study, the principal combining ability in *S. aethiopicum* was identified to be the specific combining ability. Occurrence of other than general combining ability indicated that non-additive gene action is at play in inheritance of yield related traits in *S. aethiopicum* and its relatives. Hence rejecting the hypothesis that stated that, inheritance of yield related traits in F_1-hybrids is conditioned by additive gene action. Higher specific combining ability showed the potential of hybrid vigor. Therefore, combinations with strong SCA will enable the production of *S. aethiopicum* varieties with improved yield related traits.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The first hypothesis stated that anthesis is the major contributor to incompatibility in *S. aethiopicum* and its relatives. The findings showed that anthesis, flowering time, anther dehiscence and stigma receptivity which were all significantly different among the species and flower stage are the major contributors to cross-incompatibility between *S. aethiopicum* and its relatives. Hence, the hypothesis is rejected. For instance, *S. aethiopicum* majorly has medium-styled species*; S. anguivi* has the highest proportion of exserted styles; and has a greater number of flowers with short styles. *S. aethiopicum* showed a high stigma receptivity while *S. incanum* had low receptivity. *S. aethiopicum* Shum had the shortest time to flowering whereas *S. incanum* had the longest time to flowering.

The second hypothesis stated that *S. aethiopicum* and its relatives will show crossing failure in 84% of the crosses as in S. *melongena*. The findings showed that 50% of the crosses between *S. aethiopicum* and its relatives showed incompatibility (fruit set, seed set and seed germination failures). With these findings, the hypothesis is rejected.

The third hypothesis of the study stated that, inheritance of yield related traits in F_1-hybrids is conditioned by additive gene action. From this study, the principal combining ability in *S. aethiopicum* was identified to be the specific combining ability. Occurrence of other than general combining ability indicated that non-additive gene action is at play in inheritance of yield related traits in *S. aethiopicum* and its relatives, hence rejecting the hypothesis. Higher specific combining

ability showed the potential of hybrid vigor. Therefore, combinations with strong SCA will enable the production of *S. aethiopicum* varieties with improved yield related traits.

In summary, this study showed that *S. aethiopicum* can successfully cross with other species, but some combinations do have pre- and post-zygotic barriers which hinder crossing. An understanding of interspecific compatibility barriers will support in the development of thorough breeding strategies to create new cultivars of *S. aethiopicum* with superior traits. The findings on combining ability provide new insight to the inheritance mode of traits of *S. aethiopicum* and its relatives and are beneficial in producing hybrids with desired traits.

6.2 Recommendation

Further studies are needed to explore molecular approaches in explaining causes of incompatibility. For instance, testing the presence and absence of HT-proteins (HT-A and HT-B), S-RNase binding, Na- transmitting tract specific (NaTTS) in the style, S-locus F-box proteins, and SCF ubiquitin ligase components that influence incompatibility.

Parthenocarpy in offspring of crosses between *S. Macrocarpon* and *S. aethiopicum* may be of special interest for future research. Furthermore, embryo rescue and other seed culturing techniques can be used to determine abnormal seed development and poor germination in some crosses.

Adoption of non-conventional plant breeding for introgression of important genes to produce hybrids from the failed crosses is recommended.

This study only involved three genotypes of *S. aethiopicum* with three close relatives (*S. macrocarpon, S. anguivi* and *S. incanum*). Further research on interspecific hybridization studies of *S. aethiopicum* using other wild relatives.

For evaluating the performance of F_1 hybrids, we planted them in one environment (on station) because environmental fluctuations and interactions are a major limitation for S. aethiopicum production and productivity. Genotype by environment interaction is the differential response of different genotypes across range of environments. In breeding, genotype x environment crucial, therefore multi-environment yield trials are therefore essential in assessing of genotype by environmental interaction and identification of superior hybrids and species in the final selection.

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APPENDICES

Appendix 1: Floral Characteristics of the F1 hybrids obtained from the successful crosses between *S. aethiopicum* and its wild relatives

Hybrids	Corolla color (n=300)	Sepal Color	style exertion
A1xN11	White (100%)	Green (50.3%) Green purple (47.7%)	Inserted (9.7%) Intermediate (5.3%) Exserted (85%)
A1xN4	White (57%) Purple white (43%)	Light green (50.33%), Green purple (49.7%)	Inserted (9%), Intermediate (3.7%) Exserted (87.3%)
G4xA1	White (94.3%), Purple white (5.7%)	Light green (95.7%), Green (4.3%)	Inserted (2%), Intermediate (12.3%) Exserted (85.7%)
G4xE12	Light violet (100%)	Green (100%)	Inserted (4%), Intermediate (8.7%) Exserted (87.3%)
G4xIn1	White (88.7%), purple white (11.3%)	Light green (90.3%), green 29 (9.7%)	Inserted (2%) Intermediate (7.3%) Exserted (90.7%)
G4xN11	White (99.7%), green white (0.3%)	Light green (66.7%), Green (33.3%)	Inserted (1.3%), Intermediate (1.7%) Exserted (97%)
N11xA1	Purple white (98.7), white (1.3%)	Green (54.7%), Light green (45.3%)	Inserted (0.3%), Intermediate (2%) Exserted (97.7%)
N11xE12	Light violet (100%)	Green (100%)	Inserted (0.3%), Intermediate (0%) Exserted (99.7%)
N11xG4	Green white (43%), White (57%)	Light green (90%), green (10%)	Inserted (0.7%), Intermediate (8.7%) Exserted (90.7%)
N11xIn1	Purple white (100%)	Green (99.7%), Green purple (0.3%)	Inserted (1%), Intermediate (0%) Exserted (99%)
N11xN4	Purple white (100%)	Green (94.7%), Light green (5.3%)	Inserted (3%) Intermediate (6.3%) Exserted (90.7%)
N4xA1	Purple white (100%)	Green (63.3%), green purple (36.7%)	Inserted (6.4%), Intermediate (3.3%) Exserted (90.3%)
N4xG4	Purple white (60%), white (40)	Light green (90%), green (10%)	Inserted (3%), Intermediate (1%) Exserted (97.7%)

N4xIn1	Purple (100%)	white	Green purple (100%)	Inserted (0%) Intermediate (14.3%) Exserted (85.7%)
N4xN11	Purple (88.7%), (11.3%)	white white	Green (97.3%), Green purple (2.7%)	Inserted (12%) Intermediate (1%) Exserted (87%)

Appendix 2: Vegetative traits/ features of the F1 hybrids obtained from the successful crosses between *S. aethiopicum* and its relatives

Hybrids	**No. of samples**	**Plant growth habit**	**Leaf blade colour**	**Stem color**	**Petiole colour**	**Leaf mid rib** colour	**Leaf vein** colour	**Leaf prickles**	**Leaf hairs**
A1xN11	30	30 Upright	23 green: 7 light green	28 green purple: 2 green	28 green purple: 2 green	23 green purple: 7 green	25 green purple: 5 green	very few	sparse
A1xN4	30	22 Upright: 8 intermediate	30 dark-green	30 purple	30 purple	30 green purple	30 green purple	glabrous	sparse
G4xA1	30	18 Upright: 7 intermediate: 5 mixture	30 green	30 green purple	30 green purple	30 green purple	30 green	few	sparse
G4xE12	26	14 Upright: 9 intermediates: 3 mixture	26darkgreen	26 green	26 green	26 green	26 green	medium	sparse
G4xIn1	25	14 Upright: 1 intermediate: 10 mixture	25 green	25 green purple	25 green purple	25 green purple	15 green purple: 10 green	few	Medium
G4xN11	30	24 Upright: 4 intermediate: 2 mixture	23 green: 7 light green	30 green purple	30 green purple	30 green purple	30 green	glabrous	sparse
N11xA1	30	23 Upright: 7 intermediate	30 green	20 purple: 10 green	20 purple: 10 green	16 green purple: 14 green	20 green:10 green purple	glabrous	Medium

				purple	purple				
N11xE12	30	30 Upright	30 darkgreen	30 green purple	30 green purple	17 green purple: 13 green	30 green	Medi un	sparse
N11xG4	30	30 Intermediate	20 green: 10 light green	30 green purple	30 green purple	23 green purple: 7 green	23 green purple: 7 green	glabrous	sparse
N11xIn1	28	28 Upright	28 green	28 green purple	28 green purple	28 green purple	20 green purple: 8 green	glabrous	sparse
N11xN4	30	30 Intermediate	20 green: 10 light green	30 green purple	30 green purple	30 green purple	22 green purple: 8 green	glabrous	sparse
N4xA1	30	30 Upright	21 green: 9 darkgreen	30 green purple	30 green purple	30 green purple	17 green purple: 13 purple	glabrous	mediu m
N4xG4	20	11 Upright: 9 intermediate	11 light-green: 9 green	20 green purple	20 green purple	20 green purple	20 green purple	glabrous	sparse
N4xIn1	27	27 Upright	27 green	27 green purple	27 green purple	27 green purple	27 green purple	glabrous	sparse
N4xN11	30	30 Upright	20 light green: 10 green	30 green purple	30 green purple	20 green: 10 green purple	20 green: 10 green purple	glabrous	sparse

Appendix 3: Fruit traits/ features of the F1 hybrids obtained from the successful crosses between *S. aethiopicum* and its relatives

Hybrids	No. of plants	Calyx Prickles	Fruit shape	Physiological colour	commercial	Color distribution
AxN11	300	No prickles	190 Circular, no grooves: 110 Circular few grooves	Red	Green	Striped
AxN4	300	No prickles	300 Circular, no grooves	Red	Green	Striped
N11xA	261	No prickles	261 Circular few grooves	Red	Green	290 Striped :10 uniform
N11xE12	300	No prickles	300 Circular, no grooves	Yellow-orange	Green	220 Striped: 80 uniform
N11xN4	300	No prickles	300 Circular few grooves	Red	Green	Uniform
N11xG4	299	No prickles	151 Very irregular: 148 Circular few grooves	Deep orange	Green	Striped
N11xIn1	300	No prickles	220 Circular, no grooves: 80 Circular few grooves	Deep orange	Green	
N4xA	300	No prickles	287 Circular, no grooves: 13 Circular few grooves	Red	Green	190 Striped: 110 uniform
N4xN11	300	No prickles	192 Circular, no grooves: 102 Circular few grooves	Red	Green	
N4xG4	200	No prickles	200 Circular, no grooves	Deep orange	Green-white	Striped
N4xIn1	300	No prickles	200 Circular, no grooves	Deep orange	Green	
G4xA	300	No prickles	289 Circular, no grooves: 11 Circular few grooves	Red	Green	291 Striped : 9 uniform
G4xN11	300	No prickles	160 Circular few grooves: 140 Circular, no grooves	Red	Green	160 uniforms: 140 Striped

G4xE12	260	Few prickles	150 Circular few grooves: 110 Circular, no grooves	Yellow-orange	Green	150 Striped: 110 uniform
	250	No prickles	210 Circular, no grooves: 40 Circular few grooves	Red	Green	210 Striped: 40 uniform

Appendix 3: Dissertation Correction Compliance form (Post-Viva Form)

UGANDA CHRISTIAN UNIVERSITY

A Centre of Excellence in the Heart of Africa

SCHOOL OF RESEARCH & POSTGRADUATE STUDIES

DISSERTATION CORRECTION COMPLIANCE FORM (POST-VIVA FORM)

Date: 16th/08/2023

Name of Candidate: Namutosi Winnie **Reg. No**: S20M44/001

Title of Dissertation: Cross-compatibility and combining ability between *Solanum aethiopicum* with its close relatives

S/N	COMMENTS BY EXTERNAL EXAMINER	ACTION TAKEN	INDICATOR
1.	**Chapter two:** The candidate should make an effort to look for recent research work to support the research.	-The old citations were addressed to more recent work	-Literature review, page 12 to 32 corrected.
	The accession N11 and N4 seems to have the same name though their descriptions are different.	-These are same species of *S. aethiopicum* Shum. Though they are different accession with different entries	Materials section 3.1.1. page 35.
2.	**Chapter five:** The candidate needs to know that the discussion should not be limited to comparisons with other similar works. The discussion can be improved.	-The discussions were improved basing on the implications of results, comparisons with previous studies and my opinions.	-Discussions; page 89 corrected.

3.	**Chapter Six:** The conclusion of the work was well presented although the hypothesis verification could be moved to the discussion section.	-The conclusions are done on the basis of whether to accept or to reject the hypothesis and therefore the research team asserts that they should remain at this section.	Conclusion; page 97
4.	The candidate could, if possible, think of replacing the old references. Some references need modification as suggested by the reviewer. The section can be improved.	Very old references were replaced by the new ones	References; Page 100 to 119 corrected.
5.	To what global or regional problem is the research contributing?	-Background and significance (who are the beneficiaries) shows how the research is applicable to other species or disciplines to solve some issues in the region and globally	Significance; page 8
6.	Why was this research done now and not later in the future?	-The issue was built from the background briefly then after to the justifications (potential to solve challenges of hunger).	-Justification; page 9
7.	What were the challenges faced in carrying out this study, and how were they dealt with?	-The challenges are clearly stated during discussions and added in recommendations.	-Page 89 to 91

S/N	COMMENTS BY INTERNAL EXAMINER	ACTION TAKEN	INDICATOR
1.	**Abstract**; Could move Gilo and Shum to the arrow after the *S. aethiopicum* in the third line of the abstract.	-This was addressed	Abstract page (i) corrected
2.	The manual Shows that Abstract should be 1 page with 300 words. The abstract has more than 365 words.	-The words in the abstract were reduced to 300 and is fitted in one page	Abstract page (i) corrected
3.	Could the font size be increased to match the rest of the work in Declaration and approval page	-The font size be increased to 12 as the rest of work	Declaration and approval on page (ii) and page(iii) corrected
4.	SC abbreviation has not been introduced in the text on the list of acronyms	-The SC has been included in the list of acronyms	Acronyms on page (xix) corrected

5.	**Abstract:** Introduction of the concepts of yield and yield traits for the crop in the study is missing in this chapter. Further, editing efforts in the contents could be improved.	- The yield and yield traits were included. -The editing was done as recommended for this section	-Abstract on page 5 corrected -Page 1 to 5
6.	**Objectives:** Something (genotypes) missing in the main objective Why 84% and not 50% or 90%? Justify the choice of the figure for objective two	-The word was fixed after *S. aethiopicum* -This was justified with the reference on recent work done in related species of *S. aethiopicum* (*S. melongena)*	-Main objective page 8 corrected -Specific objective two; page 8 corrected
7.	**Literature review;** Editing efforts in the contents could be improved. In many pages, the text lacks paragraphs and in some cases, there are repetitions. There is a clear need to thoroughly edit the dissertation to ensure sound scientific language is adhered to all through the write-up	The section was edited and paragraphs were included in each section	Literature review page 12 to 32 corrected
8.	Include a citation after the first sentence in section 2.5.2. Poor germination of hybrid seeds	-The citation was included	-Literature review page 31 corrected
9.	Very old citations need to be addressed (the candidate should make an effort to look for recent research work to support the research).	-The old citations were addressed apart from a few like the one of Griffings crossing design made in 1956	- Literature review page 12 to 32 corrected.
10.	Give the specifications of the instrument (haemocytometer) used	-Specification was included as shown in section 3.1.5.3-page 44	-Methods, section 3.1.5.3 page 44 corrected
11.	Clarify and justify the type of ANOVA to be used in each instance and why the proposed post hoc test.	-For ANOVA, the analysis depended on how many factors, for one factor, a one-way ANOVA was used, two factors, Two-way ANOVA was used and those with three factors, three-way ANOVA was used.	-Data analysis section Page 46 and 49 corrected

12.	Data presented for objective 1 is not congruent with what the section 3.1.5.4 promised and in an orderly fashion. Clearly outline the qualitative and quantitative floral structure traits. Then, first present qualitative followed by quantitative. Currently that orderliness is missing, there is no demarcation for quantitative data as was made for qualitative data	-This section corrected and put in order -Qualitative traits are presented and quantitative traits are presented	-Results page 46 corrected -Results; section 4.1.1 on page 53 and section 4.1.2 on page 55 corrected.
13.	In qualitative data, frequencies were promised and not delivered, there is no data on position of stigma.	-The position of the stigma was defined as stigma exsertion	-Results section 4.1.1 on page 53 and figure on page 54.
14.	Data for objective 2- mean fruit set, mean seed set as promised in 3.2.1.7 are missing. Data presented does not sufficiently meet the criteria to answer the study objective. Moreover, the candidate qualifies the results before presenting them in section 4.2.1, page 62.	-Data on the means for seed set is presented (Table 12; Column 4). Variables (Fruit set, seed set and germination) which were in section 3.2 are presented in section 4.2.1.	-Results on Page 66 and 67 corrected.
15.	Figure 6 in section 4.3.1.2 is not referred in the text! So, it is hanging	-The figure 6 is referenced	Results, section 4.3.1.2 on page 66 corrected
16.	Objective 3-data was presented in systematic and orderly way. However, there were a few typos that need to be edited and put right. There are some comments on additional data for vegetative traits that could boost the case for the study-weight of leaves, shoot weight, probably even harvest index-table 15 and 16.	-The typos were corrected and some tables were re-organized. The parameters weight of leaves and shoot weight data was missed during collection of data for some genotypes which had few crosses hence few plants (these needed to be harvested and uprooted at 7 weeks after planting which could affect fruit data).	Results on page 69 to 89 corrected
17.	The candidate should check the descriptive statistics to make sure all promised in chapter three is presented in chapter	-All the descriptive statistics described have been corrected and presented in section 4 in results	Results page 55 corrected

	four		
18.	There is a logical flow of concepts in the discussion. A few clarifications are needed	-The discussions were improved	Discussion page 89 corrected

S/N	COMMENTS BY VIVA VOCE PANEL	ACTION TAKEN	INDICATOR
1.	Explain why you chose that statistical tool you used	The tools where appropriate for the specific data according previous works.	Page 45, 47 and 49.
2.	Did you do a one-way ANOVA or a two-way ANOVA?	-For ANOVA, the analysis depended on how many factors, for one factor, a one-way ANOVA was used, two factors, Two-way ANOVA was used and those with three factors, three-way ANOVA was used.	-Data analysis section Page 46 and 49 corrected
3.	Clarity on experimental design	Each objective had its experimental design used.	Methods page 38 47 and 50.
4.	Your objective 3 is hypothesized scientifically and yet test statistically, explain	Hypothesis three was stated basing on literature and research is similar species which was a basis of the conclusions made	Hypothesis two; page 8
5.	Are the products (leaves and fruits) of the crosses palatable since *S. incanum* is involved during crossing	To produce a palatable hybrid, several back crosses are done (up to 10 seasons) until the desirable traits by consumers are obtained. This has been done in melongena and cassava	Background page 1 to 3, literature page 30

Candidate's Name: Namutosi Winnie Signature:

Supervisors

Name: **ASSOC. PROF. ELIZABETH BALYEJUSA KIZITO (PHD)**

Signature:

Name: Dr. BULYABA ROSEMARY

Signature:

NB: Post Viva compliance form is designed to capture all the corrections recommended by internal examiner (supervisor), external examiner and viva pane