UNIVERSITY OF ÇUKUROVA
INSTITUTE OF NATURAL AND APPLIED SCIENCE

PhD THESIS

Flavien SHIMIRA

ASSESSING MORPHOLOGICAL AND GENETIC DIVERSITY AMONG TRADITIONAL AFRICAN EGGPLANT LANDRACES AND DETECTING SALT TOLERANCE AND ANTHER CULTURE PERFORMANCE OF SELECTED ACCESSIONS

DEPARTMENT OF HORTICULTURE

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ÇUKUROVA UNIVERSITY

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We certify that the thesis titled above was reviewed and approved for the award of the degree of the Philosophy of Doctorate by the board of jury on 03/10/2022.

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ABSTRACT

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Scarlet eggplant (*Solanum aethiopicum* L.) is the most consumed and popular eggplant among indigenous vegetables on the African continent. Since its genetic diversity is still largely unexplored, its genetic diversity and population structure were assessed by employing the iPBS-retrotransposon markers system on a germplasm collection made of several accessions of *Solanum aethiopicum* gr. gilo. The recorded mean polymorphism information content was 0.363. The model-based structure, neighbor-joining, confirmed the existence of genetic diversity and 3 major genetic population group were obtained. This variation was also observed through phenotypic characterization by using key eggplant descriptors in two distinct experiments (greenhouse and open filed). Afterwards, *in vitro* anther culture was essayed with different treatments. Only the accession GKE12 showed satisfactory outcome with the rate of embryo formation of 0.82/100 anthers and 0.41/100 anthers corresponding to the rate of developed embryos. The sole obtained embryo/seedling was found to be doubled haploid. The morphological and salt-induced changes on *in vitro* early seedling growth of selected accessions were also assessed. The results showed that the seedling growth of all eggplant accessions was negatively affected by salt stress compared to controls. The extensive diversity of scarlet eggplant from Rwanda might be used to form the base and genetic resource of an exhaustive breeding program of this economically important African indigenous vegetable.

Keywords: Halophyte, Salt stress, *Solanum aethiopicum*, African indigenous vegetable, Germplasm characterization, Orphan crop.

ÖZ

DOKTORA TEZİ

GELENEKSEL AFRİKA PATLICAN ÜLKELERİNİN MORFOLOJİK VE GENETİK ÇEŞİTLİLİĞİNİN DEĞERLENDİRİLMESİ VE SEÇİLMİŞ ERİŞİMLERİN TUZ TOLERANSI VE BAŞKA KÜLTÜR PERFORMANSLARININ TESPİTİ

Flavien SHIMIRA

ÇUKUROVA ÜNİVESİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
BAHÇE BİTKİLERİ ANABİLİM DALI

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Scarlet patlıcanı (*Solanum aethiopicum* L.), Afrika kıtasında yerli sebzeler arasında en çok tüketilen ve popüler olan patlıcan türüdür. Genetik çeşitliliği hala büyük ölçüde keşfedilmemiş olduğundan, *Solanum aethiopicum* gr. Gilo'nun farklı aksesyonlarından oluşan bir germplazm koleksiyonu üzerinde iPBS-retrotranspozon belirteç sistemi kullanılarak genetik çeşitliliği ve popülasyon yapısı araştırılmıştır. Kaydedilen ortalama polimorfizm bilgi içeriği 0.363 olmuştur. Model tabanlı yapı, neighbor-joining, genetik çeşitliliğin varlığını doğrulamış ve 3 ana genetik popülasyon grubu elde edilmiştir. Bu varyasyon, iki farklı denemede (sera ve açık tarla) patlıcan türüne özgü tanımlama kriterleri kullanılarak fenotipik karakterizasyon yoluyla da gözlemlenmiştir. Daha sonra *in vitro* anter kültürü farklı uygulamalarla denenmiştir. Sadece GKE12, 0.82/100 anter embriyo oluşum oranı ve 0.41/100 anter gelişmiş embriyo oranı ile sonuçlanmıştır. Elde edilen tek embriyo/*in vitro* bitkinin diploid olduğu bulunmuştur. Seçilen aksesyonlarda, *in vitro* fide büyümesi üzerindeki morfolojik ve tuz kaynaklı değişiklikler de değerlendirilmiştir. Sonuçlar, tüm patlıcan aksesyonlarının fide büyümesinin, kontrole kıyasla tuz stresinden olumsuz etkilendiğini göstermiştir. Ruanda'daki scarlet patlıcan çeşitliliği, bu ekonomik açıdan önemli Afrika yerli sebzesinin kapsamlı bir ıslah programının temelini ve genetik kaynağını oluşturmak için kullanılabilir.

Anahtar Kelimeler: Halofit, Tuz stresi, *Solanum aethiopicum*, Afrika yerli sebzesi, Germplazm karakterizasyonu, Minör ürün.

EXTEND SUMMARY

Scarlet eggplant (*Solanum aethiopicum* L.) is the most consumed and popular eggplant among indigenous vegetables on the African continent. It has also been recognized as a source of genetic variation and resistance for brinjal (*Solanum melongena* L.) breeding.

Since its genetic diversity is still largely unexplored, its genetic diversity and population structure were investigated. Scarlet eggplant germplasm made of fifty-two accessions originated from two districts of Rwanda was assessed through molecular characterization by employing the iPBS-retrotransposon markers system. Twelve of the most polymorphic iPBS-retrotransposon primers were employed for molecular characterization and they yielded 329 total bands, whereupon 85.03% were polymorphic. The recorded mean polymorphism information content was 0.363 and other diversity indices such as; mean the effective number of alleles, mean Shannon's information index, and gene diversity with the following values; 1.298, 0.300, and 0.187 respectively. A superior level of diversity was noticed among accessions from Musanze district. The model-based structure, neighbor-joining, and principal coordinate analysis (PCoA) gathered scarlet germplasm in a divergence manner to their collection district. Analysis of molecular variance (AMOVA) displayed that the utmost variations (81%) in scarlet eggplant germplasm are resulting in differences within populations. The extensive diversity of scarlet eggplant in Rwanda might be used to form the basis and genetic resource of an exhaustive breeding program of this economically important African indigenous vegetable. For instance, accessions MZE53 and GKE11 might be proposed as parent candidates due to their high relative genetic distance (0.6781).

Similarly, phenotypic diversity was assessed in this study. Two major experiments (I and II) and a supplemental third experiment were established in which morphological and valuable agronomic traits were used to measure diversity

among the germplasm collection of *Solanum aethiopicum* gr. Gilo made of 57, 55 and 49 accessions, respectively. In both experiments, descriptors designated by the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the International Board for Plant Genetic Resources (IBPGR) for *Solanaceae* and eggplant were used with slight modification. In the first experiment conducted in the greenhouse located at the BATEM institute (June 2020 - January 2021), a total of 57 different accessions were able to be measured from 60 accessions initially grown. While in the second experiment, only 55 accessions were included. The said experiment was carried out in the open field located at Fidesan Fide Ltd. company (March to July 2022). For the supplemental experiment, only descriptors related to qualitative and quantitative traits of fruit were used on a total of 49 accessions grown in the greenhouse located at Çukurova University (June to December 2020). The results from descriptive statistics on quantitative traits data of plants and fruits (Experiments I and II) as well as descriptive statistics on quantitative traits of only fruits (Supplemental experiment) show a great variation among accessions of *Solanum aethiopicum* gr. Gilo. Multiple correlation analysis in the two main experiments (I and II) shows that the highly correlated variables/descriptors represented fruit quantitative traits. In the first experiment (I), the fruit weight was highly correlated to the fruit maximum diameter (r=0.937), while in the second experiment (II), the fruit length was highly correlated to the fruit maximum diameter (0.853). This means that the fruit variables/descriptors were more dependent upon one another compared to the plant variables/descriptors. Additionally, it was demonstrated that all superior elements/accessions in terms of fruit quantitative traits tend to be grouped together with regards to the phenotypic (hierarchical) clustering in the two main experiments (I and II). Finally, results from principal component analysis (PCA) confirm that the overall differences observed in the germplasm collection of *Solanum aethiopicum* gr. gilo grown in greenhouse (Experiment I) and in open

field (Experiment II) were mainly due to fruit quantitative traits. Briefly, fruit quantitative traits are decisive for phenotypic characterization of *Solanum aethiopicum* gr. Gilo eggplant.

Embryogenesis induction through *in vitro* androgenesis (anther culture) studies has been successfully employed worldwide in brinjal (*Solanum melongena* L.) and to the best of our knowledge, none has been attempted in a large germplasm of brinjal relative such as *Solanum aethiopicum*. In this study, *in vitro* anther culture was essayed in two distinct experiments on the largest germplasm collection of *Solanum aethiopicum* gr. Gilo. The study aimed to obtain an effective embryogenesis method in this relative of the brinjal eggplant that failed to engender embryos in previous research. In the first experiment, 51 different accessions were assessed on the induction medium (C medium) supplemented with 5 mg/l of 2.4-D and 5 mg/l of kinetin. The results show that the overall rate of callus induction was 36.6 calli/100 anthers. Additionally, the statistical analysis via ANOVA and LSD tests revealed the dependency of the rate of callus induction on accessions. Regrettably, in the first experiment, embryo formation was unsuccessful. The second experiment was conducted using two distinct treatments (I and II) of C medium with two concentration levels of hormones (I: 5 mg/l 2.4-D and 5 mg/l kinetin; II: 1 mg/l 2.4-D and 1 mg/l Kinetin). Only four selected accessions of *Solanum aethiopicum* gr. Gilo were used in the second experiment, and they were compared to two Turkish eggplant genotypes of *Solanum melongena*. In the first treatment (I), only the accession GKE12 showed a satisfactory outcome with the rate of embryo formation of 0.82/100 anthers and 0.41/100 anthers corresponding to the rate of developed embryos. In the second treatment (II), only controls, which are Adana and Kemer genotypes of *Solanum melongena* formed embryos with a rate of 7.26/100 anthers and 1.15/100 anthers, respectively. For the rates of developed embryos of both controls, their obtained values were slightly lower with 6.7/100 anthers and 0.57/100 anthers, respectively.

The sole obtained embryo/seedling of *Solanum aethiopicum* gr. Gilo was found to be diploid. Through this study, it was demonstrated that with the right combinations of hormones, it is possible to produce a diploid of *Solanum aethiopicum*, the world's second most popular cultivated eggplant after brinjal.

In this study, we assessed morphological and salt-induced changes on *in vitro* early seedling growth of some eggplant (*Solanum aethiopicum* gr. Gilo) landraces that originated from Rwanda, consisting of six eggplant accessions (GKE11, GKE20, MZE29, MZE44, MZE49, and MZE51), representing three population types (Pop A, Pop B, and Pop C). The *in vitro* experiment was conducted by applying several concentrations of sodium chloride (NaCl); 0 mM, 20 mM, 40 mM, 80 mM, and 160 mM during subculture, and shoots were left exposed to salt stress for 6 weeks. Afterward, various morphological parameters at the end of this time were measured. Thus, prior eggplant seeds were germinated on solidified growing medium under no-saline conditions. Salt stress effects were assessed with regard to seedling growth rate and water content. The results showed that the seedling growth of all eggplant accessions was negatively affected by salt stress compared to controls. The water content of all eggplant accessions increases slightly with salinity. Based on the results of the experiment, GKE11 and MZE44 were less sensitive to salt stress and may be useful as genetic resources for future salt tolerance studies. Our findings also suggest that *Solanum aethiopicum* may have halophyte attributes, which will need to be confirmed by additional research.

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SYMBOLS AND ABBREVIATIONS

%	: Percent
°C	: Degree Celsius
µg	: Microgram
2.4-D	: 2,4-Dichlorophenoxyacetic acid
AFLP	: Amplified fragment length polymorphism
AIVs	: African indigenous vegetables
AMOVA	: Analysis of molecular variance
ANOVA	: Analysis of Variance
BAP	: 6-Benzylaminopurine
BATEM	: Batı Akdeniz Agricultural Research Institute
Cm	: Centimeter
cm^2	: Square centimeter
CTAB	: Cetyltrimethylammonium bromide
Cum %	: Cumulative percentage
CWR	: Crop wild relatives
da	: Decare
DF	: Degrees of freedom
DH	: Doubled haploid
DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
$dS.m^{-1}$	: DeciSiemens per metre
DW	: Dry weight
ECPGR	: European Cooperative Programme for Plant Genetic Resources
FW	: Aerial fresh weight
g	: Gram
GD	: Mean Nei's genetic distance

GKE	: Gakenke district
GPS	: Global positioning system
HCA	: Hierarchical clustering analysis
He	: Gene diversity
HSD	: Tukey's honestly significant difference
I	: Shannon’s information index
IBPGR	: International Board for Plant Genetic Resource
iPBS	: Inter-primer binding site
ISSR	: Inter-simple sequence repeats
K	: Number of clusters
LRR	: Leucine-rich repeat
LSD	: Least significant difference
LTR	: Long terminal repeat
m	: Meter
M	: Mole
MAS	: Marker-assisted selection
Max	: Maximum
MCMC	: Markov chain Monte Carlo
Min	: Minimum
mm	: Millimeter
mM	: Millimole
MS	: Murashige and Skoog
MZE	: Musanze district
NAA	: 1-Naphthaleneacetic acid
NaCl	: Sodium chloride
NaOCl	: Sodium hypochlorite
NaOH	: Sodium hydroxide
NBS	: Nucleotide-binding site

Ne	: Effective number of alleles
PBS	: Primer binding site
PCA	: Principal component analysis
PCoA	: Principal coordinate analysis
PCR	: Polymerase chain reaction
PIC	: Polymorphism Information Contents
r	: Correlation coefficient
RAB	: Rwanda Agriculture Board
RAPD	: Random amplification of polymorphic DNA
RGAs	: Resistance gene analogs
RKNs	: Root-knot nematodes
ROS	: Reactive oxygen species ()
SSR	: Simple sequence repeats
STD	: Standard deviation
Taq	: *Thermus aquaticus*
TBE	: Tris-borate- Ethylenediaminetetra-acetic acid
TEs	: Transposable elements
tRNA	: Transfer ribonucleic acid
TWC	: Tissue water content ()
USA	: United States of America
w/w	: Weight for weight
µl	: Microliter

1. INTRODUCTION

1.1. Background

Scarlet eggplant is often described as African eggplant. However, African eggplant is recognized as a large group in which African native eggplants such as gboma eggplant (*Solanum macrocarpon* L.) and scarlet eggplant (*Solanum aethiopicum* L.) are comprised as well as their wild ancestors (*Solanum dasyphyllum* and *Solanum anguivi*), respectively. In African cuisine, African eggplants are appreciated mainly for their edible fruits and sometimes for their leaves (Doganlar et al., 2002; Lester and Daunay, 2003; Haliński et al., 2017; Mibei et al., 2018). Additionally, they are economically important and they are more popular among smallholder farmers in Sub-Sahara Africa, because they are easy to grow with minimum inputs and they are perfectly adapted to local conditions (Weller et al., 2015; Sseremba, 2019).

Scarlet eggplant (*Solanum aethiopicum* L.), broadly recognized as the other cultivated eggplant, is a close relative of Brinjal eggplant (*Solanum melongena*) and one of its direct wild ancestors is *Solanum anguivi.* Furthermore, a great number of wild relatives of Brinjal eggplant are found on the African continent (Doganlar et al., 2002; Knapp et al., 2013; Knapp et al., 2019). Scarlet eggplant is a diploid with 24 chromosomes (2n = 24) and also a member of the genus *Solanum* (Doganlar et al., 2002). The genus *Solanum* is vast and prosperous in species. About 1400 species are approximately estimated to exist in this rich genus. Thirteen principal clades exist within the genus such as the spiny solanums, the Leptostemonum clade and the Potato clade (which comprises of potato and tomato) just to name a few.

The scarlet eggplant (*S. aethiopicum* L.) is a member of the “Anguivi clade” (Knapp et al., 2013; Knapp et al., 2019). Scarlet eggplant is described as an herbaceous shrub with glabrous or hairy leaves. It is distinguished by its

hermaphroditic flowers that grow in clusters or separately and these flowers are self- or cross-pollinated (Kamga et al., 2015).

Scarlet and gboma eggplants are essentially cultivated and grown on the African continent, while the common eggplant is more present everywhere; including Africa, Asia, subtropics (Central America, India) and in tempered regions like Mediterranean region and southern USA (Doganlar et al., 2002; Gramazio et al., 2016; Nkansah, 2001; Zhuang et al., 2012). Apart from West and Central Africa, *S. aethiopicum* is also present in Caribbean countries, Brazil as well as South Italy. A remarkable number of wild relatives of common eggplant (*Solanum melongena*) are from Africa (Knapp et al., 2013; Gramazio et al., 2016).

Like common eggplants (brinjals), scarlet eggplants (*Solanum aethiopicum*) are consumed raw, boiled and/or fried, and also as an ingredient in stews and soups (Eletta et al., 2017). Their leaves and fruits are appreciated for their bitter taste. To a great extent, this could be attributed to the presence of alkaloids (mainly glycolalkaloids and phenolic compounds) determining their edibility. In sub-Saharan Africa, leaves are consumed as vegetables, particularly leaves of the *S. aethiopicum* Kumba and Shum groups, ones of the four groups that make up *Solanum aethiopicum*. Consumers' preference for fresh fruit relies on a few quality traits such as fruit acidity and taste, fruit color, and phenolic contents in fruit and fruit epidermis (Adeniji et al., 2012). There is increasing evidence that the intake of their leaves and fruits reduces the incidence of chronic diseases, including diabetes and atherosclerosis (Mibei et al., 2018).

The common eggplant fruit (*Solanum melongena*) has been used for the treatment of various diseases such as bronchitis, asthma, arthritis, and diabetes, as well as its nutritive properties are beneficial to the human diet (Foo et al., 2018). In the same manner, the African eggplant *Solanum aethiopicum* and *Solanum macrocarpon* have been used in traditional medicine in the cure of allergic rhinitis, asthma, constipation, dyspepsia, gastro-esophageal reflux disease, nasal catarrh,

rheumatic disease, skin infections, and swollen joint pains. They are also used in weight reduction (Eletta et al., 2017).

Eggplants have nutritional and therapeutic significance. In Nigeria, the antioxidant activity and nutritional composition of *Solanum aethiopicum* and *Solanum macrocarpon* were studied by Eletta et al. (2017). Higher moisture content was observed in *Solanum macrocarpon* L. and in *Solanum aethiopicum* with 92% and 91.20%, respectively. For protein content, *Solanum aethiopicum* has a higher percentage with 1.07% than *Solanum macrocarpon* (0.54%). Phytochemical screening of *Solanum aethiopicum* and *Solanum macrocarpon* shows a considerable presence of alkaloids which give the bitterness character to African eggplant, particularly glycoalkaloids. There is also a significant presence of saponins, tannins, and flavonoids. Saponins have antimicrobial activities that are well known and protect plants against microbial pathogens.

Kamga et al. (2015) have reported that the fruit from the three edible groups (Gilo, Kumba, and Shum) may accommodate up to 80% water, 10% protein, 2% potassium, high levels of antioxidant compounds, and carotenoids. It has been also reported by Kamga et al. (2013) that the Oforiwa variety of *Solanum aethiopicum* (originated from Ghana) contains a higher amount of carotenoids with 624.54 µg/100g in fresh weight compared to other *S. aethiopicum* eggplant varieties originated from Ghana (AB2, DB3) and one from Tanzania (N13) with 537.12 µg/100g, 299.39 µg/100g and 286.25 µg/100g, respectively. The carotenoids content in the African eggplants was two times greater than other studied vegetables such as amaranth (*Amaranthus cruentus*), jute mallow (*Corchorus olitorius*), nightshade (*Solanum scabrum*) and okra (*Abelmoschus callei*).

1.2. Problem statement

In Africa and in Rwanda particularly, fewer studies have been undertaken on the scarlet eggplant (*Solanum aethiopicum* L.). It is reported that eggplants are the fifth most produced vegetable in Rwanda after cabbages, tomatoes, fresh beans, and squashes. Additionally, they are cultivated in mixed farming system that characterize vegetable cultivation all over the country and most of the time on small plots (Larochelle and Alwang, 2014; Van Dijk and Elings, 2014; Prasad et al., 2016). Eggplants are grown from commercial seeds available within the country, and their quality is often moderate (Van Dijk and Elings, 2014). The hybrid seed market is still small and the available seed varieties are limited. Open-pollinated varieties are sold by a few private companies at affordable prices in different franchise shops of those companies all over the country (Dijkxhoorn et al., 2016; Uwamahoro et al., 2020).

The informal seed sector is still dominating, with paramount shares of farmer-saved seeds. Improved varieties' adoption in Rwanda was relatively low between 7-13%. Besides, only 3 improved varieties and breeding lines of African eggplant were recorded in the 2013 country report of genetic plant resources. Maize was the only exceptional crop with high hybrid adoption across the country. Breeds and pre-basic seeds of economically important crops such as maize, beans, rice, and wheat are being produced by the Rwanda Agriculture Board (RAB) for the most part (Gapusi et al., 2013; Context Network, 2016; Mujuju, 2018). In the national breeding programs, preferences were given only to economically important crops such as sweet potatoes, cassava, and common beans, where more pest and virus-resistant crops are being tested by the means of marker-assisted selection (MAS). Also, clones of those crops resulting from crosses between local and foreign varieties have been released in the country (Larochelle and Alwang, 2014; Dusengemungu et al., 2019; Shimira et al., 2020). In the year 2014, it was reported that there were neither African eggplant varieties nor seed products

released in Rwanda since 1990 by national agricultural research institutions. All available varieties of African eggplant are open-pollinated (Schreinemachers et al., 2017).

1.3. Objectives of the study

1.3.1. General objectives

It has been reported that developing successful varieties frequently depends on knowledge of specific genes and traits of parent crops present in the gene pool (Shah et al., 2014). Consequently, breeding research programs will necessitate a thorough comprehension of the local genetic diversity and relationships among all the genotypes commonly grown in Rwanda in order to improve yield in scarlet eggplants. Therefore, the purpose of this thesis research work is to evaluate genetic variation among sixty different landraces of scarlet eggplant (*Solanum aethiopicum* gr. Gilo) initially collected from Rwanda using a genetic diversity study based on the iPBS retro-transposons marker system. This will assist in the determination of population structure. Aside from the genetic diversity analysis, morphological characterization as well as *in vitro* studies were also carried out.

In vitro anther culture, for instance, is a useful technique for producing doubled haploid plants in economically variable crops such as eggplant. Indeed, haploid plants are very useful in breeding programs. They help with the detection of recessive mutations and the attainment of F1 hybrid vigor (Alpsoy and Şeniz, 2007). Anther culture is used in addition to conventional breeding methods to produce pure (homozygous) lines. They are produced through androgenesis by generating doubled haploid (DH) plants. Consequently, hybrid plants are produced by crossing two pure (homozygous) lines with desirable characteristics (Seguí-Simarro, 2016). As a result, embryogenesis or doubled haploid eggplant production was attempted on the largest germplasm collection made up of several accessions of *Solanum aethiopicum* gr. Gilo in this study. The salt tolerance of *Solanum*

aethiopicum was also evaluated in an *in vitro* investigation by measuring salt-induced changes in early seedling growth among a few selected accessions of *Solanum aethiopicum* gr. Gilo.

In due course, high-quality individuals identified from this study will be used as the initial generation breeding population of this particular and economically valuable crop that is essential to several small-scale farmers. Our findings will be useful to the improvement of yield, and they will greatly enable the establishment of the first breeding program at the national level for African eggplant.

1.3.2. Specific objectives

The objectives of this research study weree as follows: (i) The determination of the genetic diversity of 60 African eggplant (*Solanum aethiopicum* L.) accessions collected from Rwanda by using the latest molecular markers such as the iPBS retrotransposon markers system, (ii) The determination of the morphological diversity of same accessions through the use of several descriptors of *Solanaceae* and eggplants, (iii) The selection and recommendation of ideal and high-potential accessions for future breeding programs through morphological and molecular tools, (iv) The determination of anther culture performance and embryogenesis capacity of all *Solanum aethiopicum* gr. Gilo accessions, (v) The determination of salt stress tolerance of selected accessions using *in vitro* techniques.

2. LITERATURE REVIEW

2.1. Morphological and genetic diversity assessment

Considerable research and breeding programs have been carried out globally on brinjal (*Solanum melongena* L.) and to a smaller extent on its relatives such as *Solanum americanum*, *Solanum incanum* and *Solanum torvum* (Isshiki et al., 1998; Caguiat and Hautea, 2014). This situation prompted scarlet eggplant (*Solanum aethiopicum* L.) and other wild relatives to be embedded among the so-called "orphan crops" or underutilized crops, along with a great number of other African indigenous vegetables (Kamga et al., 2015; Weller et al., 2015; Song et al., 2019). In the breeding programs of *Solanum melongena*, scarlet eggplant is recognized as a beneficial source of variations along with *Solanum incanum* and it is also identified as cross-compatible with *Solanum melongena* (Gramazio et al., 2016). For instance, various resistances/tolerances have been identified within different materials of *Solanum aethiopicum*. It includes resistance/tolerance to some diseases and pests, as shown in Table 2.1.

Table 2.1. Acknowledged resistances/tolerances in *S. aethiopicum*

Resistance against	Description
Bacteria	*Ralstonia solanacearum* as casing agent of bacterial wilt disease
Fungi	*Fusarium oxysporum, F. solani, Pythium vexans, Phytophthora parasitica*
Insects	*Leucinodes orbonalis*
Nematodes	*Meloidogyne incognita*

Source: (Collonnier et al., 2001)

The major pests affecting the production of common eggplant or brinjal are root-knot nematodes (RKNs). Other types of eggplant, including scarlet eggplant (*S. aethiopicum* L.) and wild species such as *S. sisymbriifolium*, *S. torvum* and *S. warscewiczii* have shown resistance capability to some root-knot nematodes (*Meloidogyne* spp.) as reported by Öçal et al. (2018). Resistant and wild relative eggplants are used in the environment and in a co-effective manner to control persistent diseases (Zhuang et al., 2012). In brief, due to environmental changes in recent years, there is in breeding programs an enthusiasm for crop wild relatives (CWR) of agronomically important crops. These taxa have wide-raging relationships and identities (Knapp et al., 2013).

Depending on morphological characteristics, four cultivar groups were established within *Solanum aethiopicum* species, namely; Aculeatum, Gilo, Kumba, and Shum. Among these groups, the Gilo group is the most considerable, and it is valued for its edible oval to rounded fruits (Nkansah, 2001; Lester and Daunay, 2003; Stedje and Bukenya-Ziraba, 2003; Osei et al., 2010; Adeniji et al., 2012; Kamga et al., 2015; Gramazio et al., 2016; Haliński et al., 2017). The only group that is not edible is the Acelatum group (Kamga et al., 2015). As also described by Lester and Daunay (2003), different shapes and sizes of fruits and leaves subsist within the four cultivars of *Solanum aethiopicum* (Table 2.2).

The lack of high quality seeds limits smallholder farmers' potential to produce and benefit from scarlet eggplant and other African indigenous vegetables (AIVs). Furthermore, efforts to support AIVs are hampered by a lack of studies in genetics, breeding, and market demand (Kansiime et al., 2018). Developing successful varieties often depends on the knowledge of specific genes and traits of the parent crops present in the gene pool (Shah et al., 2014). In Rwanda, fewer studies have been undertaken on the scarlet eggplant (*Solanum aethiopicum* L.). For instance, Adeniji et al. (2012) used one accession of *Solanum aethiopicum* Gilo group originated from Rwanda in a morphological diversity study with 43 other

different accessions of scarlet eggplant (belonging to all four groups: Gilo, Kumba, Shum, and Aculeatum) originated from Africa, Asia, Europe, and South America. Similarly, Kamga et al. (2015) used three accessions of scarlet eggplant originated from Rwanda in a morphological characterization study of gboma eggplant, scarlet eggplant, and wild relative (*Solanum anguivi*) accessions originated from five other African countries.

Table 2.2. Four cultivar groups of *Solanum aethiopicum*

Group	Description
Gilo Group	• General fruit has shape and size (2-8 cm diam.) of hen's eggs. It may also exist in depressed spherical to ellipsoid shape. • No edible and hairy leaves subsist.
Shum Group	• Leaves are small and hairless (glabrous) on a short and highly branched plant. • No edible fruits, which are small (1.5 cm diam.) and excessively bitter.
Kumba Group	• The main stem is vigorous with edible and broad glabrous leaves. • Curly and large (5-10 cm diam.) fruits exist. And they are picked green or red. Most of the time eaten raw or stewed.
Aculeatum Group	• Essentially ornamental plants (in Europe) and originated from a presume hybridization between *S. anguivi* and *S. aethiopicum* Kumba Group. • They are hairy and prickly in general. • Grooved and large fruits subsist. • Known as *S. integrifolium* and very useful in disease resistance breeding.

Source: (Lester and Daunay, 2003)

During the domestication of *Solanum aethiopicum* from its ancestral parent, *Solanum anguivi*, distinctive gains or morphological diversity have been recorded. Changes in leaf sizes and shapes from broad, deeply lobed, hairy, prickly, shrubs, perennial plant and spherical fruit (1 cm diam.) to smaller, less

lobed leaves, glabrous, non-prickly, herbs, annual plants and different shaped and sizeed fruit. Obtained cultigens resulting from domestication of *Solanum aethiopicum* displays different morphological features with their ancestor and within themselves (Lester and Daunay, 2003).

This morphological divergence was also confirmed by Adeniji et al. (2012) when they carried out diversity analysis among the *Solanum aethiopicum* groups. Higher divergence was reported in different accessions within the Gilo, Aculeatum, and Kumba groups than between groups themselves. However, a little difference (minimum diversity) has been perceived when analyzing molecular markers, essentially isozymes and DNA between cultigens (Lester and Daunay, 2003).

This eccentricity was also confirmed in recent genetic diversity studies, exhibiting sequence conservation in all eggplant species. It is well-known that most plants share one large gene family pledged to embody disease-resistance genes, which consists of genes encoding the nucleotide-binding site (NBS) and leucine-rich repeat (LRR) motifs. Existing and acknowledged plant resistance genes (R genes) in this class are: Arabidopsis RPM1, flax L6, rice Pib, pepper CaMi, tobacco N, and tomato Mi-1.2. Zhuang et al. (2012) conducted a genetic diversity study of NBS–LRR class disease-resistance gene analogs in cultivated and wild eggplants; *Solanum aethiopicum* gr. Gilo, *Solanum melongena*, *Solanum linnaeanum*, *Solanum integrifolium*, *Solanum khasianum*, and *Solanum sisymbriifolium*. In order to isolate resistance gene analogs (RGAs) from those eggplants and based on conserved regions of the NBS domains from known plant resistance genes, degenerate oligonucleotide primers were designed. Results pointed out that cloned eggplant RGAs belong to the non-NBS–LRR type, which is comparable to the RGAs spotted in other plant species, mainly *Solanaceae* plants. Eight distinct families of eggplant RGAs were established, which suggests a large genetic diversity of eggplant RGAs both in interspecific and intraspecific sequences due to high substitution levels and poor recombination. Conservation of

sub-domains was noticed during sequence analysis of eggplant RGAs, indicating a common ancestral. They deduced that there was a purifying selection in studied eggplants resulting from an accumulation of mutations in NBS-encoding sequences of RGAs or a substantial variation within the sequences.

The recent genome assembly by Song et al. (2019) enabled further research in the breeding of scarlet eggplant Thus, its draft genome size was found to be 1.02 Gb and the utmost 78.9% of the sequenced genome was made up of repetitive sequences (transposable elements - TEs). A novel marker system was developed based on transposon elements by Kalendar et al. (2010), which is a PCR-based technique known as inter-primer binding site (iPBS) markers. It requires no prior knowledge of the genome sequence. Hence, this technique depends upon the existence of long terminal repeat (LTR) retro-transposons of a tRNA complement, which serve as a reverse transcriptase primer binding site (PBS). From a technical standpoint, by using its 3' terminal sequences, tRNA complement adheres to highly conserved primer binding sites (PBSs) of retrotransposons adjacent to 5' LTR. Subsequently, reverse transcription is initiated. Alternatively, primers are used to bind to those specific sequences for the purpose of diverse sequence amplification. It is the perfect tool for the determination of genetic diversity and relationships in a wide range of plant species (Baloch et al., 2015; Yıldız et al., 2015; Yaldız et al., 2018; Ali et al., 2019, Yıldız et al., 2019; Barut et al., 2020).

Several molecular markers have been employed to assess diversity in scarlet eggplant, such as AFLP, ISSR, RAPD, and SSR (Isshiki et al., 1998; Stedje and Bukenya-Ziraba, 2003; Sunseri et al., 2010; Tümbilen et al., 2011; Caguiat and Hautea, 2014). To our best of knowledge, there are no records of the use of iPBS-retrotransposon markers in eggplant breeding studies. Therefore, it was very important to collect the scarlet eggplant germplasm and to characterize it at a molecular level. The resultant information will serve as a starting point for the breeding of this important plant. Keeping this in view, our aim in this study was to

assess genetic variation among all sixty landraces of scarlet eggplant originated from Rwanda by employing the iPBS-retrotransposon marker system, which would help to determine population structure and ease the establishment of the first national breeding program of scarlet eggplant.

2.2. Embryogenesis induction through anther culture

The *in vitro* anther culture technique has been the most commonly used to induce the production of plants from microspores via direct and indirect embryogenesis. Since the 1980's, anther culture has been used in eggplant to produce double-haploid plants from microspore-derived embryos (Rotino, 2016). The generation of androgenic doubled haploid (DH) lines from haploid microspores/pollen represents an attractive alternative to conventional breeding techniques for the production of pure lines or 100% homozygous lines for hybrid seed production in high-value crops. It is well documented that DH technology is one of the most efficient and cost-effective methods for accelerating the development of pure lines from anther culture (Salas et al., 2012; Calabuig-Serna et al., 2020). DH technology is still a long way from becoming a universal method for producing pure lines on a regular basis. In some cases, DHs are obtained, confirming the recalcitrant character of eggplant species/cultivars. This technology is considered to be species dependent. It is also affected by the microspore's developmental stage and other factors such as the physical and chemical settings of the *in vitro* culture system (Salas et al., 2012).

This technique has been optimized and extensively used for more than four decades, for commercial and for experimental purposes, by which fast generation double-haploid parental lines of F1 hybrids are achieved. Furthermore, microspore-derived plants facilitate genetic analysis due to their complete homozygosis characteristics (Rotino, 2016). A handful of studies have been conducted to improve eggplant (*Solanum melongena* L.) through anther culture (Khatun et al.,

2006). Other researchers like Kumar et al. (2003), Khatun et al. (2006), Alpsoy and Şeniz (2007), Salas et al. (2011), and Başay and Ellialtıoğlu (2013) have all successfully applied anther culture to *Solanum melongena*. Salas et al. (2011) evaluated the androgenesis induction through anther culture by comparing both common eggplant accessions to other related species, including one cultivated scarlet eggplant (*Solanum aethiopicum*) accession that produced 21.5 calli/100 anthers while no embryo was observed.

It has been reported that determining the optimal development stage of microspore/pollen for successful androgenesis in different eggplant genotypes, particularly *Solanum melongena*, is more challenging. Thus, in most cases, visual references or morphological criteria differ between cultivars, or even between buds from the same plant donor. However, it has been reported that the proper growth phase from a morphological standpoint should have sepals and petals equal or petals 1-2 mm higher than sepals (Vural et al., 2019). Consequently, Salas et al. (2012) disclosed that younger anthers with mostly young and mid-microspores are preferable for anther culture. Mir et al. (2021) claim that at this younger stage, microspores are close to the first pollen mitosis, allowing embryogenesis to be induced more appropriately. However, this condition is not always met because all microspores in an anther are not always at the same stage. Different stages, on the other hand, exist side by side within the same anther. Younger anthers are also preferred because their walls are less thick, allowing media components and growth factors to diffuse and attain microspores inside the locules during *in vitro* anther culture.

In summary, several media compositions and inductive treatments have been employed to generate double haploids through anther culture in different eggplant F1 hybrids and cultivars under different experimental conditions (Başay and Ellialtıoğlu, 2013). The practical usefulness of anther culture in comparison to other androgenesis induction techniques, such as isolated microspore culture, has

made it one of the most widely used techniques in eggplant, primarily in cultivated eggplant (*Solanum melongena* L.), with the goal of obtaining double haploid parents for conventional breeding (Kashyap et al., 2003; Vural and Ari, 2020). To the best of our knowledge, there has been little research into androgenesis induction in *Solanum melongena* relatives. This is the primary reason why scarlet eggplant (*Solanum aethiopicum* L.), another cultivated eggplant that has long been neglected in scientific research (Shimira et al., 2021), was chosen for determining anther culture's performance in this study.

The objectives of this research were to determine the androgenic capacity of African eggplant using a germplasm collection of *Solanum aethiopicum* landraces from Rwanda, as well as the accessions/landraces' effects on the ability to induce haploid embryos and convert to embryo-derived plantlets.

2.3. *In vitro* salt stress assessment

Soil salinization is one of the most serious environmental disruptors affecting crop growth and crop yields around the world. This has a huge impact on the world's food security. Salinity affects more than 800 million hectares of land worldwide today, and this number is anticipated to rise in the future. High soil salinity caused the plant to consume less water, resulting in a slower growth rate. For instance, biomass production has a substantial impact on fruit yield in eggplant, making eggplant slightly sensitive to salinity (Ünlükara et al., 2010; Kamran et al., 2019; Brenes et al., 2020). Salinity induces many morphological changes, including declining water potential in the soil solution, ionic imbalance, and a greater accumulation of reactive oxygen species (ROS) (Kamran et al., 2019).

It is critical to evaluate economically viable options for improving agricultural yields and food production in order to alleviate both the direct and indirect effects of salinity stress. As a result, adopting diverse crop species or cultivars of a given crop that perform well on salinity-affected land could be a

viable solution (Brenes et al., 2020). According to reports, salt tolerance mechanisms fluctuate amongst plant species to species, implying the existence of an ambiguous salt tolerance mechanism in general (Nieves-Cordones et al., 2016).

The nutritional benefits of eggplants are well known and attributed to the accumulation of various bioactive compounds such as carbohydrates, minerals, phenolics, proteins, and vitamins, not to mention glycoalkaloids, antioxidant compounds that are a major source of their health benefits (Sharma and Kaushik, 2021). Although it has been shown that eggplants are sensitive to salinity and are not well adapted to saline soils, the threshold soil salinity values for fruit yield and vegetative dry weight have been estimated to be 1.5 and 6.7 $dS.m^{-1}$, respectively, making eggplants moderately sensitive to salinity (Ünlükara et al., 2010).

Brenes et al. (2020) reported that salt-induced growth limitation was more substantial in brinjal (*Solanum melongena*) than in its wild relative (*Solanum torvum*), particularly at extremely high salt concentrations, suggesting that the wild species has to some extent higher salt tolerance. *Solanum torvum*'s tolerance mechanisms were largely based on active transport of hazardous ions to the leaves at high salinity conditions and, consequently, revealed a superior potential to store them in the vacuoles, together with higher proline storage than in cultivated eggplant (*Solanum melongena*). Various studies have highlighted the accumulation of harmful ions (Na^+ and Cl^-) as well as K^+ and Ca^{2+} depletion as a result of salt stress on plant metabolism. Thus, under salinity stress, both ionic and osmotic effects have a variety of consequences on plant cell metabolism (Hannachi and Labeke, 2018).

More specifically, in eggplant, salinity considerably decreases K^+ concentration, increases Na^+ concentration, and does not substantially decrease Ca^{2+} content in shoots. K^+ and Ca^{2+} mobility and transport are also restricted. Furthermore, it has been reported that the decline in the K^+/Na^+ ratio is related to rising salinity stress (Fu et al., 2010), which may result in nutritional imbalances

(Ding et al., 2012). Suarez et al. (2021) reported that under salt stress, *S. melongena* restrained Na^+ transport from roots to shoots. The same researchers noted that a high salt tolerance level was strongly associated with a low leaf Na+ concentration. Furthermore, it was demonstrated that for eggplant, Na+ exclusion is a critical component trait by evaluating the gene expression of 12 different genes associated with salt tolerance. These genes included Na^+ and Cl^- transporters, and overall gene expression revealed that eggplant roots did not show general upregulation when exposed to salinity. This implies that overall gene expression in tested genes was lower in leaves than in roots.

Although it was discovered that cultivated eggplant (*Solanum melongena*) is moderately sensitive to salinity, more emphasis on salinity is needed in the production of eggplant and its close relatives (Akinci et al., 2004). Numerous salt tolerance studies on brinjal (*Solanum melongena*) have been undertaken by various researchers (Akinci et al., 2004; Yasar et al., 2006; Fu et al., 2010; Ünlükara et al., 2010; Gunalp et al., 2011; Ding et al., 2012; Hannachi and Labeke, 2018; Galal, 2019; Parkash and Singh, 2020). Nonetheless, a handful of salt tolerance studies on its close relative, scarlet eggplant (*Solanum aethiopicum* L.), have been conducted. As a matter of fact, the purpose of this study was to look at how salt stress influenced morphological alterations in chosen six eggplant (*Solanum aethiopicum* gr. Gilo) accessions during *in vitro* shoot growth. Thus, selection and breeding for salt tolerance is one of the main breeding objectives in eggplant, particularly in *Solanum melongena*.

Fortunately, one of its relatives, the long-ignored and widely consumed *Solanum aethiopicum*, has been recognized as a source of genetic variations and a source of resistance to some pest/disease for brinjal breeding (Collonnier et al., 2001; Ofori and Gamedoagbao, 2005; Gramazio et al., 2016).

Solanum aethiopicum is widely cultivated on the African continent and, to some extent, in Latin America, and it is also the second most broadly cultivated eggplant, with production exceeding 2 million tons in 2018 (Aguessy et al., 2021).

3. MATERIALS AND METHODS

3.1. Collection of plant materials

The original plant materials of *Solanum aethiopicum* gr. Gilo used in this research study were acquired in Rwanda, specifically in two districts located in the Northern Province (Gakenke and Musanze) (Figure 3.1).

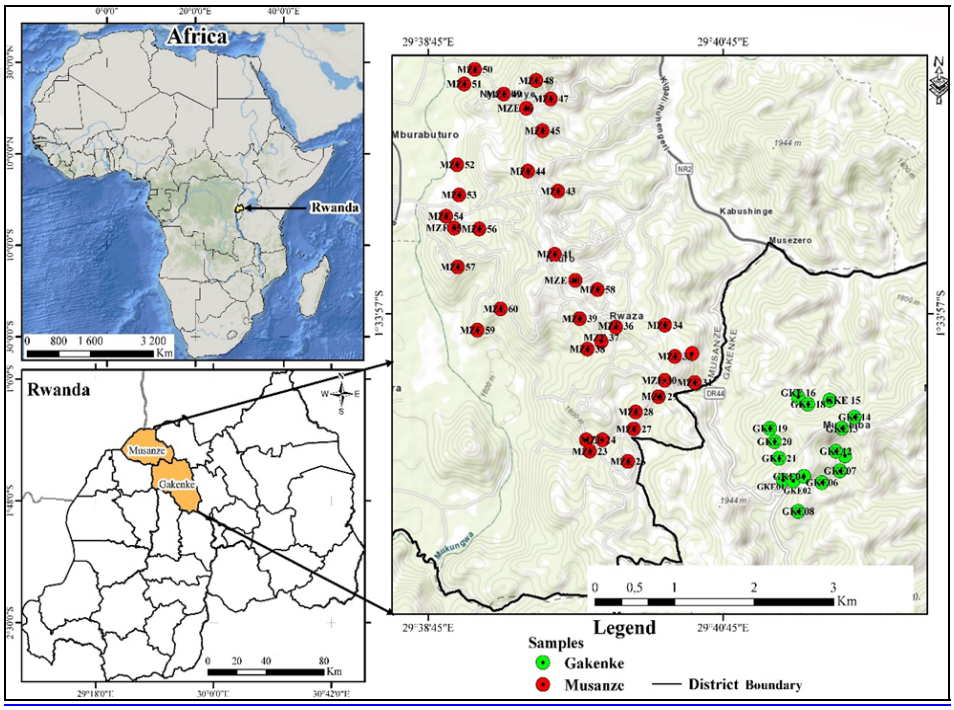

Figure 3.1. Map of Rwanda and distinct collection sites of plant materials.

Using the random sampling method, sixty distinct accessions of *Solanum aethiopicum* gr. Gilo (scarlet eggplant) were initially gathered from the local farmers' fields (Table 3.1 and Table 3.2). Samples were gathered as ripe fruits for the extraction of high-quality seeds (Figure 3.2).

Figure 3.2. Collection of plant materials.
(A & B) Local farmer's fields, (C) Collected fruits during drying process.

Table 3.1. Plant sample identifications and collection sites in Gakenke district.

N°	Sample ID number	GPS Coordinates		Elevation (m)	N°	Sample ID number	GPS Coordinates		Elevation (m)
		Latitude	Longitude				Latitude	Longitude	
1.	GKE01	-1.583976	29.685805	1867	12.	GKE12	-1.580815	29.691776	1843
2.	GKE02	-1.584058	29.686955	1876	13.	GKE13	-1.578302	29.692472	1894
3.	GKE03	-1.584876	29.687721	1883	14.	GKE14	-1.577078	29.693886	1877
4.	GKE04	-1.583553	29.688170	1911	15.	GKE15	-1.575220	29.691076	1873
5.	GKE05	-1.584615	29.688965	1885	16.	GKE16	-1.574879	29.687522	1858
6.	GKE06	-1.584234	29.690199	1851	17.	GKE17	-1.571759	29.687820	1840
7.	GKE07	-1.582951	29.692248	1826	18.	GKE18	-1.575662	29.688637	1838
8.	GKE08	-1.587383	29.687527	1864	19.	GKE19	-1.578313	29.684332	1875
9.	GKE09	-1.588903	29.687610	1820	20.	GKE20	-1.579752	29.684849	1872
10.	GKE10	-1.591151	29.689728	1851	21.	GKE21	-1.581587	29.685357	1846
11.	GKE11	-1.581309	29.692814	1820	-	-	-	-	-

Table 3.2. Plant sample identifications and collection sites in Musanze district.

N°	Sample ID number	GPS Coordinates		Elevation (m)	N°	Sample ID number	GPS Coordinates		Elevation (m)
		Latitude	Longitude				Latitude	Longitude	
1.	MZE22	-1.579563	29.663548	1750	21.	MZE42	-1.554118	29.659907	1826
2.	MZE23	-1.580789	29.663954	1725	22.	MZE43	-1.552361	29.660353	1815
3.	MZE24	-1.579562	29.665403	1754	23.	MZE44	-1.550174	29.656956	1861
4.	MZE25	-1.581738	29.665674	1776	24.	MZE45	-1.545736	29.658608	1797
5.	MZE26	-1.581925	29.668279	1804	25.	MZE46	-1.543279	29.656800	1779
6.	MZE27	-1.578372	29.668924	1764	26.	MZE47	-1.542261	29.659535	1766
7.	MZE28	-1.576508	29.669138	1813	27.	MZE48	-1.540245	29.657885	1857
8.	MZE29	-1.574831	29.671733	1807	28.	MZE49	-1.541727	29.654235	1765
9.	MZE30	-1.573059	29.672430	1856	29.	MZE50	-1.539042	29.650955	1704
10.	MZE31	-1.573290	29.675812	1781	30.	MZE51	-1.540627	29.649759	1676
11.	MZE32	-1.570113	29.675530	1819	31.	MZE52	-1.549461	29.648955	1664
12.	MZE33	-1.570431	29.673575	1879	32.	MZE53	-1.552768	29.649180	1658
13.	MZE34	-1.567012	29.672456	1842	33.	MZE54	-1.555099	29.647707	1662
14.	MZE35	-1.566824	29.668709	1816	34.	MZE55	-1.556394	29.648648	1666
15.	MZE36	-1.567221	29.666913	1814	35.	MZE56	-1.556486	29.651467	1689
16.	MZE37	-1.568786	29.665254	1786	36.	MZE57	-1.560644	29.649027	1723
17.	MZE38	-1.569627	29.663687	1765	37.	MZE58	-1.563125	29.664841	1761
18.	MZE39	-1.566292	29.662810	1808	38.	MZE59	-1.567559	29.651244	1649
19.	MZE40	-1.562108	29.662321	1799	39.	MZE60	-1.565226	29.653867	1697
20.	MZE41	-1.559252	29.659989	1843	-	-	-	-	-

The highlands that comprise this particular sampling area (both districts) are located on average 1,800 meters above sea level. The study area lies between latitudes 1°33' and 1°37' (South) and 29°38' and 29°43' (East). The two districts are part of a single agro-ecological zone referred to as the "Buberuka highlands" (Prasad et al., 2016).

3.2. Field works

For the purpose of this research study, different experimental fields (nurseries, open-field, and greenhouses) were used to establish an eggplant germplasm field in Türkiye from seeds originally collected from Rwanda. Experimental fields were set up depending on the targeted specific (molecular, morphologic, and *in vitro* tissue culture) experiments and the growing seasons (2020, 2021, and 2022). Table 3.3 shows the locations of all experimental fields.

Table 3.3. Location of experimental fields

N°	Institution	City	GPS coordinates	Types of cultivation	Growing season
1.	Çukurova University – Department of Horticulture/ The horticultural research application area.	Adana	37°01'46.1"N 35°22'02.7"E	Greenhouse	2020
2.	Batı Akdeniz Agricultural Research Institute (BATEM) - Department of Vegetable Crops and Ornamentals.	Antalya	36°55'45.7"N 30°58'47.3"E	Greenhouse	2020-2021
3.	Fidesan Fide Ltd.	Antalya	36°54'38.0"N 30°58'25.8"E	Open field	2022

3.2.1. Seeds germination and propagation

At each experimental field site, seed germination and propagation were carried out. For example, in Adana (Çukurova University), in June 2020, *Solanum aethiopicum* gr. Gilo seeds from all 60 different accessions were sown in plant growing trays (4 x 6 cells each) containing a mixture of peat and perlite (3:1, w/w).

Four different eggplant accessions were sown on each plant's growing tray (Figure 3.3). The temperature in the greenhouse was kept at 25 °C during the germination period, and the plant growing trays were frequently irrigated. Unfortunately, due to the poor quality of some seeds, all accessions did not develop

as expected. Depending on the experimental sites and growing seasons, between 52 and 57 accessions germinated successfully.

Figure 3.3. Plant growing trays at different stages
(A) At 1st week of germination, (B) Young eggplant plantlets at 3rd week of germination, (C) Plantlets at 5th week of germination, (D) Plantlets just before transfer (7th Week of germination)

3.2.2. Experimental design and plantlets transfer to the fields

In the seventh week after seed sowing and germination, well-grown eggplant plantlets were transferred from plant growing trays into a well-prepared experimental field bed with natural soil within Çukurova University's main greenhouse (37°01'46.1"N 35°22'02.7"E). Plants were grown in a completely randomized block design, with three replications of each accession arranged within eight rows. The spacing between rows was approximately 1.4 m, and the spacing within rows was approximately 0.75 m (Figure 3.4).

Figure 3.4. Experimental site at Çukurova University
(A) Newly transferred young eggplants, (B) Eleven weeks-old, (C) Thirteen weeks-old eggplants (D) Fourteen weeks-old eggplants.

Water soluble fertilizers such as Potassium Nitrate (13-0-46), Mono-Ammonium Phosphate (12-61-0), and Urea were applied to the plants once a week. Drip irrigation was adopted, and irrigation is always performed on a regular basis to maintain soil moisture. Weeds were also thoroughly removed once per week. Other agronomic management practices were carried out, such as systematic pruning for each plant and application of chemicals in order to manage pests (Spider mite, *Tuta absoluta*, whitefly) and diseases (powdery mildew and botrytis).

3.3. Methodology for genetic diversity analysis

3.3.1. Plant DNA isolation

Fresh young leaves were collected from 52 different accessions grown at Çukurova University's experimental field (greenhouse). Afterward, samples were

taken to the Department of Plant Protection (Sivas University of Science and Technology - Sivas, Türkiye) for molecular analysis.

A slightly modified CTAB (cetyltrimethylammonium bromide) protocol for DNA isolation (Doyle and Doyle, 1990) was used. The said protocol is also more detailed on Diversity Arrays Technology's website via the following link; https://www.diversityarrays.com/orderinstructions/plant-dna-extraction-protocol-for-dart/. The concentration of DNA was determined using an agarose gel (0.8%). The DNA quality and concertation were sustained by using a spectrophotometer (MaestroNano Pro MN-913A, MaestroGen Inc., Hsinchu City, Taiwan - R.O.C.) with a final concentration of five ng/L for all samples (Figure 3.5).

Figure 3.5. Spectrophotometer during DNA samples analysis.
(A) broad and (B) close-up photos

3.3.2. Genetic diversity assessment

Four scarlet eggplant accession samples were randomly selected and used to screen a total of sixty-three iPBS-retrotransposon primers developed from the research work of Kalendar et al. (2010). Only twelve of the most polymorphic primers with satisfying banding profiles were picked for this genetic diversity evaluation. Table 3.4 describes exhaustively chosen iPBS-retrotransposon primers. The annealing temperature, the percentage of guanine-cytosine (G-C) content and the primer sequence are both provided.

Table 3.4. Exhaustive description on 12 selected iPBS-retrotransposon primers

N°	Primer Name	Sequence (5'→3')	GC content %	Annealing Temperature (°C)
1.	2074	GCTCTGATACCA	50.0	50
2.	2075	CTCATGATGCCA	50.0	50
3.	2085	ATGCCGATACCA	50.0	53
4.	2222	ACTTGGATGCCGATACCA	50.0	53
5.	2229	CGACCTGTTCTGATACCA	50.0	52
6.	2239	ACCTAGGCTCGGATGCCA	61.1	55
7.	2241	ACCTAGCTCATCATGCCA	55.0	55
8.	2244	GGAAGGCTCTGATTACCA	50.0	50
9.	2277	GGCGATGATACCA	52.0	52
10.	2385	CCATTGGGTCCA	58.3	50
11.	2386	CTGATCAACCCA	50.0	50
12.	2400	CCCCTCCTTCTAGCGCCA	66.7	51

Source: Kalendar et al. (2010)

PCR amplifications were carried out in strict accordance with the method described by Kalendar et al. (2010). For each eggplant accession sample, a 20 µL reaction mixture containing 5 ng template DNA and a PCR master mix was prepared (Figure 3.6). 1× PCR buffer (Thermo Scientific), 0.2 mM for each deoxyribonucleotide triphosphate (dNTP) (Thermo Scientific, Waltham, MA, USA), 2 mM $MgCl_2$, 1 mM primer for 12/13-nucleotide primers or 0.6 mM primer for 18-nucleotide primers, and 0.2 U Taq DNA polymerase (Thermo Scientific) were included in the PCR master mix. For molecular weight marker, a 100 Base-Pair (bp+) ladder was used.

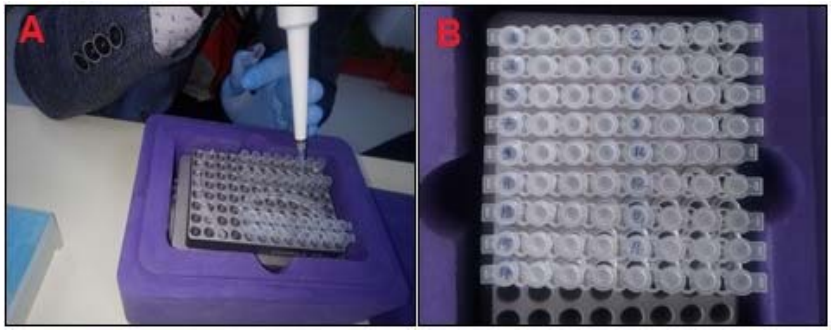

Figure 3.6. Steps of PCR analysis
(A) PCR master mix preparation (B) Ready to use PCR master mix tubes

The thermocycler phase was performed using a T100 PCR Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The denaturation phase at 95 °C (for 3 minutes) was followed by 30 cycles of denaturation at 95 °C (for 15 seconds), annealing at 50-65 °C (primer-dependent) for 1 minute, and a terminal extension step at 72 °C for 5 minutes on the PCR machine (Figure 3.7).

PCR amplicons were electrophoresed for 155 minutes on an agarose gel (2%, w/v) in a gel electrophoresis tank with 0.5 ×Tris-borate-EDTA (TBE) buffer. The electrophoresis instrument is shown in Figure 3.8A. Following the electrophoresis, the visualization step was undertaken. The gel was cautiously stained with ethidium bromide before being loaded into the Imager Gel Doc XR+ system (Bio-Rad Laboratories Inc., Hercules, CA, USA) (Figure 3.8B).

Figure 3.7. PCR machine
(A) PCR thermocycler settings monitor, (B) The PCR thermocycler in use

Figure 3.8. Electrophoresis system
(A) Electrophoresis in use, (B) Imager Gel Doc.

3.3.3. Data Analysis

A binary code was employed to score PCR products due to the dominant nature of the iPBS-retrotransposon marker system, where numbers 0 and 1 were used to designate the absence and presence of clear bands. A DNA ladder (100 bp+) was utilized to measure band sizes. The following Figure 3.9 shows gel visual results from Imager Gel Doc.

POPGENE software (version 1.32) is one of the tools used for genetic diversity assessment. With this software, diversity indices such as Shannon's information index (I), alleles number (Ne), gene diversity (He) and Nei's genetic distance were calculated (Yeh et al., 2000).

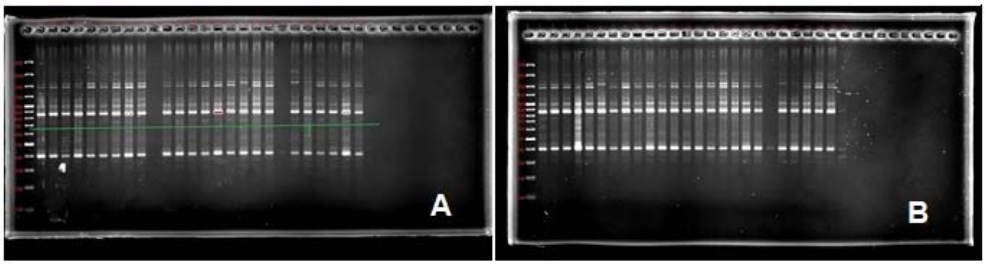

Figure 3.9. Imager Gel Doc's visual results showing DNA bands (A) DNA bands of 1-26 samples for 2386 iPBS primer (B) DNA bands of 1-26 samples for 2386 iPBS primer

Similarly, genetic diversity indicators were also computed through the same software with regard to two distinct districts in which eggplant germplasm was collected. By employing the formula by Roldán-Ruiz et al. (2000) to calculate Polymorphism Information Contents (PIC), PIC = 2fi (1-fi). The frequency of present loci of a molecular marker is denoted by "fi", while the frequency of absent loci is indicated by "1-fi".

The GenAlEx V6.5 software (Peakall and Smouse, 2012) tool was used for the calculation of molecular variance (AMOVA) and principal coordinate analysis (PCoA). In order to get a big picture of the genetic relationship among various *Solanum aethiopicum* gr. Gilo accessions used in this research study, the level of polymorphism was determined by calculating PIC and PCoA. Furthermore, a neighbor-joining analysis, which provides relationship status among 52 different *Solanum aethiopicum* gr. Gilo accessions, was carried out by employing R statistical software (version 3.4.1, Vienna, Austria).

The Bayesian clustering model was employed to obtain more details about the genetic structure of the entire *Solanum aethiopicum* gr. Gilo germplasm through the use of STRUCTURE software (version 2.3.4, Stanford, CA, USA). The most favorable number of clusters (K subpopulations) was determined utilizing a protocol proposed by Evanno et al. (2005), in which 10 independent runs were adjusted for each K value. Moreover, for each run, the preliminary burn-

in phase was adjusted to 50000 with 100,000 MCMC (Markov chain Monte Carlo) iterations. Lastly, the logarithmic probability relative to the standard deviation (ΔK) was plotted against the number of clusters (K). The degree of the membership coefficient greater than or equal to 75% was utilized to categorize individual accessions.

3.4. Methodology for morphological variation analysis

3.4.1. Experimental design

Two main experiments of morphological characterization were both carried out in Antalya.

- **The first experiment**

The first experiment of agro-morphological characterization was performed in a greenhouse at the BATEM Institute from June 2020 to January 2021. The experimental field was 0.32 da. Plant pruning was also carried out.

- **The second experiment**

The second experiment of agro-morphological characterization was carried out in an open field belonging to a private company (Fidesan Fide Ltd.) from March 2022 to July 2022 (Figure 3.10). The experimental field was 0.32 da. There was no pruning.

Figure 3.10. Open field at Fidesan Fide Ltd.
(A) During plantlets plantation (May 2022)
(B) During fruits harvesting (end July 2022)

❖ **Third experiment**

The third experiment of agro-morphological characterization was performed in a greenhouse at Çukurova University from June 2020 to December 2020. The experimental field was 0.36 da. Plant pruning was also carried out. Exceptionally, for this experiment, only fruit characterization was done.

3.4.2. Traits used for morphological variation analysis

The morphological and agronomic valuable traits assessed were mainly based on two distinct descriptors with some modifications. Those descriptors were both advanced by the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the International Board for Plant Genetic Resources (IBPGR) for *Solanaceae* and eggplant, respectively (Boyaci et al, 2020). Measurements on plant agronomic, qualitative, and quantitative traits (leaf prickliness, leaf hairiness, corolla color, plant height, etc.) were gathered according to replications. Similarly, fruit qualitative and quantitative traits (fruit color, length, weight, width, etc.) were measured using 3 ripened fruits (commercial stage) per replication of each accession. The used morphological and agronomically important traits are listed in the following tables (Table 3.5, Table 3.6, and Table 3.7). Figure 3.11, Figure 3.12, Figure 3.13, Figure 3.14 are illustrating the whole process of agro-morphological characterization.

Figure 3.11. Steps of morphological analysis
(A) & (B) Fruit samples collection in the open field of Fidesan Fide Ltd (Antalya – July 2022), (C) Fruit samples after collection.

Table 3.5. List of Descriptors employed for plant qualitative and quantitative traits

Morphological descriptor	**Abbrev.**	**Scale/Unit**	**Description**
Growth habit	GHA	[1-7] >> *1=very upright, 3=upright, 5=intermediate, 7=prostrate*	
Leaf blade lobes	LBO	[1-7] >> *1=very weak, 3= weak, 5=intermediate, 7=strong, 9=very strong*	
Anthocyanin distribution in plant	ADP	[1-7] >> *1=absent, 3=low, 5=intermediate, 7=high*	General anthocyanin distribution in apex, stem, calyx, prickles, leaf veins
Anthocyanin distribution in leaves	ADL	[1-7] >> *1=absent, 3=low, 5=intermediate, 7=high*	Anthocyanin distribution in leaf blade (intervein) as many times is different from the other tissues
Leaf prickliness	LPR	[0-9] >> *0=none, 1=very few (1-2), 3=few (3-5), 5=intermediate (5-10), 7= many (11-20) 9= very many (>20)*	3 representative fully expanded leaves.
Leaf hairiness	LHA	[0-5] >> *0=none, 1= low, 3=intermediate, 5=high*	3 representative fully expanded leaves.
Corolla color	CCO	[0-10] >> *0=yellow, 1=green, 2=greenish white, 3=white 4=rose, 5=pink, 6=dark pink 7=pale violet, 8= violet 9=dark violet ,10=blue*	
Fruit load	FLO	[0-9] >> *0= none, 1=very low, 3=low, 5=intermediate, 7=high, 9=very high*	
Stem color	SCO	[1-5] >> *1=green, 3=greenish purple, 5=purple,*	Measured in 3 representative fully expanded leaves.
Petal color	PCO	[1-5] >> *1=green, 3=greenish purple, 5=purple.*	Measured in 3 representative fully expanded leaves.
Leaf blade width	LBW	Cm	Measured in 3 representative fully expanded leaves)
Leaf blade length	LBL	Cm	Measured in 3 representative fully expanded leaves)
Total plant height	TPH	Cm	Measured in the principal stem at the end of cropping period. Average of 2 plants per rep/accession

Table 3.6. List of Descriptors employed for fruit qualitative traits

Morphological descriptor	Abbrev.	Scale/Unit	Description
Varietal type	VTY	[1-7] >> *1=long, 3=oval, 5=round, 7=striped.*	According to local description already existing
Predominant fruit color	PFC	[0-10] >> *0=dark green, 1=green, 2=milk white, 3=deep yellow, 4=fire red 5= scarlet red, 6= lilac, 7= dark lilac, 8=purple.*	
Secondary fruit color	SFC	[0-10] >> *0=dark green, 1=green, 2=milk white, 3=deep yellow, 4=fire red, 5= scarlet red, 6= lilac, 7= dark lilac, 8= purple, 9=dark purple, 10=black.*	
Fruit color distribution	FCD	[1-7] >> *1=uniform, 3=mottled, 5=netted, 7=striped*	
Fruit undercalyx colour	FUC	[0-2] >> *0= absent, 1= intermediate, 2=present*	Presence of a lighter peel color edge next to calyx
Fruit glossiness	FGL	[1-3] >> *1=opaque, 2=intermediate, 3=bright peel color*	3 representative fruits /block/ accession
Fruit curvature	FCU	[0-9] >> *0=round, 1=no curvature, 3=slightly curved, 5=curved, 7=S shaped, 9= U shaped*	3 representative fruits /block/ accession
Fruit apex shape	FAS	[3-7] >> *3= protruding, 5=smooth, 7=depressed*	3 representative fruits /block/ accession
Position of the maximum diameter	PMD	[2-8] >> *3= about ¼ way from base to tip, 5= about ½ way from base to tip, 7= about ¾ way from base to tip*	3 representative fruits /block/ accession
Fruit cross section	FCS	[1-7] *1=circular, 3=elliptic, 5=smashed, 7=very irregular*	3 representative fruits /block/ accession
Presence of grooves on fruit	PGF	[1-3] >> *1=absent, 3=present*	
Presence of hole in fruit	PHF	[1-3] >> *1=absent, 3=present*	
Fruit end button size	FEB	[0-3] >> *0=none, 1=small, 2=intermediate, 3=large*	
Fruit shape	FSH	[1-9] >> *1=broader than long, 3=as long as broad, 5=Slightly longer than broad, 7=Twice as long as broad, 8=Three time as long as broad, 9=several times as long as broad*	
Presence of clorophyll on the pistil scar	PCP	[1-3] >> *1=absent, 3=present*	
Seed content	SEC	[1-3] >> *1=absent, 3=present*	

Table 3.7. List of descriptors employed for fruit qualitative and quantitative traits

Morphological descriptor	Abbrev.	Scale/Unit	Description
Fruit weight	FWE	g	
Fruit length	FLE	Cm	
Fruit maximum diameter	FMD	Cm	
Peduncle length	PLE	Cm	
Fruit calyx prickliness	FCP	[0-9] >> 0=none, 1=Very few (<3), 3=Few (~5), 5=Intermediate (~10), 7=Many (~20), 9= Very many (>30)	Average of 3 values/block
Calyx fruit coverage	CFC	[1-5] >> 1= less than 10%, 2=10-20%, 3=20-30%, 4=30-40%, 5=50% and more	Average of 3 values/block
Locule number	LON	Count	Average of 3 values/block
Presence of a greenish ring next to the peel	PGP	[0-2] >> 0=no, 1= slight, 2=yes and markedly green	Average of 3 values/block
Average color of the flesh	ACF	[1-7] >> 1=white, 3=greenish, 5=green, 7=cream	Average of 3 values/block

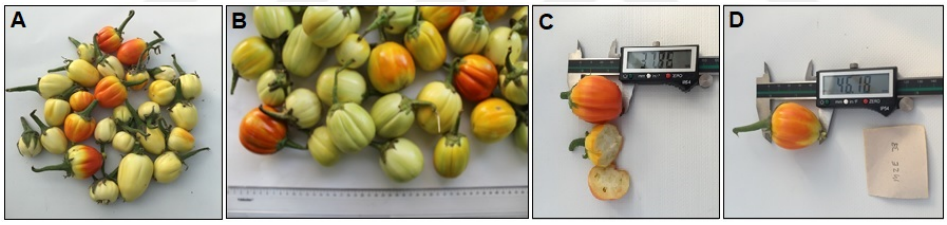

Figure 3.12. Fruit samples collected in greenhouse of BATEM Institute (January 2021) (A) Fruits presentation, (B) Fruits presentation next to a ruler, (C) Fruit height measurement with digital caliper and transverse section of fruit, (D) Fruit height measurement with digital caliper

Figure 3.13. Fruit samples collected in Antalya – July 2022 (Fidesan Fide Ltd.)

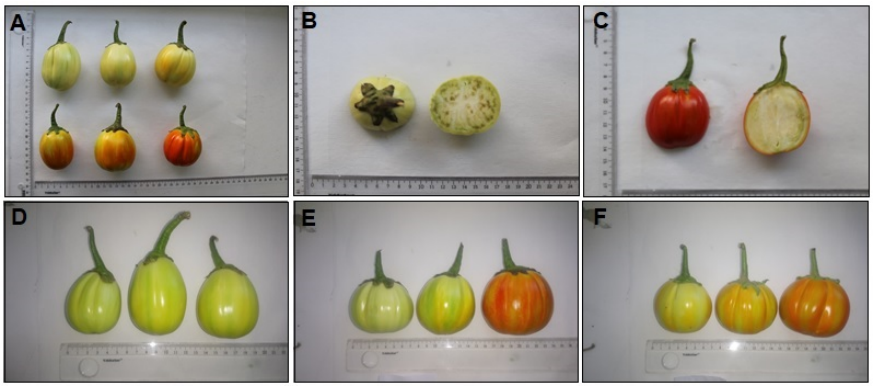

Figure 3.14. Fruit samples collected in greenhouse of Çukurova University (December 2020). (A) Fruits presentation, (B) Transverse section of fruit, (C) Longitudinal section of fruit (D) GKE7 fruit samples, (E) MZE49 fruit samples, (F) MZE47 fruit samples

3.4.3. Multivariate analysis of morphological relationship

Relationship analysis was carried out on morphological traits data from two distinct growing seasons (2020 and 2022) by employing JMP software (version 15.2.1, SAS Inc., Cary, NC, USA). Furthermore, inter-trait correlations, principal component (PCA) and cluster analyses were determined by using the same software.

3.5. Methodology for embryogenesis induction through anther culture

3.5.1. Plant materials and growing seasons

Plant materials used for *in vitro* anther culture study were from the germaplasm collection of *Solanum aethiopicum* gr. Gilo originated from Rwanda (Shimira et al., 2021) and maintained in a greenhouse located at the horticultural research application area of Cukurova University (37°01'46.1"N 35°22'02.7"E).

For the purpose of this experiment on embryogenesis induction, there were two growing seasons. The first growing season in 2020 lasted from June to December. The second growing season in 2021 lasted from May to November.

For the first growing season, the whole eggplant germplasm collection of *Solanum aethiopicum* gr. Gilo made of 60 different accessions were grown. Unfortunately, due to the quality of seeds, only 52 accessions were able to grow and provide enough flower buds for *in vitro* anther culture. Meanwhile, in the second growing season, fewer accessions of *Solanum aethiopicum* gr. Gilo (GKE 12, GKE20, MZE24 and MZE53) were grown with two commercial varieties of *Solanum melongena* (Adana and Kemer).

3.5.2. Samples collection

An *in vitro* anther culture experiment was carried out at *the Prof. Dr. Saadet BÜYÜKALACA - Tissue Culture Laboratory* (Horticulture Department, Çukurova University) in Adana, Türkiye, from October 2020 to January 2021 (first experiment), then from September 2021 to March 2022 (second experiment) to evaluate androgenesis capacity and embryo formation of scarlet eggplant (*S. aethiopicum* gr. Gilo) accessions chosen for their genetic diversity.

Flower buds were collected from donor plants in the greenhouse according to the three stages established by cytological examination (See below). In the most cases, collected flower buds were in stages 1 and 2. Ay few samples were collected every morning and immediately transported into appropriate containers as shown in Figure 3.15B.

Figure 3.15. Eggplants' flower buds (A) Flower buds in the greenhouse just before collection, (B) Flower buds in the containers after collection

❖ **Control of suitable flower bud sizes and development stage of microspore/pollen**

The optimal development stage of microspore/pollen and also the ideal size of flower buds were evaluated using microscope observations. According to Vural et al. (2019), the best stage for microspores is when they are uninucleate before the first pollen mitosis or binucleate at the start of cytokinesis. These observations permitted the connection between floral bud sizes and microspore developmental stages to be identified (Figure 3.16).

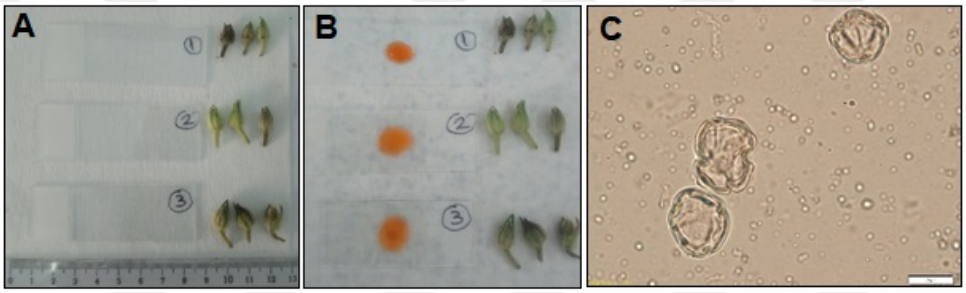

Figure 3.16. Flower bud size selection (A) Three type of flower buds depending on their size, (B) Step of microspore staining with acetocarmine on labeled microscope slides, (c) Microspores observation from microscope (Magnification 100X ~ 10 µm)

Afew samples of freshly collected flower buds of *Solanum aethiopicum* were used. Firstly, individual anthers were dissected out from flower buds and then crushed/squashed on petri dishes using a scalpel to expose micropores. Afterward, those microspores were stained using acetocarmine. The obtained mix was put on microscope slides and covered with glass slides for further cytological observation. From the cytological examinations, three suitable development stages for anther culture were established.

3.5.3. Media preparation

The protocol for media preparation used in this study was first proposed by Dumas de Vaulx et al. (1982) for anther culture in eggplants. Three distinct

nutrient media (C, R, and V3) containing different plant growth regulators at different concentrations were used (Table 3.8).

Table. 3.8. Details on C, R and V3 nutrient media (mg/L)

	C medium	R medium	V3 medium		C medium	R medium	V3 medium
Macro nutrients				**Vitamin and amino acids**			
KNO_3	2150	2150	1900	Myo-inositol	100.00	100.00	100.00
NH_4NO_3	1238	1238	1650	Pyrodoxin HCl	5.500	5.500	5.500
$MgSO_4$-$7H_2O$	412	412	370	Nicotinic acid	0.700	0.700	0.700
$CaCl_2$-$2H_2O$	313	313	440	Thyamine HCl	0.600	0.600	0.600
KH_2PO_4	142	142	170	Calcium panthotenate	0.500	0.500	0.500
$Ca(NO_3)_2$-$4H_2O$	50	50	-	Vitamin B_{12}	0.030	-	-
NaH_2PO_4-H_2O	38	38	-	Biotin	0.005	0.005	0.005
$(NH_4)_2SO_4$	34	34	-	Glycin	0.100	0.100	0.200
KCl	7	7	-				
Micro nutrients				**Chelated Irons**			
$MnSO_4$-H_2O	22.130	20.130	0.076	Na_2-EDTA	18.65	18.65	37.30
$ZnSO_4$-$7H_2O$	3.625	3.225	1.000	$FeSO_4$-$7H_2O$	13.90	13.90	27.80
H_3BO_3	3.150	1.550	1.000				
KI	0.695	0.330	0.010				
Na_2MoO_4-$2H_2O$	0.188	0.138	-				
$CuSO_4$-$5H_2O$	0.016	0.011	0.030				
$CoCl_2$-$6H_2O$	0.016	0.011	-				
$AlCl_3$-$6H_2O$	-	-	0.050				
$NiCl_2$-$6H_2O$	-	-	0.050				

The anther culture initiation is performed on C medium for the first 13 days. Then, R medium is used for sub-culturing till embryos are obtained. Lastly, V3 medium is used for embryo growth and development. Details on the different concentrations of plant growth regulators are given in Table 3.9. Figure 3.17 shows media stock preparation and the sterile bench used in this experiment.

Table 3.9. Plant growth regulators and their concentrations

Growing Medium	**Plant growth regulators**		**Sucrose**	**Agar**
	2.4-D	**Kinetin**		
C medium	5*	5*	100**	8**
	1*	1*	100**	8**
R medium	0.01	-	30**	8**
V3 medium	-	-	30**	8**
*: mg/L, **: g/L				

Figure 3.17. Nutrient media preparation (A) Three type of stock solutions used during media preparation, (B) Prepared medium and petri dishes in the bench just before dispensing the medium

3.5.4. Experimental design for *in vitro* embryogenesis

3.5.4.1. Experiment 1 (2020): "Effects of *S. aethiopicum* accessions on *in vitro* androgenesis induction"

In this experiment, the entire germplasm collection of *Solanum aethiopicum* gr. Gilo made up of 52 different accessions originated from Rwanda was essayed on C medium supplemented with 2.4-D (5 mg/l) and kinetin (5 mg/l).

The experiment was conducted in the randomized complete blocks design (RCBD) with 26 replications for each accession. Here, one accession that did not reach that same number of replications was excluded from the study.

3.5.4.2. Experiment 2 (2021): "Effects of *S. aethiopicum* accessions and 2 Turkish eggplant (*Solanum melongena*) varieties as well as 2 hormanal treatments on *in vitro* androgenesis induction".

In this experiment, the 4 accesions (GKE12, GKE20, MZE24, and MZE53) of *Solanum aethiopicum* gr. Gilo and 2 commercial eggplant (*Solanum melongena*) genotypes (Adana and Kemer) were tested on two distinct treatments and evaluated for *in vitro* endrogenesis induction on C medium. The experiment was conducted in the randomized complete blocks design (RCBD) with 31 replications for each accession/genotype. For treatment 1, C medium was supplemented with 2.4-D (5 mg/l) and kinetin (5 mg/l) while, for treatment 2, a 1 mg/l of 2.4-D and 1 mg/l of kinetin were supplemented in C medium.

3.5.5. Initiation of Anther Culture

For the disinfection step, a 20% commercial sodium hypochlorite (NaOCl) solution was used for 15 minutes, followed by four rinses with sterile distilled water (Figure 3.18).

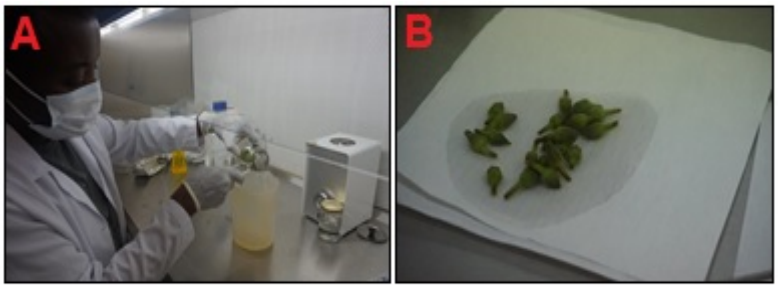

Figure 3.18. Sterilization process (A) Flower buds sterilization under the bench, (B) Sterilized flower buds on sterile tissue paper

By working under a sterile bench (laminar flow hood), the aseptic condition was maintained throughout the procedure, Başay and Ellialtıoğlu (2013). After dissecting the flower buds and carefully removing their filaments, anthers were carefully placed on the appropriate media to avoid anthers sinking below the media surface. Petri dishes are sealed before being placed into the incubator (Shimira et al., 2019). Figure 3.19 illustrates the whole process of *in vitro* anther culture.

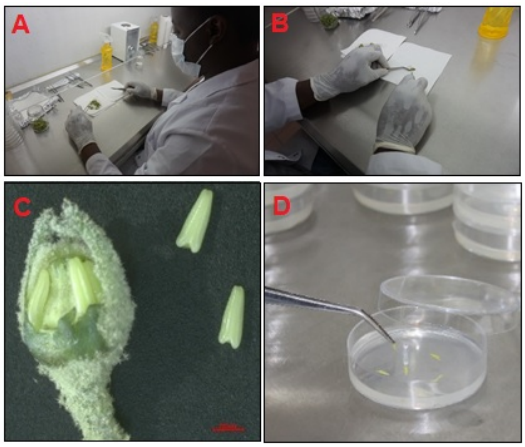

Figure 3.19. Anther culture process (A) (B) (C) Flower bud and anthers in 200 µm (D) Anther placement on media

Excised anthers were cultured in the induction medium (C), supplemented with appropriate plant growth regulators (Table 3.8 and Table 3.9), and placed at 35 °C in an incubator under darkness for 8 days, according to the protocol developed by Dumas de Vaulx et al. (1982). Figure 3.20 shows newly cultivated peti dishes in the incubator.

Afterwards, petri dishes were kept in the growth chamber at 25 °C for 16 hours under fluorescent light (50 mol/$m^{-2}.s^{-1}$). Anthers were transferred to the differentiation medium on the 13th day (R). Sub-culturing can begin once embryos appear in V3 medium (Salas et al., 2012). In this study, R medium was renewed every 30 days while waiting for embryos development.

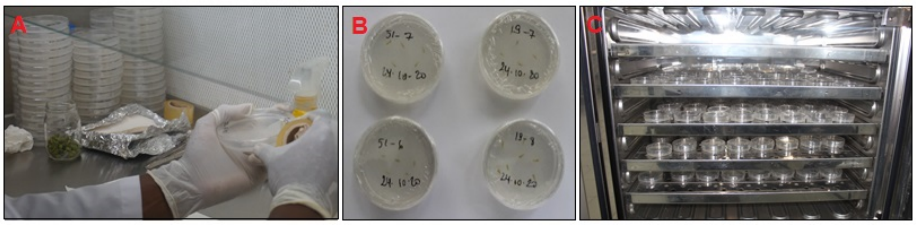

Figure 3.20. Major steps after anther culture (A) The sealing of petri dish (B) Sealed petri dishes ready to be incubated (C) Incubator containing newly cultured anthers

3.5.6. Embryo growth and development

The monitoring and observation of changes and callus development as well as embryo development were carried out by using a stereo microscope, Olympus SZ61 (Olympus Corporation, Shinjuku, Tokyo, Japan). This microscope, with high quality optics, allowed the thorough following of anthers' transformation to callus (Figure 3.21) and eventually the observation of a few embryos (Figure 3.22).

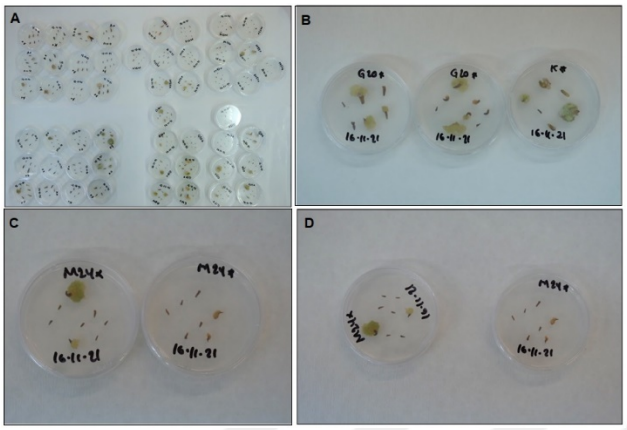

Figure 3.21. Petri dishes in the growing room (A), (B), (C) and (D) Petri dishes with some developed anthers

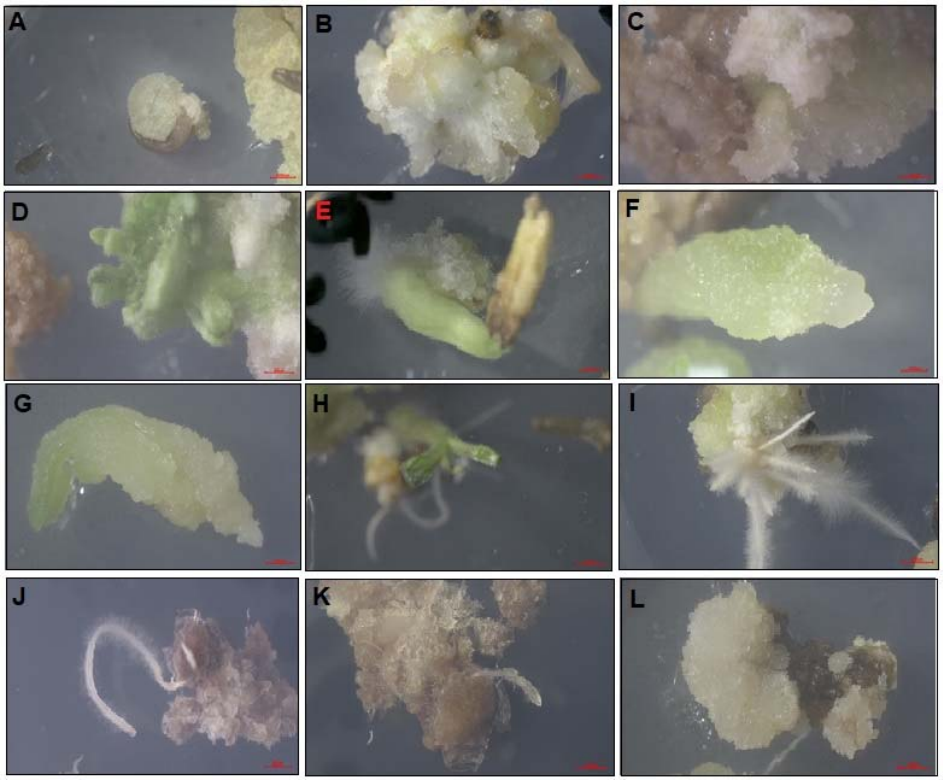

Figure 3.22. Embryo-like and embryo formations (A) to (L) Picture are shown at 300 µm

The protocol used by Vural and Ari (2020) for embryo conversion and acclimatization was slightly modified to meet the needs of our experiment. In brief, V3 medium was used after embryos were obtained to ensure their optimal growth and development. Firstly, embryos were transferred from petri dishes (R medium) to small glass flasks containing V3 medium (7 cm in height) (Figure 3.23A, Figure 3.23B, and Figure 3.23C). To obtain overall healthy embryos, grown embryos with slightly longer shoots and roots (or matured enough) were separated from their small siblings and transferred to new individual glass flasks of superior width and height (7 cm, 8.5 cm, and 13.5 cm) containing V3 medium (Figure 3.23D).

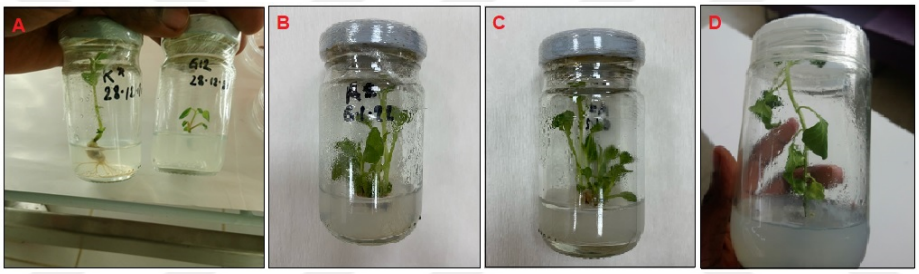

Figure 3.23. Eggplant seedlings into small grass flasks

After forming strong shoots and roots, *in vitro* embryo-derived plantlets were transferred to pots with a mixture of peat moss and perlite (3:1 v/v) for acclimatization (Figure 3.24). They were later moved to a greenhouse and subjected to a gradually decreasing humidity and gradually increasing lighting regime.

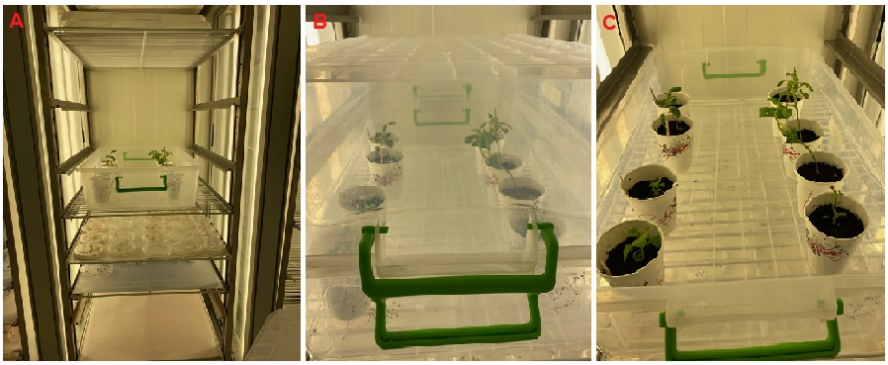

Figure 3.24. Embryo derived seedlings during acclimatization

3.5.7. Ploidy level analysis

Flow cytometry was used to determine the ploidy level. Fresh and young leaf samples from ex vivo embryo-derived plantlets (eggplant regenerants) were collected from the acclimation greenhouse and transported directly to the lab for ploidy determination. At this point, the research method used by Shimira et al. (2019) was employed. In that regard, leaf samples (0.5 cm^2 of leaf tissue per plantlet in individual petri dishes) were chopped with a harsh razor blade after adding 400 µl of extraction buffer (Figure 3.25). Afterwards, all these samples were incubated for 30 to 60 seconds. Following that, samples were filtered using a special filter (Partec 50 m Cell Trics®), and 1.6 ml of staining buffer was added to the sample tube for a brief incubation of 30 to 60 seconds before taking readings on a flow cytometer (Figure 3.26).

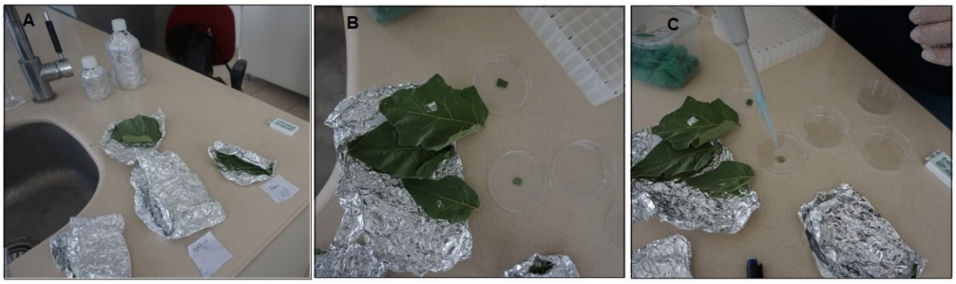

Figure 3. 25. Collected leaf samples for flow cytometry analysis

Figure 3. 26. Flow cytometry analysis

3.5.8. Data collection and analysis

The anthers were examined for callus initiation after four weeks of incubation. Furthermore, *in vitro* cultures were scored for the frequency of callus induction. The frequency was calculated as the ratio between the numbers of anthers responding to callus induction or regeneration to that of the total number of anthers inoculated as described by Kumar et al. (2003). For statistical analysis, descriptive statistics were conducted, and for advanced statistics, JMP (version 15.2.1, SAS Inc., Cary, NC, USA) statistics package software was used. An ANOVA test ($p \leq 0.05$) was conducted to evaluate global significant differences, and then a Fisher's least significant difference (LSD) test for multiple comparisons was used to group the different eggplant accessions into clusters, with significant differences at a p value < 0.05.

3.6. Methodology for *in vitro* salt stress assessment

3.6.1. Plant materials and experimental design

An *in vitro* experiment was carried out at "*the Prof. Dr. Saadet BÜYÜKALACA - Tissue Culture Laboratory*" (Horticulture Department, Cukurova University) in Adana, Türkiye, from July 2021 to November 2021 to evaluate salt stress induced morphological changes during early *in vitro* shoot growth in six different eggplant (*S. aethiopicum* gr. Gilo) accessions chosen for their genetic diversity. They were among three population groups identified by Shimira et al. (2021) when they assessed genetic diversity from fifty-two accessions of eggplants

using iPBS retrotransposon markers. Thus, population A is represented by GKE11 and MZE44, population B by GKE20 and MZE29, and population C by MZE49 and MZE51. During subculture, four salt stress treatments (20 mM, 40 mM, 80 mM, and 160 mM) and the control (0 mM) were applied to the culture medium. In this study, the experiment consisted of 660 plant shoots in which four treatments and one control were applied, with 22 replications for each accession. We investigated how traits related to growth (number of leaves, seedling length, aerial fresh and dry weight) responded during eggplant seedling growth under different levels of salt stress.

3.6.2. Preparation of seed materials sterilization

Six eggplant accessions (GKE11, GKE20, MZE29, MZE51, and MZE49) were chosen for their seeds. And only uniform and homogenous seeds without mechanical damage were preferred for surface sterilization. Furthermore, seeds were surface-sterilized with 70% ethanol and rinsed thoroughly with distilled water. Then, they were soaked in a solution of 0.5% sodium hypochlorite for 10 min with continuous stirring (Kirtiş and Aasim, 2019). Additionally, seeds were dried for a few seconds on paper filters after three rinses with sterile distilled water.

3.6.3. Seed germination

The standard growth medium, also known as Murashige and Skoog (MS) (Murashige and Skoog, 1962), was prepared with 3% (w/v) sucrose and the pH was rectified to 5.8 with 1 M NaOH before adding agar. Afterward, the medium was autoclaved and later docked into appropriate petri dishes. The well-dried seeds were germinated on agar-solidified (0.65%) MS medium in petri dishes under non-saline conditions. Figure 3.27 illustrates the germination of eggplant seeds in a petri dish.

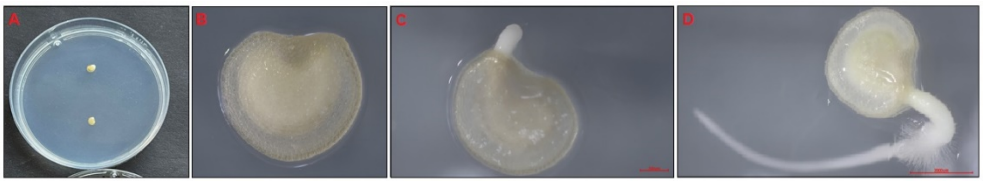

Figure 3.27. Seed germination process (A) Newly cultured seeds on MS medium, (B) Microscopic picture of the eggplant seed, (C) Radicle emergence at 5th day of germination (electronic microscope ~ 700 µm), (D) More developed radicle on the 7th day of germination (electronic microscope ~ 200 µm)

All petri dishes were later transferred to the tissue culture growing room with a photoperiodicity of 16 hours of light and 8 hours of darkness at 25°C. Germination levels were monitored. Thus, the germination was monitored at 24-hour intervals until the sub-culturing. Figure 3.28 shows petri dishes in the growing room.

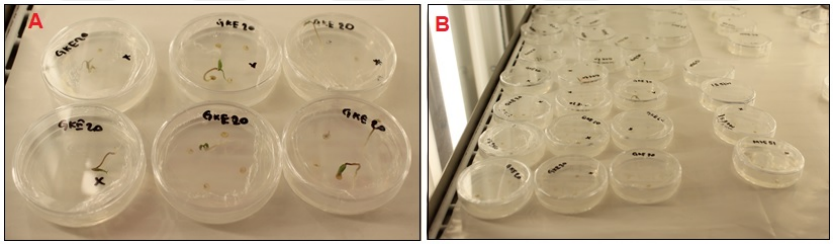

Figure 3.28. Petri dishes in the growing room (A & B) Petri dishes containing radicle with different stages of development

3.6.4. *In vitro* salt treatment of eggplant seedlings

When eggplant seedlings were at the two-true leaf stage, the sub-culturing was conducted into tubes containing salt (NaCl) treatments with different levels of concentrations. Five concentrations (treatments) of NaCl were prepared and added to the medium (MS): 0 (control), 20, 40, 80, and 160 mM. At this stage, only grown seedlings were sub-cultured in tubes (Figure 3.29).

Figure 3.29. Eggplant seedlings in tubes (A) Seedlings of MZE44 shown in different treatments, (B) Tubes into stainless steel rack, (C) Tubes in collection shelf in the growing room

For each genotype, at least 22 tubes (or replications) were prepared for each treatment (22 tubes x 5 treatments). In total, for each eggplant landrace, there were at least 110 seedlings (tubes). The seedlings were kept in a growth chamber at 28 °C with a photoperiodicity of 16 hours.

3.6.5. Measurements, data processing and analysis

After 6 weeks, all seedlings per treatment were measured. We measured parameters such as seedling length, number of leaves, internode spacing, aerial fresh weight (FW) and dry weight (DW). The aerial sections of the seedlings (shoots and leaves) were weighed (FW), then dried in a forced-draft oven at 80 °C for 24 hours before being re-weighed (DW). Moreover, tissue water content (TWC) was determined as the (FW-DW/FW) ratio by following the protocol advanced by Hannachi and Labeke (2018). Figure 3.30 depicts the use of a digital caliper to measure shoot height.

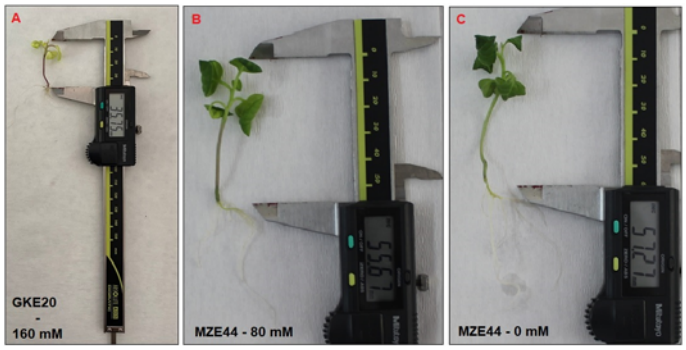

Figure 3.30. Eggplant seedling's height measurement with digital caliper (A) Seedlings of GKE20 in 160mM salt treatment, (B) Seedlings of MZE44 in 80mM salt treatment, (C) Seedlings of MZE44 in the control treatment (0mM)

Data analyses were performed with JMP (version 15.2.1, SAS Inc., Cary, NC, USA) statistics package software. To identify differences among the treatments per accession, the same software was used to perform an analysis of variance (ANOVA) followed by a post-hoc Tukey's honestly significant difference (HSD) test (P = 0.05). In order to obtain more details about the data structure and variation, the principal component analysis (PCA) based correlation matrix and hierarchical clustering analysis (HCA) were carried out using the same JMP software.

4. RESULTS AND DISCUSSION

4.1. Results and discussion for genetic diversity assessment

4.1.1. Genetic diversity and population structure analysis

A total of 329 bands with impeccable banding profiles have been generated from all twelve polymorphic iPBS-retrotransposon primers used in this study. The overall average number of bands per primer was 27.4 throughout the 52 scarlet eggplant accessions. Moreover, the highest and lowest numbers of scorable bands were found to be 38 and 13 for primers 2241 and 2400, respectively. The count of polymorphic bands was 276 out of 329 initial bands, which is equal to a percentage of 85.03. Thus, polymorphism ranged from 55.56% for the primer 2277 as the lowest to 100% for three primers; 2085, 2244 and 2386 as the highest. Therefore, each primer had an average of 23 polymorphic bands (Table 4.1).

The obtained mean value for PIC was 0.363, which is the translation of a PIC value range with the highest point at 0.581 and the lowest point at 0.155 for 2244 primer and 2239 primers, respectively. The primers 2244 and 2222 displayed the maximum and minimum number of effective alleles with 1.476 and 1.071, respectively. Besides, an average of 1.298 was observed for Ne. Additionally, Shannon's information index, a maximum of 0.473 and minimum of 0.106 were recorded for primer 2244 and primer 2222, respectively. A mean of 0.300 was also recorded for Shannon's information index.

Genetic diversity displayed a 0.305 maximum and a 0.052 minimum value for the primers 2244 and 2222, respectively. Furthermore, it was observed that the maximum Nei's genetic distance (0.6781) was obtained between accessions MZE 53 and GKE 11, while a minimum genetic distance of 0.0122 was encountered between MZE 29 and MZE 28 accessions.

Table 4.1. Details on genetic diversity indices

Primers	Total bands	Polymorphic bands	Polymorphism (%)	PIC	Ne	He	I
2074	14	13	92.86	0.519	1.420	0.252	0.387
2075	29	22	75.86	0.423	1.408	0.227	0.341
2085	29	29	100.00	0.288	1.150	0.116	0.222
2222	27	22	81.48	0.165	1.071	0.052	0.107
2229	27	25	92.59	0.555	1.402	0.250	0.393
2239	34	21	61.76	0.155	1.151	0.099	0.167
2241	38	27	71.05	0.256	1.257	0.169	0.276
2244	33	33	100.00	0.581	1.476	0.305	0.473
2277	27	15	55.56	0.207	1.260	0.153	0.238
2385	32	31	96.88	0.276	1.283	0.191	0.316
2386	26	26	100.00	0.413	1.389	0.243	0.387
2400	13	12	92.31	0.523	1.306	0.188	0.299
Mean	27.42	23	85.03	0.363	1.298	0.187	0.300
Total	329	276					

PIC: Polymorphism information content, Ne: Effective number of alleles, He: Gene diversity, I: Shannon's information index

Additionally, a broad assessment of genetic diversity was carried out at the district level (Table 4.2). A superior level of diversity was noticed among accessions from Musanze district compared to those from Gakenke district by disclosing the highest Ne (1.260), Gene diversity (0.167), and Shannon's information index (2.268). Moreover, we also determined genetic distance at the district level and it was found that Musanze has the highest (0.252) compared to Gakenke district, with the lowest genetic distance (0.154). By running the model-based STRUCTURE algorithm, the scarlet eggplant germplasm was divided into 3 major populations on the basis of a membership coefficient greater than or equal to 75% (Figure 4.1).

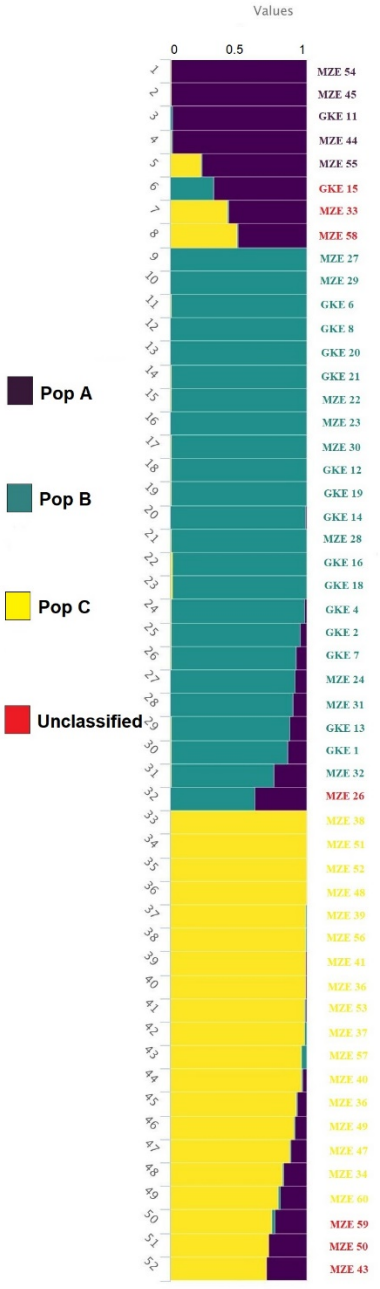

Figure 4.1. Population structure of *S. aethiopicum* gr. Gilo germplasm

Population A (Pop A) had 5 accessions (equals to 9.6% of the germplasm); population B (Pop B) had 23 accessions (equals to 44.2% of the germplasm); population C (Pop C) had 17 accessions (equals to 32.7% of the germplasm); and the remaining 13.5% of the germplasm contained only 7 accessions which were unclassified.

Table 4.2. District and population based genetic diversity

	Factor	Ne	he	I	Mean GD
Districts	Gakenke (GKE)	1.209	0.136	0.218	0.154
	Musanze (MZE)	1.260	0.167	0.268	0.252
Populations	Pop A	1.321	0.186	0.273	0.378
	Pop B	1.149	0.093	0.149	0.103
	Pop C	1.110	0.073	0.120	0.087
	Unclassified	1.370	0.225	0.342	0.266

Ne: Effective number of alleles, He: Gene diversity, I: Shannon's information index, Mean GD: Mean Nei's genetic distance.

A population-based genetic diversity analysis was also performed for the STRUCTURE based populations. A greater level of diversity was displayed among accessions from Pop A compared to the other two populations (B and C), with a superior number of Ne (1.321), He (0.186) and I (0.273). But, if we consider all accessions belonging to the "Unclassified group" as one population,.they were more diverse with higher Ne, He and I values, with 1.370, 0.225, and 0.342, respectively. Population C expressed low diversity among its accessions, with a low value number for all assessed diversity indices. Population-based genetic distance was higher in Pop A (0.378) and lower in Pop C (0.087). Neighbor-joining clustering was also used to group the whole African eggplant germplasm in accordance with the country's districts (Figure 4.2). Furthermore, PCoA was also carried out (Figure 4.3).

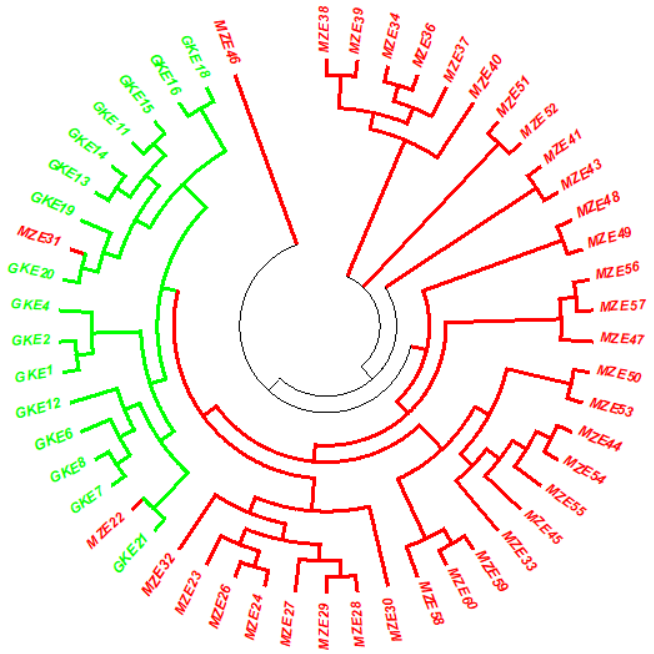

Figure 4.2. Neighbor-joining analysis of *S. aethiopicum* gr. Gilo germplasm

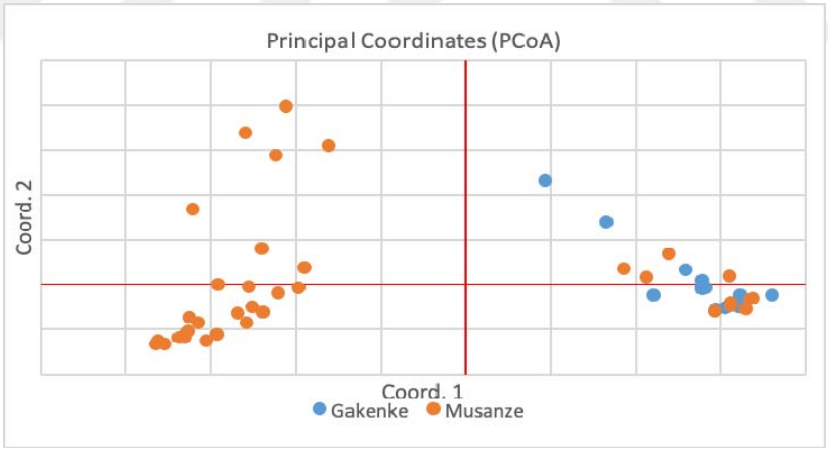

Figure 4.3. Principal coordinate analysis (PCoA) of *S. aethiopicum* gr. Gilo germplasm

Thus, the entire scarlet eggplant germplasm made by 52 accessions was divided into three core clusters that matched with the 3 populations (Pop A, Pop B and Pop C), which led to structure analysis. An analysis of variance (AMOVA) was employed to quantify total variations. Thus, the variation within populations was found to be 81% and the variation among populations was 19% (Table 4.3).

Table 4.3. Analysis of molecular variance (AMOVA)

Summary AMOVA Table

Source	df	SS	MS	Est. Var.	%
Among Pops	1	222.673	222.673	8.432	19
Within Pops	50	1793.924	35.878	35.878	81
Total	51	2016.596		44.310	

4.1.2. Discussion on the genetic diversity and population structures

❖ **Polymorphism in *Solanum aethiopicum* gr. Gilo germplasm**

The scarlet eggplant is an African indigenous plant, popular in African cuisine. Nevertheless, there is a lack of information about the characterization of scarlet eggplant germplasm using retrotransposon markers in Rwanda and on the entire African continent. The mean percentage of polymorphic bands obtained in this present study was greater compared to the 79% reported by Tümbilen et al. (2011) when they employed SSR markers to study several accessions of *S. melongena* and relative species, including scarlet eggplant.

Using RAPD markers, Stedje and Bukenya-Ziraba (2003) found a lower percentage of polymorphic bands (48%) in various populations of scarlet eggplant and *S. anguivi*. Our findings were also greater than the percentage of polymorphic bands reported by Sunseri et al. (2010) using both AFLP and SSR markers to assess the genetic diversity of scarlet eggplant germplasm from various geographic origins. The results from polymorphic information content (PIC) displayed a mean

PIC higher than what has been obtained by Caguiat and Hautea (2014) when they assessed genetic diversity among 6 different eggplant species (*S. melongena* and relatives such as *S. americanum*, *S. linnaeanum*, *S. mammosum* and *S. torvum*), including scarlet eggplant using SSR marker.

Other scholars have found lower mean PIC values using SNPs in the genetic characterization of scarlet eggplant and its close relative, *S. macrocarpon* (Borràs et al., 2015) and using SSR in brinjal (Nunome et al., 2009). An exceptionally high number of polymorphic information content (0.47) was obtained by Vilanova et al. (2012) using SSR markers on several accessions belonging to three distinct species of eggplant (brinjal, gboma, and scarlet). Usually, a high PIC value (greater than 0.5) explains the high polymorphism of used markers matching with genome distribution (Liu et al., 2018).

Additionally, PIC values between 0.25 and 0.5 suggest loci of intermediate polymorphism (Ge et al., 2013). Therefore, making iPBS retrotransposon markers adequate for genetic diversity analysis due to the obtained intermediate PIC value greater than numerous prior studies in scarlet eggplant diversity analysis.

- **Genetic diversity and population structure**

In this study, different computed diversity indices (Ne: Effective number of alleles, He: Gene diversity, I: Shannon's information index) revealed the existence of genetic variations in the scarlet eggplant germplasm originated from Rwanda (Table 4.1). The mean value of Ne obtained in this study was lower than the average Ne value (1.631) obtained by Ge et al. (2013) when they analyzed the genetic diversity of Chinese eggplant landraces (*Solanum melongena*) using SSR markers, and it was also lower than the average Ne value (2.015) obtained by Wang et al. (2010) when they studied several accessions of *Solanum melongena* and two of its wild relatives (*Solanum integrifolium* and *Solanum torvum*). Furthermore, a superior Ne value (2.60) was obtained by employing SSR markers

to study the diversity of Spanish landraces of eggplant (*Solanum melongena*) (Vilanova et al., 2012).

On the contrary, the Ne value obtained in this study was higher than that obtained in previous iPBS-retrotransposons based studies in diverse crops (Yıldız et al., 2015; Yaldız et al., 2018; Yıldız et al., 2019; Barut et al., 2020). Mean genetic diversity and Shannon's information index were also found to be higher than reported by Yıldız et al. (2015), Yıldız et al. (2019) and Barut et al. (2020) in previous studies. The likely reasons for the presence of higher values for various diversity indices might be due to the high efficiency of the iPBS-retrotransposon marker system in assessing the genetic diversity or the higher diversity might come from the nature of germplasm itself. For instance, a greater diversity of *Solanum aethiopicum* gr. Gilo was also acknowledged in the study by Tümbilen et al. (2011) using EST-SSR markers.

The recorded mean genetic distance among 52 accessions of *Solanum aethiopicum* was 0.1995. Two individuals (MZE53 and GKE11) from distinct districts displayed a maximum genetic distance (0.6781) between themselves. Since they are genetically distinct, they can be proposed as candidate parents in future breeding programs of scarlet eggplants. A minimum genetic distance (0.0122) was found between MZE28 and MZE29, which suggests a genetic similarity between the two accessions.

Thorough details on genetic variations in scarlet eggplant were checked with regard to diversity indices based on the district of collection. Accessions from the Musanze district displayed a higher level of diversity as compared to the district of Gakenke (Table 4.2). The observed higher diversity in the district of Musanze might be related to the size of samples, which was slightly higher than samples collected in the district of Gakenke. Furthermore, a model-based STRUCTURE algorithm revealed the existence of 3 population groups (Pop A, Pop B and Pop C) within the scarlet eggplant germplasm (Figure 4.1). The

groupings were expressing genetic similarity of accessions with regards to membership coefficient greater than or equal to 75%.

Population A mainly consisted of accessions from Musanze district and one accession from Gakenke, whereas in population B, accessions from Gakenke were predominantly grouped with accessions from Musanze. Briefly, there was an admixture of individuals from Musanze and Gakenke districts, both in Pop A and Pop B. However, Pop C exclusively accommodated individuals from Musanze. The obtained population structure might be explained by the existence of gene flow between population groups, which might also have originated from farm-to-farm seed diffusion among smallholder farmers and the geographic features of the Buberuka highlands. They consist of several hills with narrow valleys that do not limit winds, therefore allowing pollen dispersal. This hypothesis was further verified by determining the population-based diversity parameters. It was found that a higher level of diversity was present among accessions from Pop A and a low level of diversity was encountered in Pop C (Table 4.2).

The neighbor-joining assessment partitioned the studied germplasm into three major groups of populations, and the first two of which were dominated by accessions from Musanze, and the third of which accommodates both accessions from Musanze and Gakenke districts. Furthermore, the aforementioned individuals from Gakenke district were more clustered in one sub-group with only two exceptional individuals from Musanze (Figure 4.2).

Results from neighbor-joining clustering may be explained by the possibility of seed exchanges among farmers through the informal seed sector dominating in Rwanda and particularly in the agro-ecological zone of the Buberuka highlands. This ultimately emerged in higher gene flow in the eggplant germplasm. The obtained maximum genetic distance between accessions MZE53 and GKE11 is reaffirmed by neighbor-joining, owing to their separation into two distinct sub-

clusters. Individuals with a minimum genetic distance (MZE28 and MZE29) were grouped together.

The results from PCoA showed an admixture of all Gakenke accessions with accessions from Musanze into one distinct group (Figure 4.2). Three different reasons could be given for the obtained population structure, in which individuals collected from close proximity sites tend to be grouped together: (a) the highlands' features (hill slopes exposed to winds, microclimate variations from one hill to another and types of soils) and the self/cross-pollinated nature of scarlet eggplant; (b) multiple small plot possessions among local farmers, which are in disparate locations (hills and valleys); and (c) the predominance of the informal seed sector (on-farm seeds production) (Gapusi et al., 2013; Context Network, 2016; Mujuju, 2018; Ntihinyurwa et al., 2019).

The analysis of molecular variance (AMOVA) was also conducted and it showed the availability of higher variations among individuals within the population and the percentage of the total variance was 81% (Table 4.3). These findings are in line with Stedje and Bukenya-Ziraba (2003) as they reported more variations among the individuals within the population (90%) influence on genetic structure than among populations (10%) using RAPD markers to investigate the genetic structure and variations among 18 populations of *S. aethiopicum* gr. Gilo, *S. aethiopicum* gr. Shum and their ancestor *Solanum anguivi*. Thus, superior variation among members within the population is a reliable indicator of upper levels of subdivision and hierarchy (Barut et al., 2020). It was also confirmed by Acquadro et al. (2017) through a reduced complexity genome sequencing approach that scarlet eggplant does express a high within-population genetic diversity.

4.2. Results and discussion for morphological diversity assessment

4.2.1. Assessment of phenotypic diversity

A perceptible level of phenotypic variations has been recorded from *Solanum aethiopicum* gr. Gilo germplasm in three distinct experiments with reference to measured morphological and valuable agronomic traits.

4.2.1.1. The first experiment (I)

A summary of the descriptive statistics (means, standard deviations, and maximum and minimum values) for the first experiment conducted in 2020 is shown in Table 4.4.

Table 4.4. Summary on descriptive statistic for the studied quantitative variables in the first experiment.

Trait category	Descriptor Abbreviations	Mean	STD	Range	
				Min	Max
Qualitative traits of plant	LBW	23.51	3.59	14.75	31.75
	LBL	32.63	3.41	24.00	40.50
	TPH	139.29	39.28	70.00	240.00
Quantitative traits of fruit	FWE	25.75	9.53	10.00	45.00
	FLE	4.01	0.77	2.60	5.57
	FMD	3.50	0.51	2.50	4.70
	PLE	3.61	0.61	2.25	4.90
	LON	5.85	1.37	3.00	9.00

Descriptive analyses made from the data of quantitative traits of plants and fruits displayed a wide range of variations among all 57 accessions of *Solanum aethiopicum* gr. Gilo. For instance, the minimum leaf blade width was 14.75 cm (for accession MZE37) and the maximum was 31.75 cm (for accession MZE31); leaf blade length ranged from 24 cm (for accession MZE34) to 40.50 cm (for

accession GKE14); and total plant height was scaled from 70 cm (for MZE27) to 240 cm (for GKE21).

Besides, we also noted extent changes from fruits'qualitative traits on studied accessions. As an illustration, fruit weight ranged from 10 g (for accession GKE11) to 45 g (for accession MZE41), fruit length ranged from 2.60 cm (for accession MZE51) to 5.6 cm (for accession MZE53), fruit maximum diameter ranged from 2.5 cm (for accession MZE36) to 4.70 cm (for accession MZE41), peduncle length ranged from 2.30 cm (for accession MZE57) to 4.90 cm (for accessions MZE53 and MZE48), and locule number ranged from 3 (for accession GKE20) to 9 (for accession MZE53).

The statistical correlation test between all 8 studied quantitative traits showed corresponding correlation coefficients in Table 4.5. It was found that both positive and negative correlations prevail among morphological and agricultural valuable traits.

Table 4.5. Correlation coefficient between descriptors in the first experiment

Row	LBW	LBL	TPH	FWE	FLE	FMD	PLE	LON
LBW		0.612	0.026	0.249	0.018	0.293	-0.311	0.183
LBL			0.368	0.542	0.240	0.467	-0.031	0.077
TPH				0.339	0.221	0.281	0.157	0.109
FWE					0.801	0.937	0.588	0.336
FLE						0.741	0.616	0.262
FMD							0.554	0.423
PLE								0.198
LON								

The correlation coefficient r values were all significant at $p < 0.05$. For instance, more positive significant correlation coefficients (values above 0.6) are listed as follow: fruit weight (FWE) was the most highly correlated with fruit

maximum diameter (FMD) and fruit length (FLE) with r values of 0.937 and 0.801, respectively. Fruit length (FLE) was greatly correlated with fruit maximum diameter (FMD) and penducle length (PLE) with r values of 0.741 and 0.616, respectively. Additionally, leaf blade width (LBW) was moderately correlated with leaf blade length (LBL) with r value of 0.612.

Results from the PCA based correlation matrix yielded principal components where the two first ones weighted 67.43 % of the total variances with eigenvalues greater than 1 (Table 4.6).

Table 4.6. Details on principal components analysis (1st experiment)

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
LBW	0.15	0.64	0.26	-0.14	-0.02	0.65	0.20	-0.06
LBL	0.30	0.52	-0.24	-0.14	0.37	-0.57	0.27	0.16
TPH	0.22	0.10	-0.69	0.62	-0.13	0.26	0.00	0.01
FEW	0.50	-0.02	-0.02	-0.15	-0.03	-0.10	-0.32	-0.78
FLE	0.43	-0.23	0.01	-0.21	-0.65	-0.09	0.51	0.15
FMD	0.49	-0.01	0.12	-0.09	-0.02	0.08	-0.63	0.58
PLE	0.32	-0.50	-0.05	-0.10	0.64	0.34	0.32	0.04
LON	0.24	0.00	0.62	0.71	0.07	-0.20	0.14	-0.03
Eigenvalue	3.67	1.72	0.99	0.81	0.32	0.25	0.19	0.04
Percent	45.90	21.53	12.34	10.17	4.02	3.13	2.39	0.53
Cum %	45.90	67.43	79.77	89.94	93.95	97.09	99.47	100.00

Thus, the first principal component (PC1) obtained from the evaluation of different morphologic and agronomic valuable traits among *Solanum aethiopicum* gr. Gilo accessions expressed 45.90% of the total variance, and it was dependent on FEW, FMD, and FLE. Moreover, PC2 contributed 21.53% of the total variability and was associated with LBW and LBL (Figure 4.4).

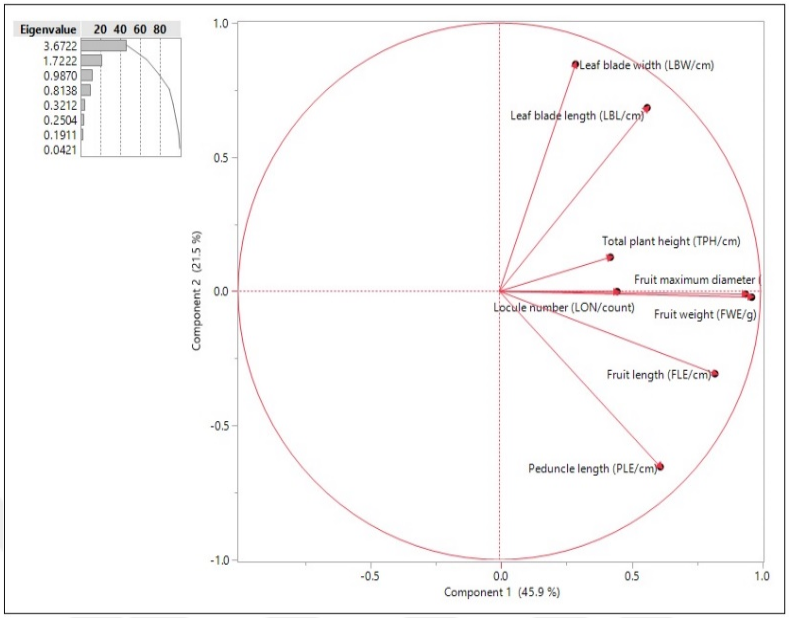

Figure 4.4. Summary plot on variables based Principal Components in the 1st experiment

The phenotypic hierarchical cluster analysis was performed to display the relationship structure among 57 accessions of *Solanum aethiopicum* gr. Gilo. Phenotypic similarity was calculated from 7 agro-morphological traits (quantitative traits) based on Ward aggregation distances. Due to the lack of fruits at maturity stage, 24 accessions were excluded from the hierarchical cluster analysis. Two major clusters were clearly identified. Thus, cluster A consisted of 18 accessions, and cluster B was slightly smaller, with only 16 accessions (Figure 4.5).

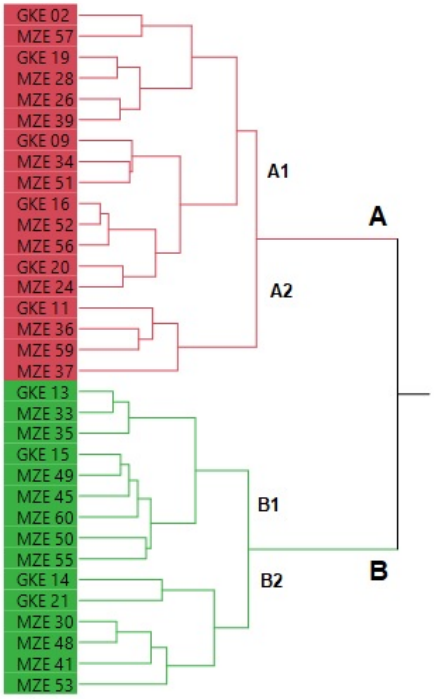

Figure 4.5. Hierarchical clustering analysis (HCA) dendrogram derived from 1st experiment.

4.2.1.2. The Second experiment (II)

A summary of the descriptive statistics (means, standard deviations, and maximum and minimum values) for the second experiment (2022) is shown in Table 4.7. Descriptive analyses made from quantitative traits data of plant and fruit displayed wide range of variations among all 55 accessions of *Solanum aethiopicum* gr. Gilo. For instance, the minimum leaf blade width was 10 cm (for accession GKE11) and the maximum was 18 cm (for accessions GKE7, GKE13 and MZE33), leaf blade length ranged from 17.5 cm (for accession GKE11) to 25.5 cm (for accession GKE20), and total plant height was scaled from 120 cm (for GKE5) to 160 cm (for MZE27).

Table 4.7. Summary on descriptive statistic for the studied quantitative variables in the second experiment.

Trait category	Descriptor Abbreviations	Mean	STD	Range	
				Min	Max
Qualitative traits of plant	LBW	14.71	1.84	10.00	18.00
	LBL	21.45	2.14	17.50	25.50
	TPH	142.60	8.54	120.00	160.00
Quantitative traits of fruit	FWE	52.97	16.92	16.67	91.00
	FLE	5.38	0.94	3.54	9.15
	FMD	4.78	0.98	2.15	8.82
	PLE	2.97	0.42	2.17	4.00
	LON	6.16	1.18	4	9

Besides, we also noted extent changes from qualitative traits of fruit on studied accessions. As an illustration, fruit weight ranged from 16.7 g (for accession MZE43) to 91 g (for accession MZE49), fruit length ranged from 3.54 cm (for accession MZE43) to 9.15 cm (for accession GKE4), fruit maximum diameter ranged from 2.15 cm (for accession MZE34) to 8.82 cm (for accession GKE4), peduncle length ranged from 2.2 cm (for accessions MZE23 and MZE41) to 4 cm (for accession GKE3), and locule number ranged from 4 (for accessions MZE23 and MZE58) to 9 (for accessions GKE19 and MZE37).

The statistical correlation test between all 8 studied quantitative traits showed corresponding correlation coefficients in Table 4.8. It was found that both positive and negative correlations prevail among morphological and agricultural valuable traits. Correlation coefficient r values were all significant at $p < 0.05$. For instance, more positive significant correlation coefficients (values above 0.7) are listed as follow; fruit length (FLE) was the highly correlated with fruit maximum diameter (FMD) with r value of 0.853. Additionally, leaf blade width (LBW) was greatly correlated with leaf blade length (LBL) with r value of 0.710.

Table 4.8. Correlation Coefficient between descriptors in the first experiment

Row	LBW	LBL	TPH	FWE	FLE	FMD	PLE	LON
LBW		0.710	0.155	-0.084	0.092	0.025	-0.164	-0.189
LBL			0.172	-0.178	0.032	-0.043	-0.105	-0.001
TPH				-0.023	0.215	0.177	0.032	0.008
FWE					0.282	0.341	-0.019	0.335
FLE						0.853	0.037	0.220
FMD							-0.115	0.355
PLE								0.011
LON								

Results from PCA based correlation matrix yielded principal components where three first ones weighted 65.65% of the total variances with eigenvalues more than 1 (Table 4.9).

Table 4.9. Details on principal components analysis (1st experiment)

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
LBW	-0.03	0.65	-0.11	0.15	-0.21	0.21	0.68	-0.05
LBL	-0.05	0.63	-0.08	0.37	-0.01	-0.13	-0.65	0.11
TPH	0.16	0.28	0.55	-0.19	0.72	0.21	0.03	0.00
FWE	0.39	-0.17	-0.28	0.29	0.03	0.80	-0.16	0.01
FLE	0.57	0.14	0.18	-0.21	-0.33	-0.11	-0.14	-0.66
FMD	0.60	0.08	0.01	-0.23	-0.18	-0.18	0.06	0.71
PLE	-0.03	-0.19	0.73	0.54	-0.35	0.06	0.06	0.13
LON	0.37	-0.14	-0.21	0.58	0.41	-0.46	0.25	-0.14
Eigenvalue	2.29	1.89	1.07	0.91	0.80	0.69	0.24	0.12
Percent	28.65	23.60	13.40	11.36	9.95	8.63	2.96	1.45
Cum %	28.65	52.25	65.65	77.01	86.95	95.58	98.55	100.00

The results of principal components analysis are shown in Figure 4.6. The first two components are displayed.

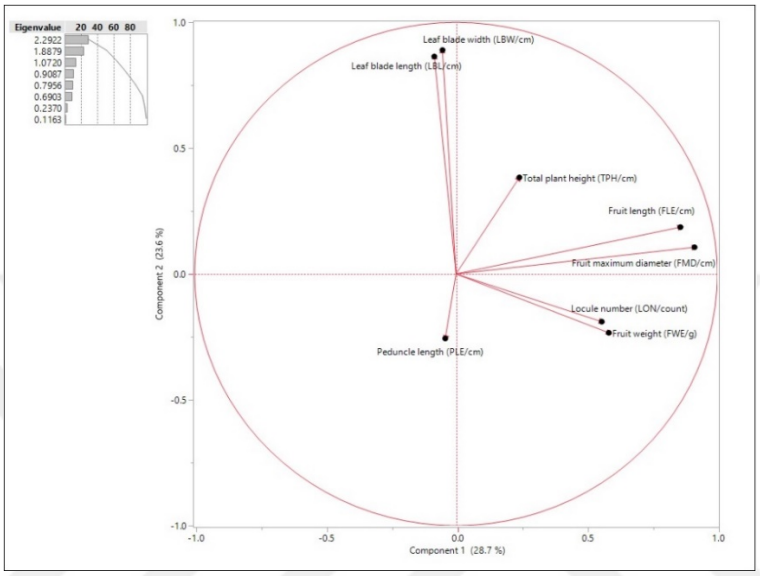

Figure 4.6. Summary plot on variables based Principal Components in the 2nd experiment

The first principal component (PC1) was obtained from the evaluation of different morphologic and agronomic valuable traits among *Solanum aethiopicum* gr. Gilo accessions expressed 28.65 % of the total variance and it was dependent on FMD and FLE. PC2 contributed 23.60 % of the total variability and was associated with LBW and LBL. The third component (PC3) accounted for 13.40 % of total variance and it is more related to PLE and TPH.

The phenotypic hierarchical cluster analysis was performed to display the relationship structure among 55 accessions of *Solanum aethiopicum* gr. Gilo. Phenotypic similarity was calculated from 7 agro-morphological traits (quantitative traits) based on Ward aggregation distances (Figure 4.7).

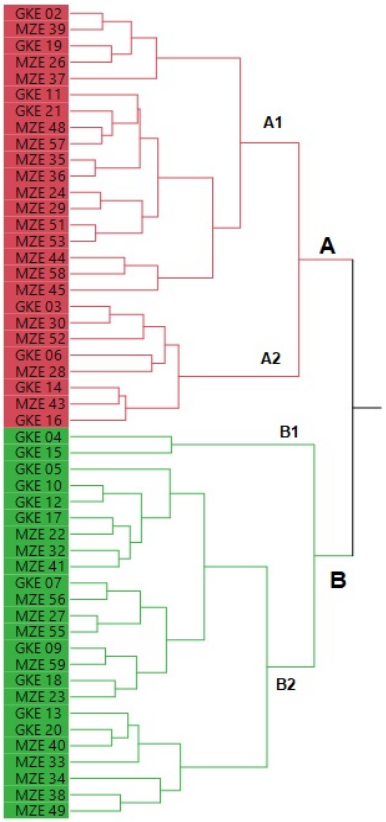

Figure 4.7. Hierarchical clustering analysis (HCA) dendrogram derived from 2nd experiment.

Five accessions were excluded in the hierarchical clustering analysis due to the lack of fruits at maturity stage. Two major clusters were clearly identified. Thus, cluster A consisted of 26 accessions, while cluster B was slightly smaller, with only 24 accessions.

4.2.1.3. The third experiment

A summary of the descriptive statistics (means, standard deviations, and maximum and minimum values) for the third experiment (2020) is shown in Table 4.10. Descriptive analyses made from only quantitative traits data of fruit displayed

wide range of variations among all 49 accessions of *Solanum aethiopicum* gr. Gilo grown in the greenhouse at Çukurova University (Only 3 accessions did not have fruits at plant maturity stage from 52 accessions). For instance, fruit weight ranged from 28 g (for accession GKE14) to 130 g (for accession GKE13), fruit length ranged from 4.38 cm (for accession MZE44) to 7.33 cm (for accession GKE13), fruit maximum diameter ranged from 3.98 cm (for accession MZE44) to 6.58 cm (for accession GKE13), and peduncle length ranged from 2.83 cm (for accession MZE52) to 4.45 cm (for accession MZE43).

Table 4.10. Summary on descriptive statistic for the studied quantitative variables in the third experiment.

Trait category	Descriptor Abbreviations	Mean	STD	Range	
				Min	Max
Quantitative traits of fruit	FWE	73.79	22.33	28.00	130.80
	FLE	5.79	0.64	4.38	7.33
	FMD	5.30	0.55	3.99	6.59
	PLE	3.58	0.35	2.83	4.45

The statistical correlation test between all 4 studied fruit quantitative traits showed corresponding correlation coefficients in Table 4.11. It was found that only positive correlations prevailed among fruit morphological traits. The correlation coefficient r values were all significant at $p < 0.05$. For instance, more positive significant correlation coefficients (values above 0.6) are listed as follow: fruit weight (FWE) was the most highly correlated with fruit maximum diameter (FMD) with r values of 0.945. Fruit length (FLE) was greatly correlated with fruit maximum diameter (FMD) with r values of 0.902. Additionally, fruit weight (FWE) was highly correlated with fruit length (FLE) with r value of 0.852.

Table 4.11. Correlation Coefficient between descriptors in the third experiment

Row	FWE	FLE	FMD	PLE
FWE		0.852	0.945	0.427
FLE			0.902	0.473
FMD				0.463
PLE				

Results from the PCA based correlation matrix yielded principal components where only the first one weighted 77.39% of the total variances with eigenvalues greater than 1 (Table 4.12).

Table 4. 12. Details on principal components analysis (3rd experiment)

Variables	PC1	PC2	PC3	PC4
FWE	0.54	-0.25	0.57	0.57
FLE	0.53	-0.15	-0.80	0.23
FMD	0.55	-0.21	0.18	-0.79
PLE	0.35	0.93	0.06	0.01
Eigenvalue	3.10	0.71	0.15	0.05
Percent	77.39	17.67	3.78	1.16
Cum %	77.39	95.06	98.84	100.00

The first principal component (PC1) was obtained from the evaluation of different fruit quantitative traits among *Solanum aethiopicum* gr. Gilo accessions expressed 77.39 % of the total variance and it was dependent on FMD, FEW, and FLE (Figure 4.8).

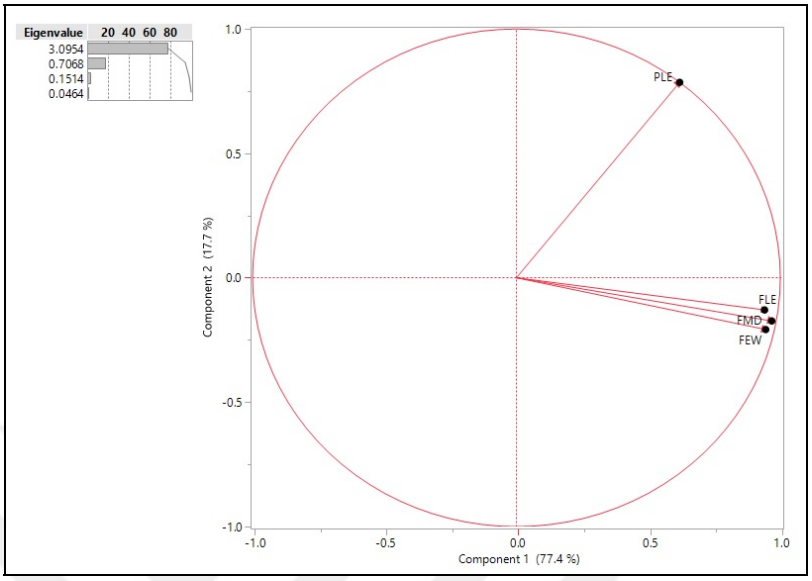

Figure 4.8. Summary plot on variables based Principal Components in the 3rd experiment

The phenotypic hierarchical cluster analysis was performed to display the relationship structure among 52 accessions of *Solanum aethiopicum* gr. Gilo. Phenotypic similarity was calculated from 4 quantitative traits of fruits based on Ward aggregation distances. Here, 3 accessions were excluded in the hierarchical cluster analysis due to the lack of fruits at maturity stage. Two major clusters were clearly identified. Thus, cluster A was the largest and consists of 35 accessions, while cluster B was smaller, with only 14 accessions (Figure 4.9).

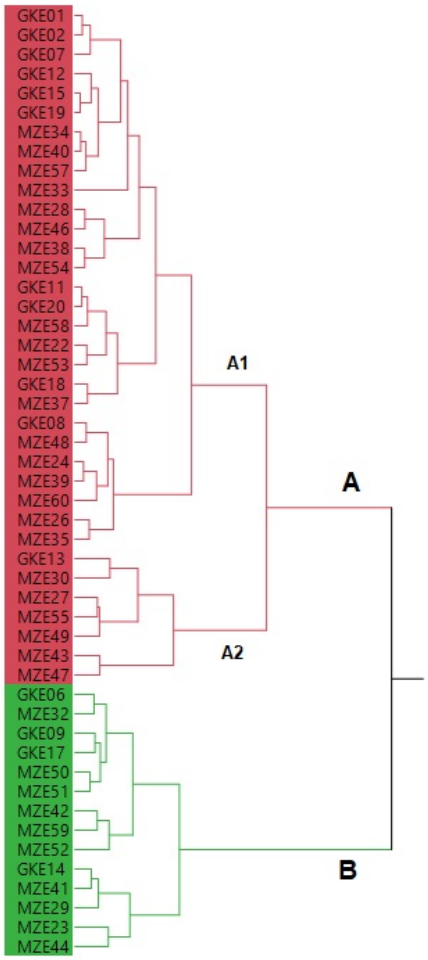

Figure 4.9. Hierarchical clustering analysis (HCA) dendrogram derived from 3rd experiment.

4.2.2. Discussion on the observed phenotypic diversity

The summary of descriptive statistics of both main experiments (I and II) of agro-morphological characterization of different accessions of *Solanum aethiopicum* gr. Gilo shows that there are significant differences between accessions. Lester and Daunay (2003) previously reported on this finding. These researchers stated that the fruits and leaves of *Solanum aethiopicum* vary in shape and size within and between cultivar groups (Gilo, Kumba, Shum, and Acelatum).

When the mean values of different quantitative agro-morphological traits were compared, it was observed that there was a significant difference between experiments (or between greenhouse and open field cultivation) in terms of plant development and yield features (Figure 4.10).

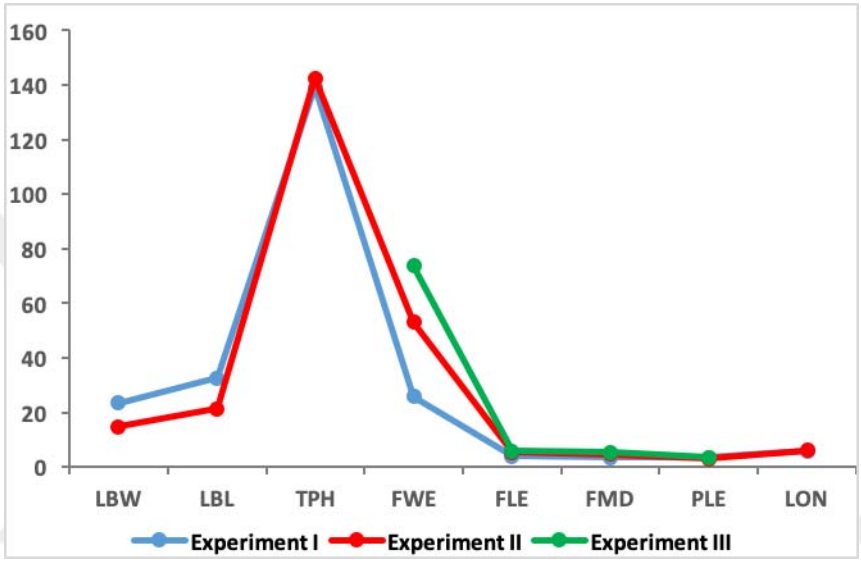

Figure 4. 10. Means comparison of experiments I and II.

The first experiment (greenhouse) produced significantly larger leaves (LBW and LBL) than the second experiment (open field). In contrast, yield characteristics (FWE, FLE, and FMD) were better in the second (open field) experiment than in the first (greenhouse) experiment. Although the obtained mean values of fruit weight in both main experiments (25.75 and 52.97 g) and also the third experiment (73.79 g) were lower than the mean value reported by Prohens et al. (2005) on the morphological diversity assessment between three accessions of *Solanum aethiopicum* gr. Gilo and other eggplants, which was 74.30 g.

Substantial variations between experiments can be identified by analyzing other morphological traits, particularly qualitative traits (Table 4.13). Some of

these variations include the distribution of anthocyanin in plants and leaves (ADP and ADL).

Table 4.13. Range and mean of qualitative traits between experiments

	Experiment I		Experiment II	
Descriptors	Range(Min-Max)	Mean	Range(Min-Max)	Mean
GHA	1-7	4.33	3-7	3.32
LBO	3-9	6.15	5-7	6.36
ADP	1-7	5.06	1-5	3.16
ADL	1-7	5	1-5	3.08
LPR	0-5	1.03	0-0	0
LHA	3-7	4.57	0-3	0.06
CC0	3-3	3	3-3	3
FLO	1-5	2.09	0-7	1.76
SCO	1-5	3.36	1-3	1.08
PCO	1-3	1.96	1-5	1.15
VTY	3-5	3.48	3-5	3.28
PFC	1-1	1	1-5	4.12
SFC	1-1	1	0-5	4.04
FCD	1-1	1	1-7	1.64
FUC	0-0	0	0-0	0
FGL	3-3	3	3-3	3
FCU	1-1	1	0-1	0.54
FAS	3-5	3.66	3-7	4.88
PMD	5-7	6.15	5-5	5
FCS	1-7	5.12	1-7	3.6
PGF	3-3	3	3-3	3
PHF	1-3	2.75	1-3	2.76
FEB	1-5	3.96	1-3	1.6
FSH	3-5	4.51	1-5	4.84
PCP	1-3	2.09	1-3	1.2
SEC	1-3	1.06	1-3	2.92
FCP	0-1	0.06	0-0	0
PGP	0-1	0.06	0-1	0.22
ACF	3-7	6.63	1-7	2.72

GHA: Growth habit, LBO: Leaf blade lobes, ADP: Anthocyanin distribution in plant, ADL: Anthocyanin distribution in leaves, LPR: Leaf prickliness, LHA: Leaf hairiness, CCO: Corolla color, FLO: Fruit load, SCO: Stem color, PCO: Petal color, VTY: Varietal type, PFC: Predominant fruit color, SFC: Secondary fruit color, FCD: Fruit color distribution, FUC: Fruit undercalyx color, FGL: Fruit glossiness, FCU: Fruit curvature, FAS: Fruit apex shape, PMD: Position of the maximum diameter, FCS: Fruit cross-section, PGF: Presence of grooves on fruit, PHF: Presence of hole in fruit, FEB: Fruit end button size, FSH: Fruit shape, PCP: Presence of chlorophyll on the pistil scar, SEC: Seed content, FCP: Fruit calyx prickliness, PGP: Presence of a greenish ring next to the peel, ACF: Average color of the flesh

For instance, plants and leaves in experiment I (greenhouse) had higher anthocyanin levels than plants and leaves in experiment II (open field). Similarly, in experiment I, the leaves had more prickles and were hairier than in experiment II.

Furthermore, in the first experiment (greenhouse), eggplants had a higher fruit load (FLO) than in the second experiment. However, the yield characteristics (quantitative traits) change in favor of the second experiment (open field) at the maturity stage. The high seed content (SEC) observed in the second experiment (open field) confirms the above. In summary, greenhouse-grown eggplants have better overall plant qualitative traits, but their fruit yield qualities are not substantial.

The findings of multiple correlation analysis were used to compare the two main experiments once more (Figure 4.11). It was discovered that highly correlated variables represented fruit quantitative traits. The first experiment had fruit weight (FWE) highly correlated to fruit maximum diameter (FMD) (r=0.937), as well as fruit weight (FWE) highly correlated to fruit length (FLE) (r=0.801), and the second experiment had fruit length (FLE) highly correlated to fruit maximum diameter (FMD) (r=0.853). This means that the fruit descriptor variables were more dependent upon one another than the plant descriptor variables.

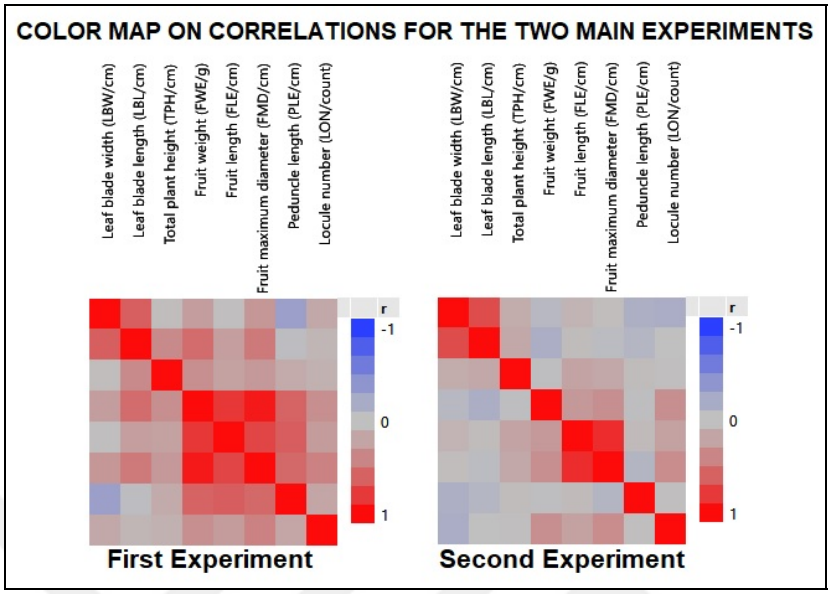

Figure 4. 11. Comparison of multiple correlation analysis.

The phenogram or hierarchical clustering analysis (HCA) generated using eight morphological descriptors based on the Ward aggregation distance clustering method (Figure 4.5 and Figure 4.7) clearly displayed the phonetic relationship among the accessions based on similarity and relatedness of eggplants.

For the first experiment (greenhouse), it separated the 33 accessions (from 57 accessions originally assessed) into two major clusters (cluster A and cluster B) and it was found that each cluster accommodated accessions from both Gakenke and Musanze districts. A thorough examination of the phenotypic clustering results (Figure 4.5) and morphological traits' mean performances (summary on descriptive statistics on quantitative traits (Table 4.4), have revealed that Cluster "B" does accommodate all superior elements in terms of fruit quantitative traits such as; fruit weight (FEW - MZE41), fruit length (FLE - MZE53), fruit maximum diameter (FMD - MZE41), peduncle length (PLE - MZE53 and MZE48) and locule number (LON, MZE53) as well as plant quantitative traits such as; leaf blade width (LBW - MZE53), leaf blade length (LBL - GKE14) and total plant height (TPH - GKE21).

Besides, cluster "A" does also contain superior accessions in regard to LBW, LBLT and LPH (qualitative traits of the plant). These results made accessions from this cluster promising donor parents for multiple traits.

For the second experiment (open field), it separated the 50 accessions (from 55 accessions originally assessed) into two major clusters (cluster A and cluster B) and it was found that each cluster accommodated accessions from both Gakenke and Musanze districts. A thorough examination on the phenotypic clustering results (Figure 4.7) and morphological traits' mean performances (summary on descriptive statistisc on quantitative traits (Table 4.7), have revealed that Cluster "B" does accommodate all superior elements in terms of fruit quantitative traits such as; fruit weight (FEW - MZE49), fruit length (FLE - GKE4), fruit maximum diameter (FMD - GKE4), as well as plant quantitative traits such as; leaf blade width (LBW - GKE7, GKE13, and MZE33), leaf blade length (LBL - GKE20) and total plant height (TPH - MZE27). Although, cluster "A" does also contain superior accessions in regard to fruit qualitative traits of the plant like peduncle length (PLE, GKE3) and locule number (LON - GKE19 and MZE37). These results made accessions from mainly cluster B promising donor parents for multiple traits.

Furthermore, the evidence based on the PCA correlation matrix in experiment I (greenhouse) has assured the significance of fruit quantitative variables (FWE, FMD, FLE) in the first principal component (PC1), which explained 45.90% of the total variance within the studied germplasm. Similarly, in experiment II, fruit quantitative variables (FMD and FLE) were substantial in the first principal component (PC1), which explained 45.90% of the total variance within the studied germplasm.

This means that the overall differences observed in *Solanum aethiopicum* gr. Gilo germplasm both in greenhouse and in open field experiments were due mainly to fruit quantitative traits. In other words, the chosen morphological

descriptors, particularly those related to yield and fruit quality, were conclusive for eggplant germplasm characterization. The phenotypic results provide new awareness on the agronomic behavior of several accessions. For future breeding programs, accessions with high average fruit weight, fruit maximum diameter, and fruit length could be taken into consideration.

In brief, this high diversity is often based on high discriminatory fruit traits of *Solanum aethiopicum* and has been highlighted in diversity studies by Adeniji et al. (2012), Plazas et al. (2014), Sakhanokho et al. (2014), and Bationo-Kando et al. (2015). It was also reported that different fruit sizes and shapes exist among individuals of *Solanum aethiopicum* gr. Gilo (Taher et al., 2017). Our results show the existence of an extensive phenotypic diversity in *Solanum aethiopicum* gr. Gilo which is in agreement with similar results by Kouassi et al. (2014). Observations from fruit traits reaffirm that the used germplasm of *Solanum aethiopicum* belongs to the Gilo group and it is more particularly similar to one of three sub-groups reported by the same authors in Côte d'Ivoire and which is known as "N'Drowa" (Figure 3.14). Thus, it was found that the N'Drowa subgroup has larger leaves and larger fruits. Several scholars pointed out the high morphological diversity of Scarlet eggplant on the African continent, as well as the existence of large germplasm collections made of several landraces in countries such as Burkina Faso, Cameroon, Côte d'Ivoire, Ethiopia, Gabon, Ghana, Kenya, Nigeria, Senegal, Tanzania, Uganda, Zambia, Zimbabwe, and also Rwanda. Furthermore, those listed countries are seen as domestication centers of *Solanum aethiopicum* (Sseremba, 2019).

4.3. Results and and discussion for *in vitro* embryogenesis induction

4.3.1. Results for *in vitro* embryogenesis induction

In vitro embryogenesis induction through anther culture was carried out in two distinctive experiments (2020 and 2021), and the obtained results are presented in this following sub-sections:

4.3.1.1. Fist experiment

The number of cultured anthers and formed calli are described in Table 4.14. Unfortunately, since no embryos were obtained, only the frequency of callus formation was calculated in percentage per accessions.

Table 4.14. Frequency of calli formation for the first experiment

N°	Accession ID	Petri Number (Repetitions)	Number of Cultured Anthers	Frequency of Calli Formation	
				Number	%
1.	GKE1	26	186	72	38.71
2.	GKE2	26	190	46	24.21
3.	GKE5	26	177	88	49.72
4.	GKE7	26	201	71	35.32
5.	GKE8	26	173	35	20.23
6.	GKE9	26	175	54	30.86
7.	GKE11	26	202	74	36.63
8.	GKE12	26	207	68	32.85
9.	GKE13	26	217	58	26.73
10.	GKE14	26	157	85	54.14
11.	GKE15	26	196	72	36.73
12.	GKE16	26	182	44	24.18
13.	GKE17	26	180	57	31.67
14.	GKE18	26	182	59	32.42
15.	GKE19	26	180	78	43.33
16.	GKE20	26	200	77	38.50
17.	MZE22	26	204	96	47.06
18.	MZE23	26	183	85	46.45
19	MZE24	26	199	97	48.74
20.	MZE26	26	183	55	30.05
21.	MZE27	26	170	64	37.65
22.	MZE28	26	197	70	35.53
23.	MZE29	26	201	90	44.78
24.	MZE30	26	202	64	31.68
25.	MZE32	26	199	71	35.68
26.	MZE33	26	195	78	40.00
27.	MZE34	26	195	109	55.90
28.	MZE35	26	191	46	24.08
29.	MZE36	26	207	62	29.95
30.	MZE37	26	200	86	43.00
31.	MZE38	26	194	59	30.41
32.	MZE39	26	182	64	35.16
33.	MZE40	26	190	65	34.21
34.	MZE41	26	189	102	53.97
35.	MZE42	26	187	61	32.62
36.	MZE43	26	203	70	34.48
37.	MZE44	26	167	59	35.33
38.	MZE46	26	181	89	49.17
39.	MZE47	26	183	40	21.86
40.	MZE48	26	207	67	32.37
41.	MZE49	26	190	68	35.79
42.	MZE50	26	207	96	46.38
43.	MZE51	26	173	53	30.64
44.	MZE52	26	180	70	38.89
45.	MZE53	26	174	64	36.78
46.	MZE54	26	205	70	34.15
47.	MZE55	26	211	49	23.22
48.	MZE56	26	177	51	28.81
49.	MZE58	26	192	73	38.02
50.	MZE59	26	199	71	35.68
51.	MZE60	26	189	75	39.68

Results show that callus formation ranged between 55.90% and 20.23%, with the highest percentage for accession MZE34 and the lowest percentage for accession GKE8. The average callus formation percentage is 36.36 calli/100 anthers for the whole germplasm of *Solanum aethiopicum* gr. Gilo.

The details of each accession, particularly the mean number of cultured anthers and their corresponding standard deviations, are given in Figure 4.12. For instance, the highest mean value of cultured anther was observed in GKE13 accession with values of 8.35±1.24 while the highest mean value of formed calli was noticed in MZE32 accession with 1.59±1.89.

The statistical analysis (one-way ANOVA) shows that accessions significantly influenced callus induction (Table 4.15). The statistical significance is shown by the small value of the P-value (compared to α=0.05). This can be taken as evidence that the means are different. Similarly, the mean comparisons through the least significant difference (LSD) test confirmed that means were statistically different (Table 4.16).

Table 4.15. Statistical anlysis on callus induction (First experiment)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Accessions	50	518.1568	10.3631	3.6274	<.0001
Error	1301	3716.846	2.8569		
C. Total	1351	4235.003			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Accessions	50	50	518.1568	3.6274	<.0001

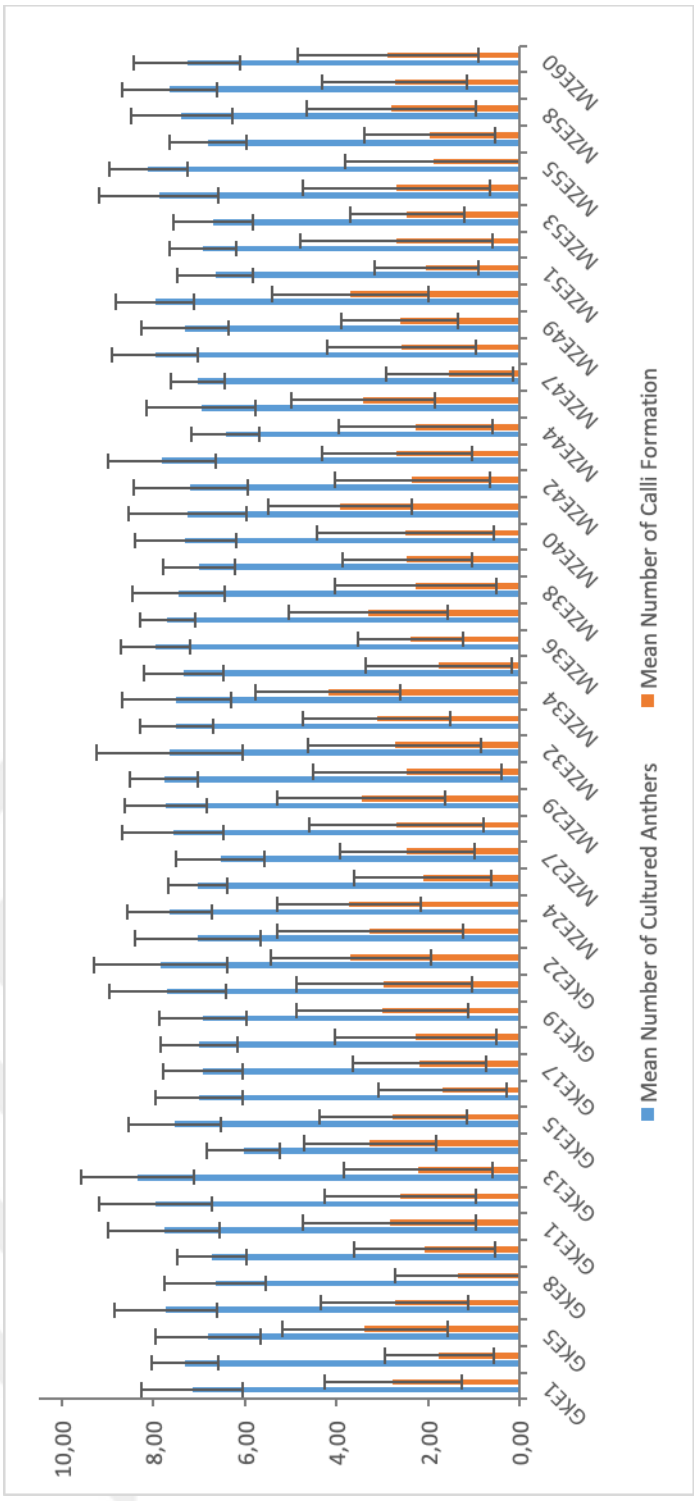

Figure 4. 12. Details on callus induction with means and their corresponding standard deviations (Experiment I)

Table 4.16. Details on LSD test (First experiment)

N°	Accessions	LSD results on the number of formed calli
1.	GKE01	2.77±1.53 EFGHIJK
2.	GKE02	1.77±1.21 MNOP
3.	GKE05	3.38±1.83 ABCDEF
4.	GKE07	2.73±1.64 EFGHIJK
5.	GKE08	1.35±1.41 P
6.	GKE09	2.08±1.57 JKLMNOP
7.	GKE11	2.85±1.93 CDEFGHIJK
8.	GKE12	2.62±1.68 EFGHIJKLM
9.	GKE13	2.23±1.66 IJKLMNOP
10.	GKE14	3.27±1.46 BCDEFGH
11.	GKE15	2.77±1.63 EFGHIJK
12.	GKE16	1.69±1.44 NOP
13.	GKE17	2.19±1.50 IJKLMNOP
14.	GKE18	2.27±1.80 IJKLMNO
15.	GKE19	3.00±1.92 CDEFGHI
16.	GKE20	2.96±1.95 CDEFGHIJ
17.	MZE22	3.69±1.78 ABCD
18.	MZE23	3.27±2.07 BCDEFGH
19	MZE24	3.73±1.59 ABC
20.	MZE26	2.12±1.53 IJKLMNOP
21.	MZE27	2.46±1.50 GHIJKLMN
22.	MZE28	2.69±1.93 EFGHIJKL
23.	MZE29	3.46±1.86 ABCDE
24.	MZE30	2.46±2.10 GHIJKLMN
25.	MZE32	2.73±1.93 EFGHIJK
26.	MZE33	3.00±1.72 CDEFGHI
27.	MZE34	4.19±1.60 A
28.	MZE35	1.77±1.63 MNOP
29.	MZE36	2.38±1.17 HIJKLMNO
30.	MZE37	3.31±1.76 ABCDEFG
31.	MZE38	2.27±1.80 IJKLMNO
32.	MZE39	2.46±1.45 GHIJKLMN
33.	MZE40	2.50±1.96 FGHIJKLMN
34.	MZE41	3.92±1.60 AB
35.	MZE42	2.35±1.72 IJKLMNO
36.	MZE43	2.69±1.67 EFGHIJKL
37.	MZE44	2.27±1.71 IJKLMNO
38.	MZE46	3.42±1.60 ABCDE
39.	MZE47	1.54±1.42 OP
40.	MZE48	2.58±1.65 EFGHIJKLMN
41.	MZE49	2.62±1.30 EFGHIJKLM
42.	MZE50	3.69±1.74 ABCD
43.	MZE51	2.04±1.15 KLMNOP
44.	MZE52	2.69±2.15 EFGHIJKL
45.	MZE53	2.46±1.27 GHIJKLMN
46.	MZE54	2.69±2.09 EFGHIJKL
47.	MZE55	1.92±1.71 LMNOP
48.	MZE56	1.96±1.46 LMNOPQ
49.	MZE58	2.81±1.88 DEFGHIJK
50.	MZE59	2.73±1.61 EFGHIJK
51.	MZE60	2.88±2.01 CDEFGHIJK

Levels not connected by same letter are significantly different

4.3.1.2. Second experiment

For the second experiment, the number of cultured anthers as well as the number of formed calli and embryos are described in Table 4.17. Both the formation frequency of calli and embryos as well as development of embryos were thoroughly calculated.

Table 4.17. Frequency of calli and embryos formation for the second experiment

Treatments	Access. IDs	Petri Number - Repetions	Number of Cultured Anthers	Frequency of Calli Formation		Frequency of Embryo Formation		Frequency of Embryo Development	
				Number	%	Number	%	Number	%
I.	GKE12	31	244	78	31.97	2	0.82	1	0.41
	GKE20	31	243	78	32.10	0	0.00	0	0.00
	MZE24	31	234	92	39.32	0	0.00	0	0.00
	MZE53	31	200	34	17.00	0	0.00	0	0.00
	Adana	31	163	44	26.99	1	0.61	0	0.00
	Kemer	31	168	53	31.55	2	1.19	0	0.00
II.	GKE12	31	213	38	17.84	0	0.00	0	0.00
	GKE20	31	231	73	31.60	0	0.00	0	0.00
	MZE24	31	234	66	28.21	0	0.00	0	0.00
	MZE53	31	204	28	13.73	0	0.00	0	0.00
	Adana	31	179	20	11.17	13	7.26	12	6.70
	Kemer	31	174	27	15.52	2	1.15	1	0.57

I: First treatment [C medium supplemented with 2.4-D (5 mg/l) and kinetin (5 mg/l)].
II: Second treatment [C medium supplemented with 2.4-D (1 mg/l) and Kinetin (1 mg/l)].

In the first treatment, results show that GKE12 accession (*S. aethiopicum* gr. Gilo) was the only accession to have a good response regarding embryo formation and development, with 0.82 formed embryos per 100 anthers and 0.41 developed embryos per 100 anthers. Although, it was not the only accession to have formed embryos. Adana and Kemer (*S. melongena*) used here as control genotypes formed embryos with 0.61 formed embryos per 100 anthers and 1.19 formed embryos per 100 anthers, respectively. Briefly, the only developed embryo/seedling was obtained from GKE12.

Regarding callus induction in the first treatment, there was a great variation within the 4 different accessions of *S. aethiopicum* gr. Gilo and the two control *S. melongena* genotypes (Adana and Kemer) as shown in Figure 4.13. For instance, the highest mean value of cultured anther and mean value of formed calli were observed in MZE24 accession with values of 2.97±1.91 and 3.23±1.58, respectively.

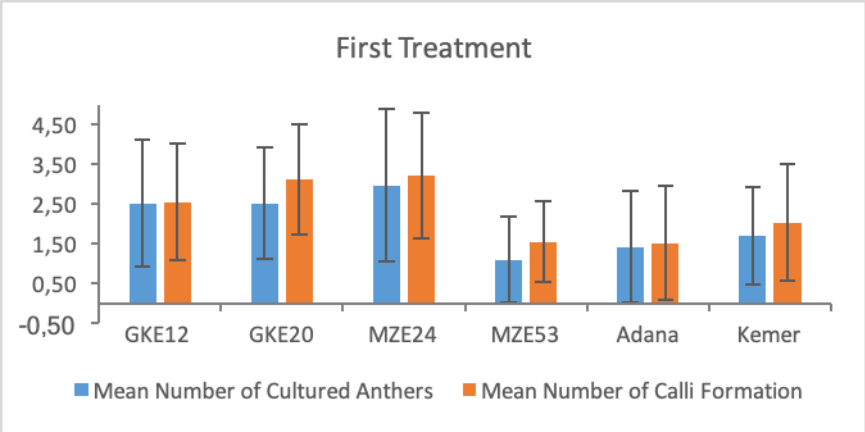

Figure 4.13. Details on callus induction with means and their corresponding standard deviations (Experiment II – First treatment)

In the second treatment, results show that none of the eggplant accessions (*S. aethiopicum* gr. Gilo) were able to form embryos. Embryo formation and development were observed in the two control *S. melongena* genotypes; Adana and Kemer, with 7.26 formed embryos per 100 anthers and 1.15 formed embryos per 100 anthers, respectively. For the frequency of developed embryos/seedlings, these values slightly decrease for Adana and Kemer, with 6.70 developed embryos per 100 anthers and 0.57 developed embryos per 100 anthers, respectively.

Regarding callus induction in the second treatment, there was a great variation within the 4 different accessions of *S. aethiopicum* gr. Gilo and the two control *S. melongena* genotypes (Adana and Kemer) as shown in Figure 4.14. For instance, the highest mean value of cultured anther was observed in GKE20 accession with values of 2.35±1.26, while the highest mean value of formed calli was observed in MZE24 accession with 2.97±1.43.

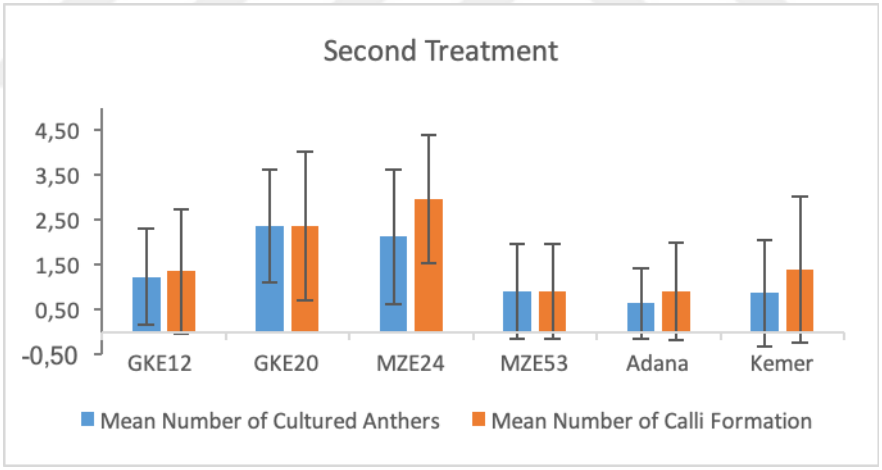

Figure 4.14. Details on callus induction with means and their corresponding standard deviations (Experiment II – Second treatment)

The statistical analysis (Two-way ANOVA) shows that accessions/genotypes and treatments (I and II) significantly influenced callus induction (Table 4.18). The statistical significance is shown by the small value of the P-value (compared to α=0.05). This can be taken as evidence that the means are different. Similarly, the mean comparisons through the least significant difference (LSD) test confirmed that means were statistically different (Table 4.19).

Table 4.18. Statistical analysis on callus induction (Second experiment)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Accessions/Genotypes	11	206.2876	18.7534	10.3803	<.0001
Error	360	650.3871	1.8066		
C. Total	371	856.6747			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatments	1	1	43.35753	23.9991	<.0001
Acc/Gen	5	5	148.4005	16.4284	<.0001
Treatments*Acc/Gen	5	5	14.52957	1.6085	0.157

Table 4.19. Details on LSD test (Second experiment)

	Level	Least Sq Mean		Level	Least Sq Mean
Accessions/ Genotypes	GKE12	1.87 ^{B}	**Genotypes *Treatments**	I, GKE12	2.52 $^{A, B}$
	GKE20	2.44 ^{A}		I, GKE20	2.52 ^{A,B}
	MZE24	2.55 ^{A}		I, MZE24	2.97 ^{A}
	MZE53	1.00 ^{C}		I, MZE53	1.10 $^{D, E, F}$
	Adana	1.03 ^{C}		I, Adana	1.42 $^{D, E}$
	Kemer	1.29 ^{C}		I, Kemer	1.71 $^{C, D}$
Treatments	I	2.04 ^{A}		II, GKE12	1.23 $^{D, E, F}$
	II	1.35 ^{B}		II, GKE20	2.35 $^{A, B, C}$
				II, MZE24	2.13 $^{B, C}$
				II, MZE53	0.90 $^{E, F}$
				II, Adana	0.65 ^{F}
				II, Kemer	0.87 $^{E, F}$

Levels not connected by same letter are significantly different

ANOVA results show that there was also a significant difference between treatments. That means that the factor "treatment" has an influence on the result obtained with regard to callus induction. At the accession/genotype level, we also found a significant difference. At the interaction level (Treatments*Accession/Genotype), we observed no significant difference.

4.3.1.3. Flow cytometric assessment of embryo-derived plantlets

Ploidy levels were measured in all obtained plantlets of *Solanum aethiopicum* gr. Gilo from the second experiment on *in vitro* androgenesis through anther culture. As shown in Table 4.20, 100% of all obtained plantlets were found to be diploid.

Table 4.20. Ploidy levels in all *S. aethiopicum* gr. Gilo plantlets

Treatments	Accession IDs	Number of embryo-derived plantlets	Haploid plantlets	Diploid plantlets
I	GKE12	1	-	1
	GKE20	-	-	-
	MZE24	-	-	-
	MZE53	-	-	-
	Adana	-	-	-
	Kemer	-	-	-
II	GKE12	-	-	-
	GKE20	-	-	-
	MZE24	-	-	-
	MZE53	-	-	-
	Adana	12	-	12
	Kemer	1	-	1

I: First treatment [C medium with 2.4-D (5 mg/l) and kinetin (5 mg/l)].
II: Second treatment [C medium with 2.4-D (1 mg/l) and Kinetin (1 mg/l)].

4.3.2. Discussion for *in vitro* embryogenesis induction

In the first experiment, the overall callus formation rate was 36.36 calli/100 anthers and there was no embryo formation. This value was greater than the value obtained by Salas et al. (2011), when they assessed androgenic capacity via anther in various genotypes of common eggplant and related species, including *Solanum aethiopicum*. They obtained 21.5 calli/100 anthers for the sole genotype of *Solanum aethiopicum* utilized in their study, and it also failed to generate embryos.

The results from ANOVA demonstrated the existence of a significant statistical difference among the entire germplasm collection of *Solanum aethiopicum* gr. Gilo with regards to callus induction.

Successful strategies for increasing the number of embryos and embryo-derived plantlets in *Solanum melongena* can be replicated in *Solanum aethiopicum*. For instance, Emrani Dehkehan et al. (2017) demonstrated that an individual supplement of 1 mg/l zeatin riboside and 10 mg/l mannitol in C medium (containing NAA and Kinetin) can positively affect anther culture outcomes. The highest embryo-derived plantlets obtained in this case were 25% and 66.6% for zeatin riboside and mannitol, respectively.

Obtaining doubled haploid in this type of eggplant, also known as the closest relative to brinjal, is a significant step forward for DH technology. This technology is reported to have the potential to speed up the process of generating new parental pure lines in several different species, including eggplant, which is considered a species moderately recalcitrant with regards to this technology. Eggplant breeding programs are primarily concerned with the release of hybrid varieties with improved traits. For instance, eggplant F1 hybrids, which are produced by crossing two parental homozygous plants, typically outperform parental lines in terms of various agronomic traits (Mir et al., 2021).

4.4. Results and and discussion for *in vitro* salt stress assessment

4.4.1. Results for *in vitro* salt stress assessment

An *in vitro* study of salt-induced changes in early seedling growth among different *Solanum aethiopicum* gr. Gilo accessions revealed that increasing salt stress had an effect on the vegetative growth of all eggplant accessions (Table 4.21 and Table 4.22). Although there was a slight increase in the number of leaves at the NaCl concentration threshold of 80 mM, all eggplant accessions showed a decrease after the same threshold as the salt stress level continued to increase. Additionally, it was observed that at the highest salinity level (160 mM), the number of leaves decreased in GKE11, GKE20, MZE29, MZE44, MZE49, and MZE51 by 4.3%, 14.9%, 1.3%, 5.1%, 8.9%, and 11.9%, respectively, compared to their respective controls (Figure 4.15).

Figure 4.15. Plots of different variables and their fluctuations (%) based on control levels

Table 4. 21. Evaluation of salt stress effects on the seedling growth of GKE11, GKE20 and MZE29 accessions

Accessions	NaCl (mM)	Number of leaves	Height(Cm)	Internode spacing(Cm)	Fresh weight(g) - FW	Dry weight(g) - DW	Water Content TWC
GKE 11	0	6.273 ± 0.827^{a,b}	4.234 ± 1.041^{a,b,c,d,e}	0.293 ± 0.153^{a}	0.147 ± 0.075^{a}	0.011 ± 0.006^{a}	13.012 ± 2.130^{b}
	20	6.136 ± 0.990^{a,b}	3.957 ± 0.568^{c,d,e}	0.247 ± 0.102^{a}	0.141 ± 0.05^{a}	0.020 ± 0.026^{a}	12.106 ± 5.902^{b}
	40	6.545 ± 1.224^{a,b}	3.816 ± 0.835^{c,d,e}	0.219 ± 0.082^{a}	0.167 ± 0.080^{a}	0.014 ± 0.016^{a}	14.185 ± 3.768^{b}
	80	6.227 ± 1.270^{a,b}	3.555 ± 0.809^{d,e}	0.215 ± 0.075^{a}	0.183 ± 0.077^{a}	0.011 ± 0.004^{a}	15.445 ± 2.661^{b}
	160	6.000 ± 0.926^{b}	3.273 ± 0.540^{e}	0.197 ± 0.065^{a}	0.199 ± 0.077^{a}	0.015 ± 0.018^{a}	15.562 ± 5.401^{b}
GKE 20	0	7.000 ± 1.877^{a,b}	5.512 ± 3.397^{a}	0.403 ± 0.402^{a}	0.198 ± 0.199^{a}	0.015 ± 0.015^{a}	12.043 ± 3.137^{b}
	20	6.636 ± 1.465^{a,b}	4.628 ± 1.181^{a,b,c,d,e}	0.321 ± 0.107^{a}	0.179 ± 0.088^{a}	0.012 ± 0.005^{a}	14.158 ± 2.617^{b}
	40	6.682 ± 1.673^{a,b}	4.536 ± 1.936^{a,b,c,d,e}	0.352 ± 0.217a	0.204 ± 0.145^{a}	0.015 ± 0.010^{a}	12.692 ± 2.915^{b}
	80	6.682 ± 1.555^{a,b}	3.941 ± 1.125^{c,d,e}	0.279 ± 0.089a	0.165 ± 0.078^{a}	0.019 ± 0.039^{a}	13.221 ± 4.274^{b}
	160	5.955 ± 1.430^{b}	3.525 ± 1.439^{d,e}	0.243 ± 0.097a	0.168 ± 0.090^{a}	0.013 ± 0.013^{a}	12.905 ± 3.588^{b}
MZE 29	0	7.091 ± 1.269^{a,b}	5.125 ± 1.109^{a,b,c}	0.357 ± 0.128a	0.192 ± 0.075^{a}	0.013 ± 0.006^{a}	12.463 ± 2.759^{a}
	20	6.955 ± 1.133^{a,b}	4.569 ± 0.857^{a,b,c,d,e}	0.320 ± 0.093a	0.169 ± 0.052^{a}	0.014 ± 0.015^{a}	14.360 ± 3.930^{b}
	40	6.636 ± 1.002^{a,b}	4.226 ± 1.174^{a,b,c,d,e}	0.267 ± 0.109a	0.178 ± 0.056^{a}	0.011 ± 0.004^{a}	15.608 ± 2.817^{b}
	80	7.227 ± 1.572^{a,b}	3.898 ± 0.852^{c,d,e}	0.250 ± 0.097a	0.188 ± 0.070^{a}	0.011 ± 0.005^{a}	15.769 ± 3.069^{b}
	160	7.000 ± 1.543^{a,b}	3.452 ± 1.097^{d,e}	0.435 ± 0.986a	0.199 ± 0.065a	0.012 ± 0.003^{a}	15.444 ± 2.183^{b}

LS Means Differences Tukey HSD (α=0,050; Q=3,7664)
Levels not connected by same letter are significantly different.

Table 4. 22. Evaluation of salt stress effects on the seedling growth of MZE44, MZE49 and MZE51accessions

Accessions	NaCl (mM)	Number of leaves	Height(Cm)	Internode spacing(Cm)	Fresh weight(g) - FW	Dry weight(g) - DW	Water Content TWC
MZE 44	0	7.182 ± 1.053^{a,b}	5.017 ± 0.997^{a,b,c}	0.326 ± 0.132a	0.177 ± 0.080^{a}	0.014 ± 0.006^{a}	11.723 ± 1.797^{b}
	20	7.273 ± 1.202^{a,b}	5.420 ± 1.129^{a,b}	0.355 ± 0.142^{a}	0.221 ± 0.065^{a}	0.016 ± 0.005^{a}	13.441 ± 2.242^{b}
	40	7.273 ± 1.241^{a,b}	4.861 ± 1.026^{a,b,c,d}	0.331 ± 0.092^{a}	0.231 ± 0.095^{a}	0.016 ± 0.006^{a}	13.838 ± 2.615^{b}
	80	7.227 ± 1.572^{a,b}	4.542 ± 1.034^{a,b,c,d,e}	0.280 ± 0.064^{a}	0.215 ± 0.072^{a}	0.014 ± 0.004^{a}	14.883 ± 2.645^{b}
	160	6.818 ± 1.181^{a,b}	3.865 ± 0.824^{c,d,e}	0.211 ± 0.070^{a}	0.205 ± 0.075^{a}	0.013 ± 0.003^{a}	14.974 ± 3.065^{b}
MZE 49	0	7.182 ± 1.097^{a,b}	5.004 ± 1.460^{a,b,c}	0.359 ± 0.139^{a}	0.202 ± 0.122^{a}	0.019 ± 0.019^{a}	11.998 ± 3.035^{b}
	20	7.227 ± 1.193a,b	5.095 ± 0.755^{a,b,c}	0.342 ± 0.070^{a}	0.194 ± 0.066^{a}	0.016 ± 0.019^{a}	13.803 ± 3.899^{b}
	40	6.409 ± 1.532^{a,b}	4.744 ± 1.243^{a,b,c,d}	0.348 ± 0.176^{a}	0.205 ± 0.121^{a}	0.014 ± 0.009^{a}	13.764 ± 3.580^{b}
	80	7.000 ± 1.604^{a,b}	4.673 ± 0.922^{a,b,c,d,e}	0.325 ± 0.075^{a}	0.201 ± 0.053^{a}	0.013 ± 0.004^{a}	15.187 ± 2.910^{b}
	160	6.545 ± 1.101^{a,b}	3.988 ± 0.555^{b,c,d,e}	0.269 ± 0.058^{a}	0.185 ± 0.059^{a}	0.012 ± 0.003^{a}	14.584 ± 3.317^{b}
MZE 51	0	7.636 ± 2.150^{a}	4.740 ± 1.754^{a,b,c,d}	0.332 ± 0.181^{a}	0.166 ± 0.082^{a}	0.016 ± 0.017^{a}	12.133 ± 3.538^{b}
	20	7.273 ± 0.985^{a,b}	4.695 ± 1.092^{a,b,c,d,e}	0.312 ± 0.123^{a}	0.180 ± 0.075^{a}	0.012 ± 0.004^{a}	13.703 ± 2.491^{b}
	40	6.636 ± 1.706^{a,b}	4.289 ± 1.497^{a,b,c,d,e}	0.330 ± 0.171^{a}	0.222 ± 0.173^{a}	0.015 ± 0.013^{a}	14.053 ± 3.847^{b}
	80	7.227 ± 1.445^{a,b}	4.565 ± 1.344^{a,b,c,d,e}	0.328 ± 0.145^{a}	0.224 ± 0.107^{a}	0.014 ± 0.007^{a}	15.665 ± 3.972^{b}
	160	6.727 ± 1.723^{a,b}	3.762 ± 0.767^{c,d,e}	0.238 ± 0.061^{a}	0.187 ± 0.055^{a}	0.011 ± 0.003^{a}	15.474 ± 4.015^{b}

LS Means Differences Tukey HSD (α=0,050; Q=3,7664)
Levels not connected by same letter are significantly different.

Furthermore, different salt stress levels also induced a shoot length reduction in all eggplant accessions. There was a progressive diminution of eggplant height from 20 mM to 160 mM. Reduction of the size of eggplant accessions was recorded in GKE11, GKE20, MZE29, MZE44, MZE49, and MZE51, which amounted to 22.7%, 36.1%, 32.6%, 23.0%, 20.3%, and 20.6%, respectively, in regard to their respective controls (Figure 4.15).

Moreover, a progressive reduction of internode spacing was observed as the salt stress level increased. In contrast, only MZE29 displayed an increase of 22% at the highest salt stress level (160 mM) compared to its control. Additionally, it was also noticed that five eggplant accessions (GKE20, MZE29, MZE44, MZE49, and MZE51) showed a modest decrease in dry biomass (DW) in comparison to their respective controls. The most important decrease with regard to control was observed in MZE49 (38.1%), followed by MZE51 (26.9%), MZE44 (9.5%), GKE20 (7.8%), and MZE29 (5.4%). Only GKE11, on the other hand, shows a high increase when compared to control, with a ratio of 46.8%.

In addition, a significant increase in water content was recorded with regard to the highest concentration of NaCl (160 mM) when compared to the respective control in eggplant accessions; MZE44 (27.7%), followed by MZE51 (27.5%), MZE29 (23,9%), MZE49 (21.6%), GKE11 (19.6%), and GKE20 (7.16%) (Figure 4.15). Briefly, MZE51 produced a high number of leaves (7.636) under salt stress. Furthermore, MZE40 showed the highest shoot height (5.420 cm) and MZE29 presented high internode spacing (0.435 cm), while MZE44 showed the highest fresh weight (0.231 g), GKE11 gave the highest dry weight (0.020 g) and MZE29 presented high water content (19.206 g) under salt stress. Statistical results revealed significant differences between the means and they are represented by separate letters in the same column in Table 4.21 and Table 4.22.

The results from the PCA based correlation matrix yielded principal components where the first two principal components (PC1 and PC2) weighed 72.45% of the total variances with eigenvalues greater than 1 (Table 4.23).

Table 4.23. Principal components analysis from *in vitro* salt stress assessment.

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
NOL	0.45463	0.29552	-0.14543	0.73538	0.26574	-0.27098
HGT	0.54732	0.00867	-0.20172	0.03623	-0.60264	0.54331
AIC	0.50229	0.09864	-0.22422	-0.57012	0.59300	0.10496
FW	0.25441	0.54484	0.59099	-0.28306	-0.29263	-0.35127
DW	0.26174	-0.48248	0.71846	0.18580	0.25840	0.28500
WCT	-0.32897	0.61092	0.14976	0.13500	0.24931	0.64479
Eigenvalue	2.728	1.6193	0.7534	0.4571	0.3072	0.1349
Percent	45.467	26.989	12.557	7.618	5.121	2.248
Cum Percent	45.467	72.456	85.013	92.631	97.752	100

NOL: Number of leaves, **HGT**: Height (Cm), **AIC**: Average Internode spacing(Cm), **FW**: Fresh weight(g) – FW, **DW**: Dry weight(g) – DW, **WCT**: Water Content - (FW-DW/FW)

Thus, the first principal component (PC1) that was obtained from the *in vitro* salt stress evaluation of different measured variables of *S. aethiopicum* accessions expressed 45.46% of the total variance and it was dependent on FW, NOL, AIC, and HGT. Moreover, PC2 contributed 26.98% of the total variability and was associated with WCT (Figure 4.16).

Additionally, hierarchical cluster analysis (HCA) was performed to display the relationship structure among six eggplant accessions (GKE11, GKE20, MZE29, MZE44, MZE49, and MZE51) of *S. aethiopicum* gr. Gilo subjected to five different levels of salinity stress. Similarity was calculated from 6 morphological variables based on Ward aggregation distances.

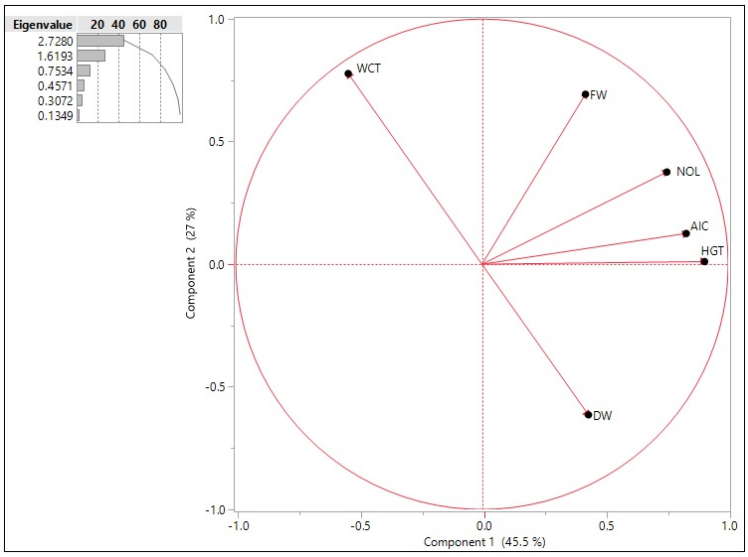

Figure 4.16. Summary plot on variables based Principal Components in the salt stress experiment.

Two major clusters were obtained by setting a suitable number based on a scree plot of Eigenvalues (Figure 4.17).

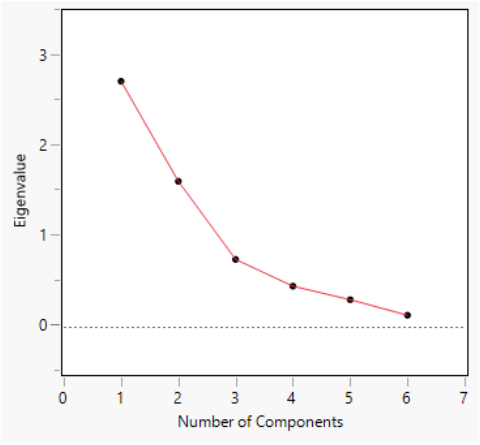

Figure 4.17. Scree plot of Eigenvalues.

Thus, cluster A consisted of 18 elements, while cluster B was the smallest, with only 12 elements (Figure 4.18). Thus, all 30 individual accession elements (6 accessions * 5 salt stress levels) were divided into two major clusters.

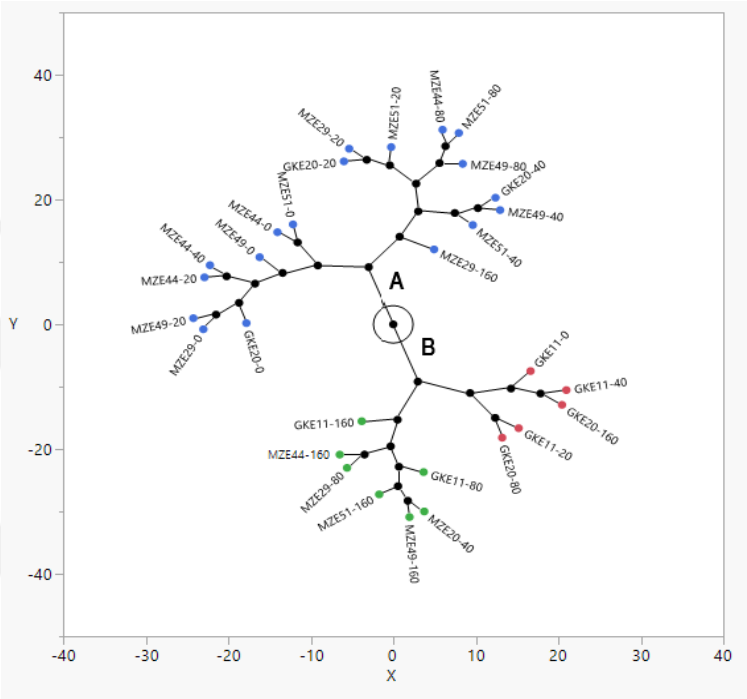

Figure 4. 18. Hierarchical clustering analysis (HCA)'s constellation plot

4.4.2. Discussion for *in vitro* salt stress assessment

Our findings differ from other eggplant salt tolerance studies in terms of water content retention. In fact, as the salt stress level increased, so did the water content. This might be due to the possible halophyte nature of *Solanun aethiopicum*. Obviously, it was reported that halophyte plants can withstand salinity levels above 200 mM (Flowers et al., 2015). These observations are consistent with what is observed in the chenopod (*Beta macrocarpa* Guss), known for its salt tolerance (Hamouda et al., 2015), and in *Asteriscus maritimus*,

Halimione portulacoides, and *Limonium cossonianum* (Jiménez-Becker et al., 2019).

An alternative explanation for the increase in water content during salt stress could be due to salinity adaptability mechanisms such as osmotic adjustment and compartmentation of ions, which are two of the most important physiological adaptations linked to salt tolerance in plants. Besides, it was found that dicotyledonous salt-tolerant plants maintain high concentrations of Na^+ and Cl^- in the central vacuolar to assist with osmotic regulation (Flowers and Colmer, 2008; Flowers et al., 2015).

Previous studies in eggplant have highlighted its ability to manage specific ions, particularly Na^+ during salinity stress (Akinci et al., 2004; Suarez et al., 2021). Moreover, the accumulation of Na^+ in both roots and shoots has been linked to a reduction in cumulative salt-induced growth in eggplant (Shaheen et al., 2013). Considering the fact that eggplant (*Solanum melongena*) is moderately sensitive to salinity (Ünlükara et al., 2010), our results confirm the high salt tolerance of *Solanum aethiopicum* over *Solanum melongena* because all 6 eggplant accessions used in this study managed to retain and increase water potential during salt treatment. Despite the fact that when the salt concentration rises and the water potential drops, cell division and elongation are obstructed, followed by stomatal closure and a reduction in photosynthetic activity.

All of the aforementioned consequences end up causing the plants to lose biomass, which is greatly exacerbated by poor or negative growth (Akinci et al., 2004). However, our results from 5 of 6 eggplant accessions were in conformity with findings by Galal (2019) that reported that different levels of salt stress to eggplant seedlings reduce the length of shoots and dry weights.

In summary, only the following eggplant accessions from population A, GKE11 and MZE44, performed well. Thus, they demonstrated high performance in terms of dry weight, fresh weight, and water content despite the rise in salinity

stress. These findings were also confirmed by the HCA results. HCA, which is based on the Ward aggregation distance clustering method, clearly displayed the relationship between eggplant accessions and their respective levels of salinity (Figure 4.18). However, each cluster accommodated accessions subjected to diverse stress levels. Eggplant accessions GKE11 and MZE44 were perfectly separated into two distinct clusters with respect to all levels of salinity stress. Expect one element of the MZE44 accession (MZE44-160) grouped in the opposite cluster B. The evidence from the PCA correlation matrix has confirmed the importance of the following variables (FW, NOL, AIC, and HGT) in defining our data. In other words, they were effective at differentiating all elements.

Soil salinity has a detrimental effect on agricultural yield. They are characterized by metabolic changes that have an impact on plant growth and development (Brenes et al., 2020). Recently, several strategies have been implemented to alleviate the effects of saline soil on eggplant and plants in general, including the use of biochar, which significantly improves stomatal conductance and eggplant photosynthesis rate, as well as reduces electrolyte leakage and leaf temperature in plant leaf tissues. Thereafter, better shoot growth, root growth, and fruit yield of eggplant can be obtained (Parkash and Singh, 2020).

Nano-fertilizers and sugar alcohols such as Sorbitol and lithovit®-guano25, which are known to have significant results by reducing the adverse effects caused by salinity, are examples of other applications used to mitigate the detrimental effect of saline soils on eggplant (*Solanum melongena* L) crops (Issa et al., 2020). *In vitro* experiments also showed that applying Zn to eggplant seedlings at diverse concentrations (5, 10 or 20 mg/L) raised the length of root and shoot as well as their dry weights even under saline conditions (Galal, 2019).

All of the above extrinsic mitigation strategies will not have the same impact as the use of salt-tolerant eggplant landraces. Plant landraces have always been heterogeneous and the geographical adaptations of cultivated plants give

genetic resources (resistant/tolerant genes) the capability to meet long-term farming difficulties related to global climate change in unfavourable environments such as salinity stress (Dwivedi et al., 2016).

5. CONCLUSION AND RECOMMENDETIONS

Landraces are crucial in contemporary plant breeding programs due to their extensive genetic diversity compared to modern varieties. As a direct consequence, they may be useful to expand the genetic base of modern cultivars. In this study, the genetic and phenotypic diversity of the largest germplasm collection of *Solanum aethiopicum* gr. Gilo accessions from Rwanda was assessed using the most recent molecular markers, the iPBS retrotransposon markers system for genetic diversity, and a couple of descriptors for eggplant morphological characterization. Furthermore, a study on embryogenesis induction through anther culture aimed to produce doubled haploid eggplants that are useful in breeding. Finally, *in vitro* salt stress was performed using the genetic groups obtained to evaluate salt stress induced morphological changes during early *in vitro* shoot growth in six different *Solanum aethiopicum* gr. Gilo accessions.

The use of iPBS-retrotransposon based markers system was successful and more accurate in establishing the current genetic diversity and relationships among scarlet eggplant accessions in Rwanda. Molecular characterization analyses have acknowledged the main population groups of eggplant germplasm. It includes Pop A, Pop B, and Pop C. Thus, high genetic diversity was pinpointed among those population groups. Local populations are often known to be valuable to breeders because they are naturally adapted to the local environment. We highly recommend the use of more genetically distant accessions as future parent candidates in the forthcoming breeding programs at the national level or at the continental level. In this regard, accessions MZE53 and GKE11 might be the implacable parent candidates. The obtained high relative genetic distance (0.6781) among two extreme accessions shows a superior level of diversity in the current germplasm, which could be the ample genetic base to explore in eggplant breeding and to establish the country’s first germplasm collection of African eggplant.

Similarly, phenotypic diversity analysis revealed a high level of diversity among *Solanum aethiopicum* gr. Gilo accessions. Multiple correlations disclosed that plant and fruit-related descriptors diverge enough to distinguish between germplasm populations. Fruit variables/descriptors, for instance, were more interdependent than plant variables/descriptors. Furthermore, phenotypic (hierarchical) clustering shows that clusters were formed primarily on the basis of fruit quantitative traits rather than plant quantitative traits. This means that fruit quantitative traits are critical for phenotypic characterization of *Solanum aethiopicum* gr. Gilo eggplant.

This study demonstrated that embryogenesis induction through androgenesis can be successfully performed in one of the brinjal relatives such as *Solanum aethiopicum* at a lower rate. *Solanum aethiopicum* accessions had significantly lower androgenic potential than *Solanum melongena* varieties. These findings highlight potential limitations for the widespread adoption of anther cultures in brinjal (*Solanum melongena* L.) relatives. Only the GKE12 accession responded and produced one embryo. The species was discovered to be the limiting factor in androgenic response. The knowledge on androgenic responses gained in this study about local landraces of *Solanum aethiopicum* from Rwanda will be used in the future to develop doubled haploids to support ongoing efforts in the common eggplant (brinjal) breeding program as well as other breeding efforts in other cultivated eggplant.

In this study, eggplant (*Solanum aethiopicum* gr. Gilo) landraces originating in Rwanda and comprised of six distinct accessions demonstrated potential for salt tolerance. The water content of all eggplant accessions increases slightly with salinity. The findings imply that *Solanum aethiopicum* may have halophyte attributes. Our findings recommend that the eggplant accessions GKE11 and MZE44 (both from Population A) be investigated further *in vivo* assessment at different salinity stress levels. Thus, they demonstrated high performance in terms

of tested parameters, specifically the water content, fresh weight, and dry weight of the aerial parts of the seedlings (shoots and leaves). When compared to controls, they remained higher and quite positive for the majority of them under various levels of salt stress. Following the identification of a population with a high tolerance to salt, future research might also focus on gaining a thorough understanding of the mechanisms associated with salt tolerance. For instance, high-throughput sequencing will be helpful in identifying salt-responsive miRNAs and their target genes for the breeding of salt-tolerant scarlet eggplant cultivars.

REFERENCES

Acquadro, A., Barchi, L., Gramazio, P., Portis, E., Vilanova, S., Comino, C., Plazas, M., Prohens, J., and Lanteri, S., 2017. Coding SNPs analysis highlights genetic relationships and evolution pattern in eggplant complexes. PLoS ONE, 12(7): 1–20. https://doi.org/10.1371/journal.pone.0180774

Adeniji, O. T., Kusolwa, P. M., and Reuben, S. O. W. M., 2012. Genetic diversity among accessions of *Solanum aethiopicum* L. groups based on morpho-agronomic traits. Plant Genetic Resources, 10(3): 177-185. https://doi.org/10.1017/S1479262112000226

Aguessy, S., Idossou, R., Dassou, A. G., Yêyinou, L. E. L., Yelome, O. I., Gbaguidi, A. A., Agre, P. A., Dansi, A., and Agbangla, C., 2021. Ethnobotanical characterization of scarlet eggplant (*Solanum aethiopicum* L.) varieties cultivated in Benin (West Africa). Journal of Agriculture and Food Research, 5:100173. https://doi.org/10.1016/j.jafr.2021.100173

Akinci, I. E., Akinci, S., Yilmaz, K., and Dikici, H., 2004. Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. New Zealand Journal of Crop and Horticultural Science, 32: 193–200. https://doi.org/10.1080/01140671.2004.9514296

Ali, F., Yılmaz, A., Nadeem, M. A., Habyarimana, E., Subaşı, I., Nawaz, M. A., Chaudhary, H. J., Shahid, M. Q., Ercişli, S., Zia, M.A.B., Chung, G., and Baloch, F. S., 2019. Mobile genomic element diversity in world collection of safflower (*Carthamus tinctorius* L.) panel using iPBS-retrotransposon markers. PloS One, 14(2): e0211985. https://doi.org/10.1371/journal.pone.0211985

Alpsoy, H.C., and Şeniz, V., 2007. Researches on the *in vitro* Androgenesis and Obtaining Haploid Plants in Some Eggplant Genotypes. Acta Horticulturae, 729: 137–141. https://doi.org/10.17660/ActaHortic.2007.729.21

Baloch, F.S., Alsaleh, A., de Miera, L.E.S., Hatipoğlu, R., Çiftçi, V., Karaköy, T., Yıldız, M., and Özkan, H., 2015. DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey. Biochemical Systematic and Ecology, 61: 244–252. https://doi.org/10.1016/j.bse.2015.06.017

Barut, M., Nadeem, M. A., Karaköy, T., and Baloch, F. S., 2020. DNA fingerprinting and genetic diversity analysis of world quinoa germplasm using iPBS-retrotransposon marker system. Turkish Journal of Agriculture and Forestry, 44: 479–491. https://doi.org/10.3906/tar-2001-10

Başay, S., and Ellialtıoğlu, Ş.Ş., 2013. Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L.). Turkish Journal of Biology, 37: 499–505. https://doi.org/10.3906/biy-1210-38

Bationo-Kando, P., Sawadogo, B., Nanema, K. R., Kiebre, Z., Sawadogo, M., and Zongo, J. D., 2015. Characterization of *Solanum aethiopicum* (Kumba group) in Burkina Faso. International Journal of Science and Nature, 6(2): 169–176.

Borràs, D., Plazas, M., Andújar, I., Gramazio, P., Herraiz, J. F., Prohens, J., and Vilanova, S., 2015. Molecular characterization of scarlet and gboma eggplants based on single nucleotide polymorphisms. Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Horticulture, 72(2): 1–3. https://doi.org/10.15835/buasvmcn-hort:11408

Boyaci, H. F., Prohens, J., Unlu, A., Gumrukcu, E., Oten, M., and Plazas, M., 2020. Association of Heterotic Groups with Morphological Relationships and General Combining Ability in Eggplant. Agriculture, 10(203): 1–13. https://doi.org/10.3390/agriculture10060203

Brenes, M., Pérez, J., González-Orenga, S., Solana, A., Boscaiu, M., Prohens, J., Plazas, M., Fita, A., and Vicente, O., 2020. Comparative Studies on the Physiological and Biochemical Responses to Salt Stress of Eggplant (*Solanum melongena*) and Its Rootstock *S. torvum*. Agriculture, 10: 328. https://doi.org/10.3390/agriculture10080328

Caguiat, X. G. I., and Hautea, D. M., 2014. Genetic diversity analysis of eggplant (*Solanum melongena* L.) and related wild species in the Philippines using morphological and SSR markers. SABRAO Journal of Breeding and Genetics, 46(2): 183–201.

Calabuig-Serna, A., Porcel, R., Corral-Martínez, P., and Seguí-Simarro, J. M., 2020. Anther Culture in Eggplant (*Solanum melongena* L.) (M. Bayer editor). Plant Embryogenesis. Methods in Molecular Biology (Volume 2122), Humana Press, New York, p. 283-293.

Collonnier, C., Mulya, K., Fock, I., Mariska, I., Servaes, A., Vedel, F., Siljak-Yakovlev, S., Souvannavong, V., Ducreux, G., and Sihachakr, D., 2001. Source of resistance against *Ralstonia solanacearum* in fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L. Plant Science, 160(2): 301–313. https://doi.org/10.1016/S0168-9452(00)00394-0

Context Network (2016). Rwanda Early Generation Seed Study: Country Report. Nairobi, Kenya: Africa Lead (US Agency for International Development).

Dijkxhoorn, Y., Saavedra Gonzalez, Y., and Judge, L. O., 2016. Horticulture and floriculture in Rwanda; Identification of focus areas for sector development, In LEI Memorandum 2015-161, LEI Wageningen UR, Wageningen.

Ding, H. D., Zhu, X. H., Zhu, Z. W., Yang, S. J., Zha, D. S., and Wu, X. X., 2012. Amelioration of salt-induced oxidative stress in eggplant by application of 24-epibrassinolide. Biologia Plantarum, 56: 767–770. https://doi.org/10.1007/s10535-012-0108-0

Doganlar, S., Frary, A., Daunay, M. C., Lester, R. N., and Tanksley, S. D., 2002. A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics Society of America, 161(4): 1697–1711. https://doi.org/10.1093/genetics/161.4.1697

Doyle, J.J., and Doyle, J.L., 1990. Isolation of plant DNA from fresh leaf tissue. Focus, 12: 13–15.

Dumas De Vaulx, R., and Chambonnet, D., 1982. Culture *in vitro* d'anthères d'aubergine (*Solanum melongena* L.): stimulation de la production de plantes au moyen de traitments à +35°C associés à de faibles teneurs en substances de croissance. Agronomie, 2(10): 983–988.

Dusengemungu, L., Igiraneza, C., and Uwimbabazi, S., 2019. Plant biotechnology: A key tool to improve crop production in Rwanda. African Journal of Biotechnology, 18(3): 68–76. https://doi.org/10.5897/ajb2018.16662

Dwivedi, S. L., Ceccarelli, S., Blair, M. W., Upadhyaya, H. D., Are, A. K., and Ortiz, R., 2016. Landrace Germplasm for Improving Yield and Abiotic Stress Adaptation. Trends in Plant Science, 21: 31–42. https://doi.org/10.1016/j.tplants.2015.10.012

Eletta, O. A. A., Orimolade, B. O., Oluwaniyi, O. O., and Dosumu, O. O., 2017. Evaluation of Proximate and Antioxidant Activities of Ethiopian Eggplant (*Solanum aethiopicum* L) and Gboma Eggplant (*Solanum macrocarpon* L). Journal of Applied Sciences and Environmental Management, 21(5): 967–972. https://doi.org/10.4314/jasem.v21i5.25

Emrani Dehkehan, M., Moieni, A., and Movahedi, Z., 2017. Effects of zeatin riboside, mannitol and heat stress on eggplantn (*Solanum melongena* L.) anther culture. Iranian Journal of Genetics and Plant Breeding, 6(1): 16–26.

Emrani Dehkehan, M., Moieni, A., and Movahedi, Z., 2017. Effects of zeatin riboside, mannitol and heat stress on eggplant (*Solanum melongena* L.) anther culture. Iranian Journal of Genetics and Plant Breeding, 6(1): 16–26.

Evanno, G., Regnaut, S., and Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14 (8): 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Flowers, T. J., and Colmer, T. D., 2008. Salinity tolerance in halophytes. The New Phytologist, 179: 945–963.

Flowers, T. J., Munns, R., and Colmer, T. D. 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Annals of Botany, 115(3): 419–431. https://doi.org/10.1093/aob/mcu217

Foo, P. C., Lee, Z. H., Chin, C. K., Subramaniam, S., and Chew, B.L., 2018. Shoot Induction in White Eggplant (*Solanum melongena* L. Cv. Bulat Putih) using 6-Benzylaminopurine and Kinetin. Tropical Life Sciences Research, 29(2): 119–129. https://doi.org/10.21315/tlsr2018.29.2.9

Fu, Q., Liu, C., Ding, N., Lin, Y., Guo, B., 2010. Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. Agricultural Water Management, 97: 1994–2000. https://doi.org/10.1016/j.agwat.2010.02.003

Galal, A., 2019. Exogenous application of zinc mitigates the deleterious effects in eggplant grown under salinity stress. Journal of Plant Nutrition, 42: 915–927. https://doi.org/10.1080/01904167.2019.1584221

Gapusi, R. J., Isibo, T., Nyirigira, R. A., Gashamura, R. F., Sirikari, S., Uwimana, J. d-A., Byamushana, C., Bizeye, B., and Hakizimana P., 2013. The state of plant genetic resources for food and agriculture in Rwanda, Country Report, Rwanda Agriculture and Animal Resources Development Board (RAB), Kigali.

Ge, H., Liu, Y., Jiang, M., Zhang, J., Han, H., and Chen, H., 2013. Analysis of genetic diversity and structure of eggplant populations (*Solanum melongena* L.) in China using simple sequence repeat markers. Scientia Horticulturae, 162: 71–75. https://doi.org/10.1016/j.scienta.2013.08.004

Gramazio, P., Blanca, J., Ziarsolo, P., Herraiz, F. J., Plazas, M., Prohens, J., and Vilanova, S., 2016. Transcriptome analysis and molecular marker discovery in *Solanum incanum* and *S. aethiopicum*, two close relatives of the brinjal eggplant (*Solanum melongena*) with interest for breeding. BMC Genomics, 17(1): 300. https://doi.org/10.1186/s12864-016-2631-4

Gunalp, B., Horasan, O., Yasar, F., Kusvuran, S., Tipirdamaz, R., and Ellialtioglu, S., 2011. Effects of jasmonic acid and salt applications on antioxydative enzyme activities of the eggplant seedlings grown *in vitro* culture. Current Opinion in Biotechnology, 22: S141. https://doi.org/10.1016/j.copbio.2011.05.465

Haliński, Ł. P., Samuels, J., and Stepnowski, P., 2017. Multivariate analysis as a key tool in chemotaxonomy of brinjal eggplant, African eggplants and wild related species. Phytochemistry, 144: 87–97. https://doi.org/10.1016/j.phytochem.2017.09.001

Hamouda, I., Badri, M., Mejri, M., Cruz, C., Siddique, K. H. M., Hessini, K., 2015. Salt tolerance of *Beta macrocarpa* is associated with efficient osmotic adjustment and increased apoplastic water content. Plant Biology, 18: 369–375. https://doi.org/10.1111/plb.12419

Hannachi, S., and van Labeke, M. C., 2018. Salt stress affects germination, seedling growth and physiological responses differentially in eggplant cultivars (*Solanum melongena* L.). Scientia Horticulturae, 228: 56–65. https://doi.org/10.1016/j.scienta.2017.10.002

Issa, D. B., Alturki, S. M., Sajyan, T. K., and Sassine, Y. N., 2020. Sorbitol and lithovit-guano25 mitigates the adverse effects of salinity on eggplant grown in pot experiment. Agronomy Research, 18: 113–126.

Isshiki, S., Uchiyama, T., Tashiro, Y., and Miyazaki, S., 1998. RFLP analysis of a PCR amplified region of chloroplast DNA in eggplant and related *Solanum* species. Euphytica, 102, 295–299. https://doi.org/10.1023/A:1018304308608

Jiménez-Becker, S., Ramírez, M., and Plaza, B. M., 2019. The influence of salinity on the vegetative growth, osmolytes and chloride concentration of four halophytic species. Journal of Plant Nutrition, 42: 1838–1849. https://doi.org/10.1080/01904167.2019.1648666

Kalendar, R., Antonius, K., Smýkal, P., and Schulman, A.H., 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theoretical and Applied Genetics, 121: 1419–1430. https://doi.org/10.1007/s00122-010-1398-2

Kamga, R.T., Kouamé, C., Atangana, A.R., Abdulai, M.S., and Tenkouano, A., 2015. Characterization of African eggplant accessions for morphological and yield parameters in the bimodal rainfall agroecology of Cameroon. Acta Horticulturae, 1102: 109–120. https://doi.org/10.17660/ActaHortic.2015.1102.13

Kamga, R.T., Kouamé, C., Atangana, A.R., Chagomoka, T., and Ndango, R., 2013. Nutritional Evaluation of Five African Indigenous Vegetables. Journal of Horticultural Research, 21(1): 99-106. https://doi.org/10.2478/johr-2013-0014

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M. S., Saleem, M. H., Adil, M., Heidari, P., and Chen, J-T., 2019. An Overview of Hazardous Impacts of Soil Salinity in Crops, Tolerance Mechanisms, and Amelioration through Selenium Supplementation. International Journal of Molecular Sciences, 21: 148. https://doi.org/10.3390/ijms21010148

Kansiime, M. K., Ochieng, J., Kessy, R., Karanja, D., Romney, D., and Afari-Sefa, V., 2018. Changing knowledge and perceptions of African indigenous vegetables: the role of community-based nutritional outreach. Development in Practice, 28 (4): 480–493. https://doi.org/10.1080/09614524.2018.1449814

Kashyap, V., Kumar, S. V., Collonnier, C., Fusari, F., Haicour, R., Rotino, G. L., Sihachakr, D., and Rajama, M. V., 2003. Biotechnology of eggplant. Scientia Horticulturae, 97 (1): 1–25. https://doi.org/10.1016/S0304-4238(02)00140-1

Khatun, F., Meah, M. B., and Nasiruddin, K. M., 2006. Regeneration of eggplant through anther culture. Pakistan Journal of Biological Sciences, 9: 48–53.

Kirtiş, A., and Aasim, M., 2019. Thidiazuron (TDZ) Induced *in vitro* Axillary Shoot Regeneration of Desi Chikpea (*Cicer arietinum* L.). Journal of Applied Biology Science, 13: 17–20.

Knapp, S., Aubriot, X., and Prohens, J., 2019. Eggplant (*Solanum melongena* L.): Taxonomy and Relationships (M. Chapman editor). The Eggplant Genome. Compendium of Plant Genomes, Springer, Cham. p. 11–22.

Knapp, S., Vorontsova, M.S., and Prohens, J., 2013. Wild Relatives of the Eggplant (*Solanum melongena* L.: Solanaceae): New Understanding of Species Names in a Complex Group. PLoS ONE, 8(2): e57039. https://doi.org/10.1371/journal.pone.0057039

Kouassi, A., Béli-Sika, E., Tian-Bi, T., Alla-N'Nan, O., Kouassi, A., N'Zi, J-C, N'Guetta, A. S-P., and Tio-Touré, B., 2014. Identification of three distinct eggplant subgroups within the *Solanum aethiopicum* gilo group from Côte d'Ivoire by morpho-agronomic characterization. Agriculture, 4(4): 260–273. https://doi.org/10.3390/agriculture4040260

Kumar, S., Singh, M., Prabhavathi, K., Mathews, A., Kumar, S., Singh, M., and Mathews, A., 2003. *In vitro* induction of haploid in eggplant (*Solanum melongena* L.). Capsicum and Eggplant Newsletter, 22: 147–150.

Larochelle, C., and Alwang, J. R., 2014. Impacts of Improved Bean Varieties on Food Security in Rwanda Impacts of Improved Bean Varieties on Food Security in Rwanda, In: 2014 Annual meeting - Agricultural and Applied Economics Association (AAEA) Conferences-2014 Annual Meeting, Minneapolis, pp. 1–27.

Lester, R. N., and Daunay, M. C., 2003. Diversity of African vegetable *Solanum* species and its implications for a better understanding of plant domestication (H. Knüpffer and J. Ochsmann editors). Proceedings of a symposium dedicated to the 100th birthday of Rudolf Mansfeld, 8-9 October 2001, Gatersleben, Zentralstelle für Agrardokumentation und -information (ZADI), pp. 136–152.

Liu, J., Yang, Y., Zhou, X., Bao, S., and Zhuang, Y., 2018. Genetic diversity and population structure of worldwide eggplant (*Solanum melongena* L.) germplasm using SSR markers. Genetic Resources and Crop Evolution 65: 1663–1670. https://doi.org/10.1007/s10722-018-0643-4

Mibei, E. K., Owino, W. O., Ambuko, J., Giovannoni, J. J., and Onyango, A. N., 2018. Metabolomic analyses to evaluate the effect of drought stress on selected African Eggplant accessions. Journal of the Science of Food and Agriculture, 98(1): 205–216. https://doi.org/10.1002/jsfa.8458

Mir, R., Calabuig-Serna, A., and Seguí-Simarro, J. M., 2021. Doubled haploids in eggplant. Biology, 10(7): 685. https://doi.org/10.3390/biology10070685

Mujuju, C., 2018. Identifying Leading Seed Companies in Western and Central Africa, Access to Seeds Foundation, Harare.

Murashige, T., and Skoog, F., 1962. A revised medium for rapid growth and bioassays. Physiologia Plantarum, 15: 473–497.

Nieves-Cordones, M., Martínez, V., Benito, B., Rubio, F., 2016. Comparison between Arabidopsis and Rice for Main Pathways of K+ and Na+ Uptake by Roots. Frontiers in Plant Science, 7: 992. https://doi.org/10.3389/fpls.2016.00992

Nkansah, G.O., 2001. Some physiological features of the African eggplant, *Solanum aethiopicum* group 'Gilo.' Scientia Horticulturae, 90 (1-2) : 181–186. https://doi.org/10.1016/S0304-4238(00)00254-5

Ntihinyurwa, P. D., de Vries, W. T., Chigbu, U. E., Dukwiyimpuhwe, P. A., 2019. The positive impacts of farm land fragmentation in Rwanda. Land Use Policy 81: 565–581. https://doi.org/10.1016/j.landusepol.2018.11.005

Nunome, T., Negoro, S., Kono, I., Kanamori, H., Miyatake, K., Yamaguchi, H., Ohyama, A., and Fukuoka, H., 2009. Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theoretical and Applied Genetics, 119(6): 1143–1153. https://doi.org/10.1007/s00122-009-1116-0

Öçal, S., Özalp, T., and Devran, Z., 2018. Reaction of wild eggplant *Solanum torvum* to different species of root-knot nematodes from Turkey. Journal of Plant Diseases and Protection, 125: 577–580. https://doi.org/10.1007/s41348-018-0167-3

Ofori, K., and Gamedoagbao, D. K., 2005. Yield of scarlet eggplant (*Solanum aethiopicum* L.) as influenced by planting date of companion cowpea. Scientia Horticulturae, 105:305–312. https://doi.org/10.1016/j.scienta.2005.02.003

Osei, M. K., Banful, B., Osei, C. K., and Oluoch, M. O., 2010. Characterization of African eggplant for morphological characteristics. Journal of Agricultural Science and Technology, 4(3): 33–37.

Parkash, V., and Singh, S., 2020. Potential of Biochar Application to Mitigate Salinity Stress in Eggplant. HortScience, 55: 1946-1955.

Peakall, R., and Smouse, R. P. P., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research: An update. Bioinformatics 28(19): 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Plazas, M., Andújar, I., Vilanova, S., Gramazio, P., Javier Herraiz, F., and Prohens, J., 2014. Conventional and phenomics characterization provides insight into the diversity and relationships of hypervariable scarlet (*Solanum aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplant complexes. Frontiers in Plant Science, 5: 1–13. https://doi.org/10.3389/fpls.2014.00318

Prasad, P. V. V., Hijmans, R. J., Pierzynski, G. M., and Middendorf, J. B., 2016. Climate Smart Agriculture and Sustainable Intensification: Assessment and Priority Setting for Rwanda. Feed the Future Sustainable Intensification Innovation Lab (Kansas State University), Manhattan, Kansas, USA.

Prohens, J., Blanca, J., and Nuez, F., 2005. Morphological and molecular variation in a collection of eggplants from a secondary center of diversity: Implications for conservation and breeding. Journal of the American Society for Horticultural Science, 130(1): 54–63. https://doi.org/10.21273/JASHS.130.1.54

Roldán-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A., and De Loose, M., 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding, 6: 125–134. https://doi.org/10.1023/A:1009680614564

Rotino, G. L., 2016. Anther Culture in Eggplant (*Solanum melongena* L.) (M. Germana and M. Lambardi editors), *In vitro* embryogenesis in higher plants, Methods in Molecular Biology (Volume 1359), Humana Press, New York, NY.

Sakhanokho, H. F., Islam-Faridi, M. N., Blythe, E. K., Smith, B. J., Rajasekaran, K., and Majid, M. A., 2014. Morphological and cytomolecular assessment of intraspecific variability in Scarlet eggplant (*Solanum aethiopicum* L.). Journal of Crop Improvement, 28(4): 437–453. https://doi.org/10.1080/15427528.2014.913280

Salas, P., Prohens, J., and Seguí-Simarro, J.M., 2011. Evaluation of androgenic competence through anther culture in common eggplant and related species. Euphytica, 182: 261-274. https://doi.org/10.1007/s10681-011-0490-2

Salas, P., Rivas-Sendra, A., Prohens, J., and Seguí-Simarro, J. M., 2012. Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther cultures. Euphytica, 184(2): 235–250. https://doi.org/10.1007/s10681-011-0569-9

Schreinemachers, P., Sequeros, T., and Lukumay, P. J., 2017. International research on vegetable improvement in East and Southern Africa: Adoption, impact, and returns. Agricultural Economics, 48: 707–717. https://doi.org/10.1111/agec.12368

Seguí-Simarro, J.M., 2016. Androgenesis in Solanaceae (M. Germana, and M. Lambardi editors). *In Vitro* Embryogenesis in Higher Plants. Methods in Molecular Biology, vol 1359. Humana Press, New York, NY, pp. 206–244.

Shah, M. A., Khan, A. I., Awan, F. S., Sadaqat, H. A., Bahadur, S., Rasheed, A., and Baloch, F. S., 2014. Genetic Diversity of Some Tomato Cultivars and Breeding Lines Commonly Used in Pakistani Breeding Program. Turkish Journal of Agriculture - Food Science and Technology, 3(3): 126–132. https://doi.org/10.24925/turjaf.v3i3.126-132.249

Shaheen, S., Naseer, S., Ashraf, M., and Akram, N. A., 2013. Salt stress affects water relations, photosynthesis, and oxidative defense mechanisms in *Solanum melongena* L. Journal of Plant Interactions, 8: 85–96. https://doi.org/10.1080/17429145.2012.718376

Sharma, M., and Kaushik, P., 2021. Biochemical composition of eggplant fruits: A review. Applied Science, 11: 7078. https://doi.org/10.3390/app11157078

Shimira, F., Afloukou, F., and Maniriho, F. 2020. A review on challenges and prospects of potato (*Solanum tuberosum*) production systems in Rwanda. Journal of Horticulture and Postharvest Research, 3: 97–112. https://doi.org/10.22077/jhpr.2020.2854.1099

Shimira, F., Boyaci, H. F., Çilesiz, Y., Nadeem, M. A., Baloch, F. S., and Taşkin, H., 2021. Exploring the genetic diversity and population structure of scarlet eggplant germplasm from Rwanda through iPBS-retrotransposon markers. Molecular Biology Reports, 48 (9): 6323-6333. https://doi.org/10.1007/s11033-021-06626-0

Shimira, F., Keleş, D., Taşkın, H., and Abak, K., 2019. The assessment of androgenic response of two nematode resistant pepper (*Capsicum annuum* L.) genotypes. Turkish Journal of Agriculture-Food Science and Technology, 7(12): 2103-2110. https://doi.org/10.24925/turjaf.v7i12.2103-2110.2828

Song, B., Song, Y., Fu, Y., Kizito, E. B., Kamenya, S. N., Kabod, P. N., Liu, H., Muthemba, S., Kariba, R., Njuguna, J., Maina, S., Stomeo, F., Djikeng, A., Hendre, P. S., Chen, X., Chen, W., Li, X., Sun, W., Wang, S., Cheng, S., Muchugi, A., Jamnadass, R., Shapiro, H-Y., Van Deynze, A., Yang, H., Wang, j., Xu, X., Odeny, D. A., and Liu, X., 2019. Draft genome sequence of *Solanum aethiopicum* provides insights into disease resistance, drought tolerance, and the evolution of the genome. GigaScience, 8: 1–16. https://doi.org/10.1093/gigascience/giz115

Sseremba, G., 2019. Genetic diversity and breeding of *Solanum aethiopicum* shum group for drought tolerance. University of Ghana PhD Thesis, Accra, Ghana.

Stedje, B., and Bukenya-Ziraba, R., 2003. RAPD variation in *Solanum anguivi* Lam. and *S. aethiopicum* L. (Solanaceae) in Uganda. Euphytica, 131(3): 293–297. https://doi.org/10.1023/A:1024079208879

Suarez, D. L., Celis, N., Ferreira, J. F. S., Reynolds, T., and Sandhu, D., 2021. Linking genetic determinants with salinity tolerance and ion relationships in eggplant, tomato and pepper. Scientific Reports, 11: 16298. https://doi.org/10.1038/s41598-021-95506-5

Sunseri, F., Polignano, G. B., Alba, V., Lotti, C., Bisignano, V., Mennella, G., Alessandro, A. D., Bacchi, M., Riccardi, P., Fiore, M.C., and Ricciardi, L., 2010. Genetic diversity and characterization of African eggplant germplasm collection. African Journal of Plant Science, 4(7): 231–241. https://doi.org/10.5897/AJPS.9000128

Taher, D., Solberg, S. Ø., Prohens, J., Chou, Y-Y., Rakha, M., and Wu, T-H., 2017. World vegetable center eggplant collection : Origin, composition, seed dissemination and utilization in Breeding. Frontiers in Plant Science, 8: 1–12. https://doi.org/10.3389/fpls.2017.01484

Tümbilen, Y., Frary, A., Daunay, M. C., and Doğanlar, S., 2011. Application of EST-SSRs to examine genetic diversity in eggplant and its close relatives. Turkish Journal of Agriculture and Forestry, 35: 125–136. https://doi.org/10.3906/biy-0906-57

Ünlükara, A., Kurunç, A., Kesmez, G. D., Yurtseven, E., and Suarez, D. L., 2010. Effects of salinity on eggplant (*Solanum melongena* L.) growth and evapotranspiration. Irrigation and Drainage, 59: 203–214. https://doi.org/10.1002/ird.453

Uwamahoro, F., Yuen, J., Berlin, A., Bucagu, C., and Bylund, H., 2020. *Ralstonia solanacearum* causing potato bacterial wilt: Host range and cultivars' susceptibility in Rwanda. Plant Pathology 69(3): 559–568. https://doi.org/10.1111/ppa.13140

Van Dijk, N., and Elings, A., 2014. Horticulture in Rwanda: Possibilities for Further Development, BoP Innovation Center – 28 (Wageningen University), Wageningen, the Netherlands.

Vilanova, S., Manzur, J. P., and Prohens, J., 2012. Development and characterization of genomic simple sequence repeat markers in eggplant and their application to the study of diversity and relationships in a collection of different cultivar types and origins. Molecular Breeding, 30(2): 647–660. https://doi.org/10.1007/s11032-011-9650-2

Vural, G. E., and Ari, E., 2020. Triple synergistic effect of maltose, silver nitrate and activated charcoal on high embryo yield of eggplant (*Solanum melongena* L.) anther cultures. Scientia Horticulturae, 272: 109472. https://doi.org/10.1016/j.scienta.2020.109472

Vural, G. E., Ari, E., Zengin, S., and Ellialtioglu, S. S., 2019. Development of androgenesis studies on eggplant (*Solanum melongena* L.) in Turkey from past to present (M. Hasanuzzaman, M. Fujita, M. C. M. Teixeira Filho, T. A. R. Nogueira, F. S. Galindo editors), Sustainable Crop Production, BoD–Books on Demand, Sustainable Crop Production, IntechOpen, London, UK.

Wang, Q., Zhao, F., Sun, Q., and Yang, A., 2010. Genetic Diversity of Eggplant Revealed by SSR Markers. In: 4th International Conference on Bioinformatics and Biomedical Engineering (iCBBE 2010), IEEE Xplore, Chengdu, 229–232.

Weller, S. C., Van Wyk, E., and Simon, J. E., 2015. Sustainable production for more resilient food production systems: Case study of African indigenous vegetables in eastern Africa. Acta Horticulturae, 1102: 289–297. https://doi.org/10.17660/ActaHortic.2015.1102.35

Yaldız, G., Camlica, M., Nadeem, M. A., Nawaz, M. A., and Baloch, F. S., 2018. Genetic diversity assessment in *Nicotiana tabacum* L. with iPBS-retrotransposons. Turkish Journal of Agriculture and Forestry, 42: 154–164. https://doi.org/10.3906/tar-1708-32

Yasar, F., Ellialtioglu, S., and Kusvuran, S., 2006. Ion and lipid peroxide content in sensitive and tolerant eggplant callus cultured under salt stress. European Journal of Horticultural Science, 71(4): 169–172.

Yeh, F. C., Yang, R., Boyle, T. J, Ye, Z., and Xiyan, J. M., 2000. PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

Yıldız, M., Koçak, M., and Baloch, F. S., 2015. Genetic bottlenecks in Turkish okra germplasm and utility of iPBS retrotransposon markers for genetic diversity assessment. Genetics and Molecular Research, 14: 10588-10602. https://doi.org/10.4238/2015.September.8.20

Yıldız, M., Koçak, M., Nadeem, M.A., Cavagnaro, P., Barboza, K., Baloch, F.S., Argün, D., and Keleş, D., 2019. Genetic diversity analysis in the Turkish pepper germplasm using iPBS retrotransposon based markers. Turkish Journal of Agriculture and Forestry, 44: 1–14. https://doi.org/10.3906/tar-1902-10

Yücel, N. K., 2017. Natural and *in vitro* hybridization studies between *Solanum melongena* L. and *Solanum torvum* SW., Cukurova University PhD Thesis, Adana.

Zhuang, Y., Zhou, X., and Wang, S., 2012. Genetic diversity of NBS-LRR class disease-resistance gene analogs in cultivated and wild eggplants. Plant Systematics and Evolution, 298(7): 1399–1406. https://doi.org/10.1007/s00606-012-0645-1

RESUME

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