GENETIC DIVERSITY AND BREEDING OF *Solanum aethiopicum* SHUM GROUP FOR DROUGHT TOLERANCE

By

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Solanum aethiopicum is one of the most important *Solanum* species, with four morphological groups. Two of the groups, Gilo and Shum, are mainly cultivated because of their nutritional value and income generating potential for farmers in developing countries. Of focus for this study was the Shum, a leafy morphological group whose productivity and quality is directly affected by drought. Global limitations on water resource availability call for the need to develop productive varieties that are drought tolerant. This research was aimed at: (i) determining the genetic distinctiveness between Shum and its progenitor, *S. anguivi* (SAN); (ii) evaluating genetic diversity within Shum germplasm; (iii) identifying parental material for development of drought tolerant *S. aethiopicum* Shum varieties; and (iv) determining the combining ability of selected Shum group germplasm for drought tolerance. Twenty-five accessions, five of which were wild progenitors, were evaluated for morphological attributes. Similarly, clustering was used to identify structure within 20 accessions of Shum based on 61 morphological variables. Further, Shum germplasm were evaluated to discover accessions (G) which excelled across water deficit regimes (WLs) where a split-plot arrangement was used. In order to determine the mode of gene action and combining ability for drought resistance among accessions, 24 F1 hybrids from a North Carolina II mating design were evaluated at five moisture regimes premised on crop growth stage and applied moisture as a percentage of field capacity of potting substrate. Five distinct clusters were identified; the progenitor accessions for Shum were grouped in their own cluster; and days to germination and emergence provided the best separation between Shum and SAN. Four distinct clusters were obtained within Shum where it was established that genotype discrimination is possible at seedling (seedling vigor), vegetative (leaves per plant, harvest index and plant growth habit) and reproductive (for instance basing on petal length and seed color) stages. From drought

screening study, highly significant effect ($p < 0.05$) of at least two WLs on performance among at least two genotypes for majority of the traits was obtained; in addition to very highly significant ($p < 0.001$) G x WL interactions for leaf relative water content (LRWC), leaves per plant (LPP) and plant height (PH). Basing on LRWC, superior and most stable genotypes were identified as E6 followed by E12, E15, E18 and E14GP. The broad sense heritability for each measured trait for water deficit stress tolerance breeding was > 0.9 and expected genetic advance as per cent of grand mean ranged from 16.68 (for LRWC) to 70.38 % (for PH) per generation; indicating a prospective response to selection. Effects of specific combining ability (SCA) were significant across and within moisture regimes for all traits studied unlike for general combining ability (GCA) where significant effects were obtained with chlorophyll content (CHL) only. In the narrow sense (h^2), the most highly heritable traits were identified as LPP, CHL, leaf fresh yield (LYF) and leaf dry yield (LYD) while leaf area (LA), leaf mass area (LMA) and LRWC were least heritable. Broad sense heritability (H^2) was however, > 0.80 for all measured traits, indicating that nonadditive gene action exceeded additive gene effects (V_A) for moisture deficit stress tolerance in Shum. Female parent E11 had the best GCA effects for CHL. The crosses with best SCA effects were identified as E10xE20 (for LA under well-watered), E3HxE15 (for LYF across watering environments, LRWC under drought stress and CHL under drought recovery), and E11xE4 (for LMA under drought recovery). This research established that morphological markers are useful for distinguishing the Shum from its progenitor; as well as within Shum genotypes at any growth stage. Also, genotypes with good breeding value and promising specific crosses offer vital information as basis for establishment of a breeding programme for the crop.

DEDICATION

To you the stakeholder in leafy vegetable value chain

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LIST OF ACRONYMS

AVRDC	Asian Vegetable Research and Development Center
CCI	Chlorophyll content index
CHL	Chlorophyll content
CSAA	Crop Scientists for Africa Agriculture
CSS	Stability superiority coefficient
DAAD	Germany Academic Exchange Service
DF	Discriminant function
DT	Drought tolerance
DR	Drought recovery
DS	Drought stress
FAO	Food and Agriculture Organization
FC	Field capacity
GA	Genetic advance
GCA	General combining ability
GCV	Genotypic coefficient of variation
GLM	Generalized linear mixed model
IBPGR	International Board on Plant Genetic Resources
Intra-ACP	Intra – African, Caribbean, and Pacific group of States
LDA	Linear discriminant analysis
LM	Mixed model
LMA	Leaf mass area

LRWC	Leaf relative water content
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MANOVA	Multivariate analysis of variance
MAS	Marker assisted selection
NARO	National Agricultural Research Organization
PCA	Principal component analysis
QTLs	Quantitative trait loci
RE	Re-watered
SAN	*Solanum anguivi*
SCA	Specific combining ability
Shum	*Solanum aethiopicum* Shum group
swi	Shannon-weaver index
TWAS	The World Academy of Sciences
UPGMA	Unweighted pair group method with arithmetic mean
USAID	United States Agency for International Development
WorldVeg	World Vegetable Center
WACCI	West Africa Centre for Crop Improvement
WUE	Water use efficiency
WW	Well-watered

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PUBLICATIONS

The following publications are outputs of this thesis (in reverse order):

1) **Sseremba G**, Tongoona P, Eleblu JSY, Danquah EY, Kizito EB (2018). Heritability of drought resistance in *Solanum aethiopicum* Shum group and combining ability of genotypes for drought tolerance and recovery. Scientia Horticulturae 240:213–220. https://doi.org/10.1016/j.scienta.2018.06.028

2) **Sseremba G**, Tongoona P, Eleblu JSY, Danquah EY, Kaweesi T, Baguma Y, Masanza M, Kizito EB (2018). Stability of *Solanum aethiopicum* Shum accessions under varied water deficit stress levels and identification of pertinent breeding traits for resistance to water shortage. Euphytica 214(11):1-11. doi: https://doi.org/10.1007/s10681-017-2097-8

3) **Sseremba G**, Tongoona P, Eleblu JSY, Danquah EY, Kizito EB (2018). Linear discriminant analysis of structure within African eggplant 'Shum'. African Crop Science Journal 26(1):37-48. doi: https://www.ajol.info/index.php/acsj/issue/view/16652

4) **Sseremba G**, Tongoona P, Eleblu JSY, Danquah EY, Kabod PN, Kizito EB (2017). Morphological distinctiveness between *Solanum aethiopicum* Shum group and its progenitor. Journal of Plant Breeding and Crop Science 9(8):118-129. https://doi.org/10.5897/JPBCS2017.0663

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

The Shum group, a subspecies of *Solanum aethiopicum* Lam. (2n=24, section Oliganthes, sub-family Solanoideae, family Solanaceae), consists of leafy vegetable crops that are indigenous in tropical Africa; characterized by high rainfall and/or watering supplementation (Abukutsa-Onyango *et al*., 2010; Adeniji *et al*., 2012; FAO, 2005; Plazas *et al*., 2014). Other morphological groups are mainly grown for fruits and ornamentals, and they include the Gilo, Kumba and Aculeatum (Adeniji *et al*., 2013, 2012). Specifically, Aculeatum, Gilo, Kumba and Shum are cultivated for ornamental, fruits, both fruits and leaves, and leaves, respectively (Sękara *et al*., 2007; Kubie, 2013; Plazas *et al*., 2014). These subspecies can be distinguished based on either morpho-agronomic traits (Adeniji *et al*., 2013; Kubie, 2013; Prohens *et al*., 2013), phenomics in combination with morphological traits (Prohens *et al*., 2013) or using DNA markers (Adeniji *et al*., 2013). In tropical Brazil, the Gilo fruits are important as a market vegetable, with at least 7,000 hectares being cultivated; with statistics for sub-Saharan Africa being unavailable (http://www.prota4u.org/protav8.asp?p=Solanum+aethiopicum). However, rough estimates of annual fruit production for Senegal, Côte d'Ivoire and Burkina Faso are 8,000 t, 6,000 t and 4,500 tonnes, respectively. *S. aethiopicum* Shum group (Shum) is a popular leafy vegetable in East Africa, particularly Uganda, where it is called 'nakati' (Ssekabembe and Odong, 2008). It is one of the most important local vegetable species in terms of providing income and food in urban and peri - urban areas of Uganda where it ranks better than coffee; the country's most important cash crop (Ssekabembe *et al*., 2003; Ssekabembe and Odong, 2008; MAAIF, 2013). Over 200 t of Shum are traded weekly in major markets fetching at least US$104 per value chain player on a weekly

basis in Uganda (Jagwe *et al*., 2016). The crop is also popular in Central and West Africa, mainly in Cameroon, Burkina Faso and Nigeria (Adeniji *et al*., 2013). Leaves of Shum are especially popular in Cameroon, south-eastern Nigeria and Uganda (http://www.prota4u.org/protav8.asp?p=Solanum+aethiopicum).

S. aethiopicum is one of the most important local vegetable particularly in urban areas owing to its high marketability (Horna and Gruere, 2006; Czarnecki *et al*., 2008; USAID, 2013; Cernansky, 2015). According to FAO (2005), indigenous vegetables significantly contribute to income generation and subsistence for the poor in Cameroon and Uganda; owing to the vegetables' relatively lower prices compared to other food items. In addition to playing ceremonial roles and essentially contributing to traditional dishes; and hence species' popularity in both rural and urban areas, Shum vegetable markets offer opportunity for poor and unemployed women for earning a living (FAO, 2005; USAID, 2013; Cernansky, 2015; Pincus, 2015; Von Grebmer *et al*., 2015).

There are efforts towards increased cultivation of the species owing to its nutritional, medicinal, ceremonial and market value (Ssekabembe and Odong, 2008; Chinedu *et al*., 2011; Cernansky, 2015; Bisamaza and Banadda, 2017). Commercial production of *S. aethiopicum*, dominated by small-scale farmers at over 80%, is on a promising course in many sub-Saharan Africa countries such as Cameroon, Tanzania, Ethiopia, Senegal, Kenya, Gabon, Zimbabwe, Ghana, Zambia, Rwanda, Burkina Faso, Côte d'Ivoire, Nigeria and Uganda (Abukutsa-Onyango *et al*., 2010; Kubie, 2013; USAID, 2013Ebert, 2014; Cernansky, 2015). For instance, the total export value of vegetables increased from US$0.464 million in 2008 to US$2.17 million in 2012 in Uganda (MAAIF, 2013). It is rich in fibre, minerals and carotene (Ojiewo *et al*., 2013; Padulosi *et al*., 2013; Pincus, 2015; Bisamaza and Banadda, 2017; Farzana *et al*., 2017). According to Chinedu *et al*. (2011), every 100 g of fresh fruits of *S. aethiopicum* contains 89.27 g, 2.24 g, 0.52g, 0.87 g,

2.96 g, 4.14 g, 498.47 mg, 1.98 mg and 1.02 mg of moisture, protein, fat, ash, crude fiber, carbohydrate, calcium, magnesium, and iron, respectively (Chinedu *et al*., 2011; Padulosi *et al*., 2013; Bisamaza and Banadda, 2017; Farzana *et al*., 2017). It also contains phytochemicals of therapeutic importance namely alkaloids, saponins, flavonoids, tannins, ascorbic acid, terpenoids, steroids; additionally potentiating for provision of drug synthesis precursors (Chinedu *et al*., 2011; Agoreyo *et al*., 2012; Cernansky, 2015). The species is thus important in curing a number of disease conditions as well as preventing nutrient deficiency diseases especially in developing countries (Abukutsa-Onyango *et al*., 2010; Grubben, 2004; USAID, 2013; Horna and Gruere, 2006; Ebert, 2014).

With its average dry matter yield of over 7.5 t/ha (Ssekabembe, 2008; Ssekabembe and Odong, 2008) from less than 0.25 acres per household that grows the vegetable (Ssekabembe, 2007) is good indication of the crop's potential to immensely contribute towards poverty alleviation, reduced hunger and improved food security (Abukutsa-Onyango *et al*., 2010; FAO, 2005; USAID, 2013; Ebert, 2014; Cernansky, 2015). According to von Grebmer (2015), close to 35% of Uganda's population is food insecure and this is partly attributable to the people's oblivion to the food usability pillar of food security (Von Grebmer *et al*., 2015). Leafy vegetables, "nakati" inclusive are fundamental to improved food security owing to their high fibre content and mineral nutrients. Although there is increasing interest in cultivation of *S. aethiopicum* Shum group owing to its food and economic value, the crop is faced with various production constraints (Ssekabembe and Odong, 2008; Pincus, 2015; Sseremba *et al*., 2017). Among the long-standing constraints affecting the species productivity include low seed germination, pests, diseases, soil infertility and drought (Ssekabembe, 2007; Abukutsa-Onyango *et al*., 2010; Ebert, 2014). Other constraints include: high incidence of pests and diseases, high cost of manure and pesticides, occasional

flooding in swamps, lack of adequate market and low/unstable prices, postharvest deterioration, limited knowledge about crop agronomic practices, and lack of extension service (Cernansky, 2015; Pincus, 2015; Ssekabembe and Odong, 2008; USAID, 2013). Drought or water stress is increasingly becoming an issue of concern in vegetables, amidst climate change (Condon, 2004; Muthomi *et al*., 2009; De Pascale *et al*., 2011; Kumar *et al*., 2012; Nakanwagi *et al*., 2018). Drought and soil infertility are reported to be the worst crop productivity constraints (Richards *et al*., 1993; Danquah and Blay, 1999; Dhanda *et al*., 2004; Kumar *et al*., 2012; Fanaei *et al*., 2015). Drought or moisture stress and lack of irrigation water was raised by farmers as one the major "nakati" production constraints (Ssekabembe, 2007; Anjum *et al*., 2011); most of these constraints are either directly or indirectly related to climate change and one of its major consequences, the drought stress (Condon, 2004; Kumar *et al*., 2012).

Water deficit stress is defined as insufficiency of available water in quantity and distribution which includes rainfall and soil capacity to store moisture, during crop growth that impacts on expression of genotype potential (Danquah and Blay, 1999; Anjum *et al*., 2011; Kumar *et al*., 2012; Fanaei *et al*., 2015). The consequence to drought stress is mainly manifested in reduced crop yields or even plant death for non-tolerant varieties (Cattivelli *et al*., 2008; Bahadur *et al*., 2009; Palada *et al*., 2011; Yoshida *et al*., 2014). Yield losses due to water stress can occur at wide ranges of 0 to 100% depending on the type of the crop species, harvestable part and stage at which the stress sets in (Bayoumi *et al*., 2008; McKenzie *et al*., 2009; Fang and Xiong, 2015; Said, 2014). For grain crops such as maize, yield losses of up to 100% for water stress at anthesis stage is common (Jeanneau *et al*., 2002; McKenzie *et al*., 2009). In leafy vegetables, water stress impairs the quality and leaf area of the crops thus affecting their marketability; rolled/wilted and yellowed leaves are not acceptable (Rubaihayo, 1996, 2002; Ssekabembe *et al*., 2003; Rubaihayo *et al*., 2003; Ssekabembe

and Odong, 2008). Drought modifies the LRWC, rate of photosynthesis, water potential of leaves, and gas exchange; this leads destabilized membrane structure / permeability, and affects protein structure and function, with a consequence of reduced yields, total crop failure, or cell and tissue death (Swarthout *et al*., 2009; Kumar *et al*., 2012; Yoshida *et al*., 2014; Fanaei *et al*., 2015). *S. aethiopicum* Shum Group is predominantly a water-intensive crop which suggests that yield potential of its varieties cannot be achieved until water supply is not limiting (Danquah and Blay, 1999; Kumar *et al*., 2012; Nakanwagi *et al*., 2018). The Shum group has been commonly grown in areas of either high rainfall, irrigated gardens, or under swampy conditions. It is thus more sensitive to drought than any other group in the species. The Gilo group of *S. aethiopicum* is reported to carry tolerance factors for drought or water stress (Rubaihayo *et al*., 2003; Ssekabembe, 2007; Ssekabembe, 2008; Ssekabembe *et al*., 2003; Ssekabembe and Odong, 2008).

Drought tolerance, a collective term that refers to the adaptive mechanisms by which plants survive drought, can be categorized into three. Firstly, drought escape which refers to ability of crop plant to complete its life cycle before onset of severe soil water insufficiencies (Danquah and Blay, 1999; Blum, 2005; Palada *et al*., 2011); involves fast phenological development like early flowering and quick maturity. Secondly, drought avoidance; the ability of the plant to afford dehydration postponement by enduring periods without significant rainfall; entails maintaining high water potential amidst shortage of soil moisture, through whole-plant physiological mechanisms such as reduction in leaf area and canopy tolerance, closure of stomata and formation of wax cuticle, alteration of root depth and density, altering root hair development, root depth and density and root hydraulic conductance for adjustment of sink/source relationships. Because vegetables are more sensitive to water stress, yield improvement under such conditions is an indispensable breeding effort (Blum, 2005; Abukutsa-Onyango *et al*., 2010; Kumar *et al*., 2012;

Cernansky, 2015; Nakanwagi *et al*., 2018). In Shum, a leafy vegetable, this yield improvement takes the form of increased dry or fresh weight of marketable leaves per unit volume of water uptake; a phenomenon referred to as water use efficiency (WUE) (Richards *et al*., 1993; Blum, 2005; Tuberosa *et al*., 2007; Swarthout *et al*., 2009; Kumar *et al*., 2012). The WUE is calculated by measuring photosynthetic rate divided by transpiration rate or the performance divided by quantity of water used (Blum, 2005, 2009). The purpose of this study is therefore to improve of WUE and drought tolerance in Shum through a dedicated analysis of genetic mechanisms that underpin associated trait(s).

1.2 Problem statement

No research effort has been committed to identify and utilize genetic diversity within Shum group to bring about yield and other traits improvement in the subspecies (Rubaihayo, 1996; Ssekabembe, 2007). Vital among the traits are resistance to water deficit stress (drought tolerance). Germplasm sources for drought tolerance improvement in the Shum group are currently unknown. Similarly, the genetic mechanisms that govern the drought resistance traits have not been investigated. Quantitative trait loci (QTLs), mode of inheritance, gene action and combining ability for drought/water stress tolerance in *S. aethiopicum* Shum are currently unknown. In effect, no breeding activities have been dedicated to adapting the subspecies to the realities of declining water resource and unreliable rainfall as some of the major aspects of climate change. In addition, no validation of the most important farmer and other stakeholder constraints that should direct demand-led breeding objectives for the crop has been carried out in Uganda since 2008 (Ssekabembe, 2007; Ssekabembe and Odong, 2008; Sseremba *et al*., 2017).

Current varieties of the Shum group subspecies of *S. aethiopicum* are high water resource-intensive since they are mainly grown in waterlogged valleys or swampy areas for good production (Rubaihayo, 1996; Ssekabembe, 2007). There is however, decreasing swampland area due to human activities and climate change that lead to drying up of valleys; partly manifested as increasing water stress as a global concern (Danquah and Blay, 1999; Kumar *et al*., 2012; Sagare and Mohanty, 2012). Among the indicators of climate change is the reduced water availability in valleys due to reduced and irregular rainfall (Danquah and Blay, 1999; Bayoumi *et al*., 2008; Cattivelli *et al*., 2008; Sagare and Mohanty, 2012). There is currently no dedicated research effort to improve the subspecies for adaptation to reduced water supply or drought through improved water-use efficiency (WUE), or drought tolerance in general. As a consequence to inadequate research attention, the subspecies productivity could significantly lessen. Yet, there is limited information on germplasm diversity and genetic resources about the Shum group and its progenitor, *S. anguivi* (Sękara *et al*., 2007; Gramazio *et al*., 2017a, 2017b, 2016; Prohens *et al*., 2013). Within-group genetic diversity for Shum germplasm as well as diversity from its progenitor is currently not known. The lack of information on genetic sources as parents that could combine well for lead traits in *S. aethiopicum* such as yield, drought tolerance and WUE curtails crop improvement initiatives. In addition, the mode of inheritance or segregation patterns for drought tolerance traits, and quantitative genetic loci (QTLs) associated with resistance to water stress are unknown (Danquah and Blay, 1999). Until information on the genetic mechanisms governing water deficit stress tolerance associated traits is availed, it is difficult to establish an effective genetic improvement programme for the crop.

On the other hand, marker assisted selection (MAS), a molecular breeding technique that has potential for increasing efficiency and effectiveness of selection relies on availability of markers

which are tightly linked to the QTLs conditioning the trait(s) of interest. There is limited information on known loci associated with drought tolerance and WUE in *S. aethiopicum*. Although previous researchers pointed to possible resistance/tolerance associated loci in the Gilo, Kumba and Aculeatum groups (Sagare and Mohanty, 2012; Gramazio *et al*., 2016, 2017a, 2017b), this is yet to be ascertained in the Shum group.

1.3 Objectives and hypotheses

1.3.1 Main objective

The main objective of this research is to improve drought tolerance in Shum for increased crop productivity.

1.3.2 Specific objectives

This thesis research was based on the following specific objectives:

i. To assess the genetic distinctiveness between Shum and its progenitor.
ii. To assess the genetic diversity within Shum group.
iii. To identify parental material for the development of drought tolerant Shum varieties.
iv. To determine the combining ability of selected Shum group germplasm for drought tolerance.

1.3.3 Hypotheses

This thesis research was based on the following hypotheses:

i. The Shum group can be clearly distinguished from its progenitor based on morphological attributes.
ii. There exists within-group selectable variability among Shum germplasm.

iii. The Shum group genotypes differ in drought tolerance.

iv. The Shum group genotypes vary in their combining ability for drought tolerance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology, origin, diversity and importance of *Solanum aethiopicum*

2.1.1 Biology

Solanum aethiopicum complex is a diploid (2n = 2x = 24) (Borràs *et al*., 2015; Acquadro *et al*., 2017) that can be considered as either annual or semi-annual crop depending on morphological group and cultivation purpose (Adeniji *et al*., 2012). While the Gilo group is fruit type and semi-annual (staying in production for about 12 consecutive calendar months), the Shum is a leafy type that is normally harvested at 6 to 8 weeks after planting (WAP) (Ssekabembe, 2008). The Shum group is a flowering plant and the flowers can bear different colors (from white to violet colored petals) depending on variety (IBPGR, 1988). The crop is mainly self-pollinating though ≥30% natural cross-pollination (mainly aided by bumblebee) is possible (Sękara *et al*., 2007). According to Wikipedia, a bumblebee is one of more than 250 species in genus *Bombus* (family Apidae, one of the bee families). The genus Bombus is the only extant group in tribe Bombini, in addition to some extinct related genera known from fossils (https://en.m.wikipedia.org/wiki/Bumblebee/). The bumblebee is thus of economic importance as far as production of quality seed is concerned (Rémi *et al*., 2005).

The leaves of the Shum are entire to slightly lobbed, depending on variety (IBPGR, 1988; Sękara *et al*., 2007). The crop comes into flowering at 8 WAP, and fruit maturity occurs 2 months from flowering (Sseremba *et al*., 2017). It can thus be concluded that the Shum crop takes 4 months from planting to fruit ripening; though this depends on variety and growth conditions (Adeniji *et*

al., 2012). The fruits are 1.0-3.0 cm in longitudinal and cross-sectional diameter, depending on variety. Unripe fruits can take on different colors; white, variegated white/green, green, and variegated green/deep-green depending on variety (Adeniji *et al*., 2012). Most fruits are brick-red when ripe though pale-yellow variants also exist (Kabod *et al*., 2018).

2.1.2 Origin and diversity

Scarlet eggplant (*Solanum aethiopicum* L.) belongs to section Oliganthes, genus Solanum of family Solanaceae (Sękara *et al*., 2007). The common name, eggplant, is shared by three related species; the other two being binjal eggplant / aubergine (*S. melongena* L.) and gboma eggplant (*S. macrocarpon* L.) (Sękara *et al*., 2007). The scarlet and gboma eggplants are believed to have been domesticated about the 20th century in Africa from two wild progenitors, *S. anguivi* and *S. dasyphyllum*, respectively (Sunseri *et al*., 2010). As such, high morphological and genetic diversity of the *S. aethiopicum* is evident in Africa (Sękara *et al*., 2007; Sunseri *et al*., 2010; Grubben, 2004; Keding *et al*., 2007; Czarnecki *et al*., 2008).

Because the centre of domestication and diversity point to areas in Africa (Syfert *et al*., 2016), germplasm collections can be made as landraces from such countries as Tanzania (Adeniji *et al*., 2012; Grubben, 2004; Keding *et al*., 2007; Czarnecki *et al*., 2008), Ethiopia, Kenya, Zimbabwe, Zambia, Rwanda, Cameroon, Gabon, Burkina Faso (Bationo-Kando *et al*., 2015), Côte d'Ivoire, Senegal, Ghana (Kubie, 2013), Nigeria and Uganda (Rubaihayo, 2002; Anjum *et al*., 2011; Adeniji *et al*., 2012; Gramazio *et al*., 2016). However, other continents like Asia, Europe and South America are also reported to have appreciable diversity. For instance, some authors hold a contradictory view that the domestication of *S. aethiopicum* could have occurred in Asia. The World Vegetable Center, previously known as Asian Vegetable and Research Development Centre

(AVRDC, recently the abbreviation changed to WorldVeg) is a major source of germplasm for vegetable species (http://avrdc.org/african-eggplant-solanum-aethiopicum/). Its headquarters are in Taiwan and a regional office for East and Southeast Asia in Thailand. There is also a regional office for West and Central Africa in Mali (Samako Research Station, Bamako Mali), and a liaison office in Cameroon (Yaoundé, Cameroon) (Rubaihayo *et al*., 2003; Srinivasan, 2009; AVRDC, 2010). WorldVeg also has a regional office for East and Southern Africa, which is located in Tanzania (Arusha, Tanzania) (Grubben, 2004; Keding *et al*., 2007; Czarnecki *et al*., 2008). Elite germplasm can be requested from any of the WorldVeg's gene banks at either the headquarters – the Genetic Resources and Seed Unit (GRSU) in Taiwan, or from Thailand or Tanzania. In Europe, a gene bank at the French Institute for Agricultural Research (INRA) is also known for conservation of numerous accessions of *S. aethiopicum* germplasm (Adeniji *et al*., 2012). Most genetic diversity studies have utilized collections from Africa. For instance, Adeniji *et al*. (2013) used 44 *S. aethiopicum* accessions; of which the Gilo, Kumba, Aculeatum and Shum were 21, nine, seven and seven, respectively (Adeniji *et al*., 2013). The Shum germplasm used in this research was obtained from Uganda where popularity and apparent diversity among farmers is reportedly high.

Earlier, characterization of geographically diverse scarlet eggplant germplasm categorized the species into four morphological groups namely the Gilo, Kumba, Aculeatum and Shum, based on morphological traits (Grubben, 2004; Keding *et al*., 2007; Czarnecki *et al*., 2008) and DNA markers (Sunseri *et al*., 2010; Bationo-Kando *et al*., 2015). The groups differ morpho-agronomically and genetically, as well as in purpose for their cultivation. The Gilo, Kumba, Aculeatum and Shum are cultivated for their fruits, fruits and leaves, ornamental purpose, and leaves only, respectively (Adeniji *et al*., 2012; Prohens *et al*., 2013). African eggplant carry useful

traits such as resistance to *Fusarium* wilt in contrast to the two other important solanaceous species, potato and tomato and (Syfert *et al*., 2016). However, numerous other constraints that require species improvement efforts through breeding to prevail on the under productivity of the crop species exist (Oyelana and Ugborogho, 2008; Srinivasan, 2009; Agona and Muyinza, n.d.).

2.1.3 Morpho-agronomic diversity

Adeniji *et al*. (2012) evaluated 44 *S. aethiopicum* accessions from gene banks at WorldVeg and INRA; representing collections from Africa, Asia, South America and Europe (Adeniji *et al*., 2012). Elsewhere, Bationo-Kando *et al*. (2015) used 95 accessions composed of collections from Burkina Faso for characterization with 22 agro-morphological descriptors (Bationo-Kando *et al*., 2015); Agoreyo, Obansa and Obanor (2012) and Chinedu *et al*. (2011) sampled various varieties of *S. melongena* for nutritional and physiochemical analyses (Agoreyo *et al*., 2012; Chinedu *et al*., 2011); while Sunseri *et al*. (2010) collected 70 accessions from USDA-ARS National Germplasm Resources Laboratory, Radbound University's Botanical Garden at Nijimegen in Netherlands, Plant Genetics and Crop Plant Research at Gatersleben in Germany, Montfervet Avignon in France, and more other locations (Sunseri *et al*., 2010). Some of the biochemical variables for characterization of *S. melongena* varieties include contents of moisture; crude protein; crude lipid; fibre; ash; carbohydrates; phytate; oxalate; saponin; alkaloids; tannin; and minerals using methods of oven; Kjeldahl; Soxhlet extraction; igniting in furnace at 550^{o}C for 90 minutes after treatment with H_2SO_4, NaOH, and HCl; similar procedure as for crude fibre but heating for 6 hours; subtracting a summed percentage of other constituents from 100; spectrophotometry; titrimetry; gravimetry; same as for saponin; same as for phytate; and an ignition in a furnace at 550^{o}C for 6 hours, respectively (Agoreyo *et al*., 2012; Chinedu *et al*., 2011). The experiments for

morphological characterization were carried in a replicated trial in the field over three years (Adeniji *et al*., 2012). Sunseri *et al*. (2010), Adeniji *et al*. (2012) and Bationo-Kando *et al*. (2015) measured a number of morphological traits including days to first flower (FLW), PH, PL, number of petals per flower, petal width, sepal width, sepal length, number of sepals per flower, number of fruits per plant, fruits per fructescence, fruit fructescence per plant, fruit calyx length, fruit calyx width, total acidity of fruits at commercial ripening, total acidity of fruits at physiological maturity, fruit browning, fruit yield, seed yield, total soluble solids (TSS) at commercial ripeness, TSS at physiological maturity (Adeniji *et al*., 2012; Sunseri *et al*., 2010; Adeniji *et al*., 2013; Bationo-Kando *et al*., 2015b). This research utilized both vegetative and reproductive traits since it was not yet apparent which variables can sufficiently discriminate among Shum and *S. anguivi* accessions.

2.1.4 Molecular diversity

Unlike morphological descriptors, molecular differences are not affected by the environment thereby leading to a more definitive discrimination among study accessions. Adeniji *et al*. (2013) studied 35 accessions of *S. aethipicum* collected from Africa, Asia, South America and Europe; by obtaining leaf samples from a field establishment in Arusha, Tanzania, and sixteen markers based on simple sequence repeats (SSR) (Adeniji *et al*., 2013). Similarly, Sunseri *et al*. (2010) carried out an amplified fragment length polymorphism (AFLP) analyses on extracted genomic DNA and

Sunseri *et al*. (2010) identified two large clusters and several small clusters of the *S. ethiopicum* accessions; basing on a combined SSR and AFLP matrix of similarity coefficients ($r = 0.506$, $p = 0.421$) (Sunseri *et al*., 2010). However, the 35 accessions studied by Adeniji *et al*. (2013) were grouped into six (6) clusters at 70% coefficient of similarity (Adeniji *et al*., 2013). It is notable in

either case; different morphological groups of *S. aethiopicum* were lumped up in one study. In this thesis, effort was made to focus on within group diversity for Shum though the characterization was done with morphological markers as motivated by Adeniji *et al*. (2012). Elsewhere, additional diversity study resources for *S. aethiopicum* and related crops have been reported (Pucholt *et al*., 2015; Gramazio *et al*., 2016; Gramazio *et al*., 2017). Molecular markers were not used in this research though by highlighting them indicates an appreciation of their relevance as compared to morphological descriptors.

2.1.5 Importance of drought tolerant *S. aethiopicum* Shum genotypes

Climate change, a challenge of the 21st century, is partly characterized by prolonged drought and increased atmospheric and soil temperature; or the phenomenon of global warming. This leads to increased rates of transpiration from crop plants. Once the escalating transpiration rates are not compensated with corresponding water supply for root uptake from the soil, the plants exhibit water stress. With the reduction in water supply due to climate change and human activities such as industrialization which competes for the available water, it is imperative that varieties of high drought tolerance are developed. According to Kumar *et al*. (2012), drought tolerance is the ability of a plant to produce its economic product with minimum loss under water deficit environment in relation to the water-constraint-free management. In the case of Shum, the marketable plant parts are the leaves; which implies the leaf cells must remain turgid to ensure vegetable marketability as fresh or verdant leaves (Ssekabembe, 2007; Ssekabembe, 2008).

Not a single strategy to improve the water use productivity in irrigated and rain-fed agriculture can be adequate. Thus, the available strategies that are deemed effective not in isolation, include water management of the water resource, changes in crop management practices, and breeding crop

varieties that are more efficient in their water use (Condon, 2004; Kumar *et al*., 2012; Kesiime, 2014). It is important that crop varieties are able to efficiently utilize the available water from either irrigation or rainfall (Condon, 2004; Blum, 2005; Kumar *et al*., 2012). According to Condon *et al*. (2004), breeding for high WUE involves moving more of the available water through the crop rather than it being wasted through evapotranspiration from the soil surface or drainage beyond the root zone or being left behind the root zone at harvest. The breeding for high WUE involves acquiring more carbon or biomass in exchange for the water transpired by the crop, i.e. improving crop transpiration efficiency (Condon, 2004). It also involves partitioning more of the achieved biomass into the harvested product (Condon, 2004; Blum, 2005, 2009). Information generated in this study regarding genetic mechanisms and QTLs underpinning water-use efficiency and drought tolerance is useful in developing high yielding and climate resilient varieties through both conventional and molecular approaches (Danquah and Blay, 1999; Kumar *et al*., 2012). Once water-use efficient or drought tolerant varieties of *S. aethiopicum* are developed and released to commercial and subsistence farmers, this can improve their standards of living, enhance local diets nutritionally and aid increase foreign currency earnings through exports (Ssekabembe, 2007).

Considerable research on the genetic diversity of *S. aethiopicum* groups, in general, has been carried out which distinguishes the species into four distinct groups. Most studies employed morpho-agronomic traits such as fruit calyx length, fruit calyx pigmentation, fruit length, leaf length, leaf surface prickles, prickles underneath the leaves (Adeniji *et al*., 2013; Plazas *et al*., 2014). In addition, phenomics for fruit traits using Tomato Analyzer (Prohens *et al*., 2013; Plazas *et al*., 2014), and molecular markers (simple sequence repeats, SSRs) have also been successfully carried out; and provided high discrimination among the four groups (Adeniji *et al*., 2013). Because the cultivars from the different groups are cross-compatible, this provides species

improvement opportunities either through trait introgression from other groups into the Shum group by backcrossing or hybridization among genetically diverse yet intra-specific individuals for exploitation of potential heterosis. The high genetic diversity among the four groups as earlier reported by Adeniji *et al*. (2013) and Plazas *et al*. (2014) emphasizes the possibility for heterosis or hybrid vigor from crosses among groups; a concept that is well exploited in other crops such as maize (*Zea mays*). The value in terms of suitability for use by consumers of the resulting F_1 offspring from an inter-group cross is uncertain, or likely undesired. For instance, it can be expected that a cross between a fruit yielding group such as Gilo and the Shum group would produce F_1 hybrids that are unexclusively for fruit yield nor leafy vegetable; rather an uncertain intermediate. Such uncertainty prompts for a need to search for within-group diverse germplasm for assured market acceptability of the resulting hybrids; on the premise that hybrids can offer the best alternative in terms of returns to growers' investment due to better yields than open pollinated varieties.

However, considering that *S. aethiopicum* group members are cross-compatible (Lester and Thitai, 1989; Sekara *et al*., 2007), backcross introgression of the drought tolerance trait(s) could be carried out. This is not precisely achievable until markers linked to relevant QTLs have been identified and validated in the available germplasm; although appreciable progress can as well be made through conventional approaches (Sękara *et al*., 2007; Adeniji *et al*., 2012; Prohens *et al*., 2013; Kubie, 2013; Plazas *et al*., 2014). This research focused on exploration of the conventional breeding approaches using morphological attributes. The breeding program for *S. aethiopicum* improvement is yet to be established in many sub-Saharan Africa countries, including Uganda. Two contrasting parents for drought tolerance and WUE can be obtained considering the high variability recently reported among *S. aethiopicum* groups on a number of traits, some related to

drought tolerance (Kumar *et al*., 2012; Adeniji *et al*., 2013; Prohens *et al*., 2013; Plazas *et al*., 2014; Gramazio *et al*., 2016, 2017a, 2017b). Identification of markers (morphological markers, in this case) can pave the way for their subsequent introgression into high yielding yet drought susceptible varieties of the Shum group.

Tremendous progress has been made in improvement of cereals for drought tolerance and high yield for example drought tolerant maize for Africa (DTMA) by the International Maize and Wheat Improvement Centre, and the upland rice (NERICA) by AfricaRice, unlike in majority of vegetable crops. Among the *Solanaceae* vegetables, some achievements are notable in tomato and potato (Kesiime, 2014), for instance drought tolerance sources have been reported (Kumar *et al*., 2012). Among the *S. aethiopicum* groups, it is reported that Gilo cultivars are generally drought tolerant (Ssekabembe, 2007; Kumar *et al*., 2012). Similar progress is yet to be achieved in the Shum group. Although it is reported that various breeding approaches are plausible for genetic gain or progress in selection for drought tolerance among well studied crops such as maize, rice, wheat and cassava, none has been optimized for the Shum group of *S. aethiopicum* (Ssekabembe, 2007). Kumar *et al*. (2012) suggested three possible approaches, in general, for drought tolerance improvement as follows: (i) breeding for high yield under optimum or irrigated condition where the maximum genetic potential of yield is expected to be realized in optimum condition with a high positive association for performance in optimum and stress conditions; (ii) breeding under actual drought condition; and (iii) improving drought tolerance in high yielding genotypes through integration of breeding methods based on morphological and physiological mechanisms of drought tolerance (Kumar *et al*., 2012). This research relied on a combination of the three approaches. On the overall, the outcome of this study was to lay a foundation for establishment of the Nakati breeding program in Uganda through generation of segregating populations such as the F_2 were generated in addition

to unveiling the genetic mechanisms that underpin key traits namely drought tolerance and leaf quality. The information generated about the genetic mechanisms for drought tolerance is applicable in bringing about genetic improvement in the rest of the groups of *S. aethiopicum*; and other members of the genus *Solanum* in general.

Statistics show that close to 35% of the 34.9 million people constituting Uganda's population is undernourished (Von Grebmer *et al*., 2015), with 48 % of the estimated total population being below 14 years of age. The children of below 5 years of age as well as pregnant women are at the greatest risk of malnutrition. Additionally, the commercial cultivation of *S. aethiopicum* Shum group is reportedly dominated by small-scale farmers who are mainly the poor and unemployed women (FAO, 2005; Ssekabembe and Odong, 2008). The subspecies' nutrient and commercial value is high, yet it is very vulnerable to drought stress and climate change effects in general. The development of climate-resilient varieties in the leafy vegetable can create a great impact towards alleviation of hunger in many sub-Saharan Africa countries since the children (and pregnant women) are often the most vulnerable to food and nutritional security (FAO, 2005; Abukutsa-Onyango *et al*., 2010; Ebert, 2014; Von Grebmer *et al*., 2015).

2.2 Cultivation practices for *S. aethiopicum* Shum

The *S. aethiopicum* Shum (also locally known as "Nakati" in Uganda), like most crops, requires well-drained fertile soils rich in organic matter. The use of both manure and mineral fertilizers, a phenomenon referred to as integrated soil fertility management, is highly recommended (Pincus, 2015; Nanyanzi *et al*., 2018). Manures as cowdung bio-slurry and poultry manure can increase yield and quality of the leaves (Nanyanzi *et al*., 2018), in addition to sustaining soil health and care for the environment when growth of soil biomes is promoted by the organic manures (Chemura,

2014). Being a leafy vegetable, the crop productivity and leaf quality greatly declines when drought sets in. During drought, leaf size reduces and wilting sets in, leading to loss of market value (Ssekabembe, 2007; Ssekabembe and Odong, 2008). Therefore, maintaining adequate soil moisture is essential for the crop; ultimately, drought tolerant varieties should be developed and made available to farmers (Ssekabembe, 2007; Nakanwagi *et al*., 2018).

2.3 Constraints to productivity of *S. aethiopicum* Shum

Solanum aethiopicum productivity is influenced by pests, diseases and abiotic constrains (AVRDC, 2010). Some of the pests include spider mites (mainly *Tetranychus evansi*), whiteflies (*Bemisia* spp.), thrips, snails, coleopteran and lepidopteran leaf miners (Srinivasan, 2009). Most of the diseases are fungal such as late blight casued by *Phytopthora infestans* (AVRDC, 2010; Erselius *et al*., 1997; Nelson, 2008; Seidl Johnson *et al*., 2014) and viral such as tomato chlorotic virus disease (Fonseca *et al*., 2016). Low soil fertility, salt stress, heat stress, cold stress, and drought stress are among the abiotic or environmental constraints (AVRDC, 2010; Anjum *et al*., 2011; Kumar *et al*., 2012; Kappachery *et al*., 2013; Kesiime, 2014). Other key constraints include poor seed viability and postharvest deterioration (Rubaihayo *et al*., 2003; Ssekabembe, 2007; Ssekabembe *et al*., 2003; Ssekabembe and Odong, 2008); the latter being especially critical in leafy crops such as *Amaranthus* spp. and Shum group (AVRDC, 2010; Anjum *et al*., 2011; Kumar *et al*., 2012; Kappachery *et al*., 2013).

As highlighted, low soil fertility, pests and diseases, post-harvest deterioration, and water stress are key challenges to African eggplant productivity (Ssekabembe, 2007; Kumar *et al*., 2012). The constraints pose greater effects in the developing sub-Saharan Africa countries than in developed countries in Europe and developing countries in South America and Asia (FAO, 2013). Of the

constraints, water deficit stress is the most important in leafy vegetables during field production especially in the Shum group where leaf quality deterioration potentially renders up to 100% loss to farmers and market vendors due to the produce's non-marketability (Ssekabembe and Odong, 2008). It is believed however, that a wide genetic base for drought tolerance improvement in solanaceous species exists (Arvin and Donnelly, 2010; Grubben, 2004; Kappachery *et al*., 2013). For instance, within *S. aethiopicum*, Gilo is reported to be more tolerant to water deficit stress than Shum (Sękara *et al*., 2007; Ssekabembe, 2007; Syfert *et al*., 2016). The existing germplasm diversity could be utilized to search for genotypes that carry drought tolerance related traits such WUE (Condon, 2004; Sagare and Mohanty, 2012; Syfert *et al*., 2016). Once contrasting genotypes on WUE are identified, their breeding values can be ascertained in combining ability studies using appropriate mating designs. Additionally, quantitative trait loci (QTLs) for drought tolerance can be mapped using recent genotyping advances for markers especially SNPs and SSRs, and phenotyping techniques for drought tolerance (Arvin and Donnelly, 2010; Barchi *et al*., 2011; Farshadfar *et al*., 2011; Golparvar, 2012; Kappachery *et al*., 2013; Kesiime, 2014; Said, 2014). This study, however, did not include QTL analysis.

2.4 Drought stress as a constraint to performance of leafy vegetables

Drought is a major constraint that impairs crop productivity (Jeanneau *et al*., 2002; Anjum *et al*., 2011; Kappachery *et al*., 2013; Yoshida *et al*., 2014; Amelework *et al*., 2015). Water deficit stress effects to crop production are more pronounced in sub-Saharan Africa than anywhere else (Jeanneau *et al*., 2002; Farshadfar *et al*., 2011; Said, 2014; Fang and Xiong, 2015; Von Grebmer *et al*., 2015). There are morphological, biochemical, physiological and molecular responses which characterize drought stress; with the ultimate effect of yield reduction or even plant death (Danquah and Blay, 1999; Kumar *et al*., 2012; Yoshida *et al*., 2014; Amelework *et al*., 2015).

Morphologically, as drought stress increases the root-shoot ratio also increases in maize and rice, until some point when severe drought impairs the root tip meristematic cell division and expansion (McKenzie *et al*., 2009; Amelework *et al*., 2015). Other morphological responses to water stress include plant height, internode length, leaf size and leaf colour (Arvin and Donnelly, 2010; Sagare and Mohanty, 2012; Amelework *et al*., 2015;). Physiologically, a number of signaling pathways such as the Ca^{2+} and ABA stimulate the closing/opening of inward/ outward pores for K+ movement out of the guard cells, which leads to stomata closure (Amelework *et al*., 2015). Gas exchange (CO2/O2) becomes affected when stomata (McKenzie *et al*., 2009; Yoshida *et al*., 2014). Consequently, internal CO_2 reduces and O_2 increases; a situation favoring photorespiration at expense of photosynthesis, in the end free radicals of oxygen (or peroxides) accumulate. The free radicals of oxygen are known as reactive oxygen species (ROS) (Kumar *et al*., 2012; Fang and Xiong, 2015). ROS causes oxidative stress characterized by bleaching of thylakoids (chlorophyll membranes) where light reactions take place (Yoshida *et al*., 2014; Amelework *et al*., 2015).

Water deficit stress affects plant morphological traits, physiological, biochemical and molecular functioning in varied ways depending on severity of drought stress, timing/duration of exposure, crop species/genotype, growth/development stage (Amelework *et al*., 2015). Some of the widely reported water deficit stress effects include reduced growth/yield, modified root-shoot ratio, reduced photosynthetic rate, reduced chlorophyll content, and changed plant-water relations (McKenzie *et al*., 2009; Kumar *et al*., 2012; Yoshida *et al*., 2014; Amelework *et al*., 2015). In SHUM group, interference on biomass yield and leaf quality traits are the most diShumtrous effects of drought stress, considering that crop's harvestable part are the leaves (Ssekabembe and Odong, 2008; Pucholt *et al*., 2015).

2.5 Drought effects and plant response

2.5.1 Gene expression

Crop response to drought is a complex process where multiple genes as well as signaling pathways are involved (Yoshida *et al*., 2014). Drought stimuli are generally perceived and/or captured by sensors on cell membranes, and the signals are transmitted through a hierarchy of transduction pathways, ultimately leading to expression of certain genes which condition adaptation to the drought (Kumar *et al*., 2012; Osakabe *et al*., 2014; Amelework *et al*., 2015; Pucholt *et al*., 2015). Secondary messengers LIKE Ca_2^+, ABA, ROS, phosphoglycerol and diacylglycerol, and transcriptional regulators contribute to signal transmitting pathways (Osakabe *et al*., 2014; Fang and Xiong, 2015;). Drought - responsive genes are under complex governance, including transcriptional cascades/steps. Table 1 includes a summary of some of drought stress response associated genes as extracted from Kumar *et al*. (2012).

Table 1: Genes conferring drought tolerance and their salient features

Genes	Function	Mechanism
DREBs/CBFs; *ABF3*	Transcriptional factors (TFs) induced by stress	Increased expression of downstream genes brings aout drought or salt or cold tolerance. If over expressed, can lead to stuntedness.
SNAC1	TFs induced by stress	Its expression will reduce water loss which increases stomata sensitivity to abscisic acid (ABA)
OsCDPK7	Calcium-dependent protein kinase induced by stress	Increased expression of genes for stress responsive
Farnesyl-transferase (*ERA1*)	ABA sensing negative regulator	The gene's down regulation increases Plant response to ABA
Mn-SOD	Manganese superoxide dismutase	Over expression enhances stress tolerance
AVP1	Vacuolar hydrogen (H^+) pyrophosphatase	Over expression leads to auxin fluxes which increases root growth
HVA1; OsLEA3	Stress induced late embryogenesis abundant (LEA) proteins	Over accumulation of LEA enhances drought tolerance

ERECTA	Putative leucine-rich repeat (LRR) receptor-like kinase; major contributor to a locus for D on Arabidopsis chromosome 2	Regulator of transpiration efficiency affecting stomata density, epidermal cell expansion, proliferation in mesophyll cells and cell-to-cell contact.
otsA and *otsB*	*Escherichia coli* (*E. coli*) trehalose biosynthetic genes	Increase in trehalose correlates with higher soluble carbohydrate levels, enhanced photosynthetic capacity and enhanced tolerance to damage by oxidative stress.

Extracted from Kumar *et al*. (2012).

2.5.2 Physiological response

2.5.3.1 Reduced photosynthetic rate

Similar to other environmental stresses, drought directly affects photosynthetic apparatus of mesophyll cells by damaging thylakoid membranes (thylakoid electron transport) where light reactions take place (Kumar *et al*., 2012). Drought also influences the assimilation efficiency of the dark reactions: stomatal control of carbon dioxide (CO_2) supply and the reduction cycle for carbon, carbohydrates accumulate, destruction of lipids by peroxides and water balance disturbance (Blum, 2005; De Pascale *et al*., 2011). The effect of reduced assimilation efficiency is a reduction in photosynthetic products. Angum *et al*. (2011) reported that ability of crop plants to adapt to specific environments is related to ability to acclimate at level of photosynthesis; thereby affecting physiological and biochemical processes which then influence overall growth and yield of the plant (Anjum *et al*., 2011; Yoshida et al., 2014). Water deficit stress negatively affects conductance parameters of crop plants as a result of premature leaf death, reduction in leaf expansion, incapacitated photosynthetic apparatus, oxidative damge of chloroplast lipids, and changes in structure of proteins and pigments (De Pascale *et al*., 2011; Amelework *et al*., 2015; Fang and Xiong, 2015). In maize, water stress is cited, in comparison to a control, to cause a 33.22% reduction in net photosynthesis, 37.84% reduction in transpiration rate, 25.54% reduction

in stomatal conductance, 5.86% reduction in intercellular CO_2, 11.58% reduction in intrinsic WUE, and 50.87% reduction in WUE (Jeanneau *et al*., 2002; Blum, 2009). It is believed that genotypes of a crop species, *S. aethiopicum* not an exception, exhibit selectable variation for WUE under limited soil moisture content (Condon, 2004; Blum, 2009; Kumar *et al*., 2012).

Based on photosynthetic pathway, plants exhibit a C3, C4, and/or crassulacean acid metabolism (CAM) to assimilate atmospheric CO_2 (Juenger *et al*., 2005; De Pascale *et al*., 2011; Fang and Xiong, 2015). Plants using C4 and CAM pathways are more adapted to drought situations (Fanaei *et al*., 2015; Fang and Xiong, 2015). C3 plants open their stomata during day and close their stomata at night. However, the C_3 pathway is deficient when plants are faced with drought because such plants does not retain moisture under drought stress (Condon, 2004). In contrast, C4 plants concentrate CO_2 in bundle sheath cells, and perform the fixation of CO_2 in mesophyll cells and bundle sheath cells separately (Pucholt *et al*., 2015); which brings about higher WUE in the C4 plants. This leads to higher survival chances in arid areas (McKenzie *et al*., 2009). For CAM, plants open their stomata at night, and close their stomata during the day. Hence, CAM can greatly increase WUE and it is proposed to be a robust/flexible adaptation to extremely dry environments (Kumar *et al*., 2012). Under drought, facultative CAM plant species are capable of switching their photosynthetic metabolism from C3 to CAM mode (Bayoumi *et al*., 2008). An enzyme, phosphoenolpyruvate carboxylase, is regulated by drought stress in the CAM pathway (Bayoumi *et al*., 2008; Said, 2014; Yoshida *et al*., 2014). Majority of crops in the Solanaceae family including *S. aethiopicum* group use the C3 photosynthetic pathway which predisposes the crop to excessive water loss (Ripley *et al*., 2007; Taylor *et al*., 2011).

2.5.3.2 Reduced chlorophyll content

A decline in chlorophyll content is considered an indication of oxidative stress (Kumar *et al*., 2012). Oxidative stress results from photo-oxidation and chlorophyll degradation (Blum, 2009; Kumar *et al*., 2012). Generally, photosynthetic rate correlates positively with chlorophyll content (CHL). Drought stress outcomes (the ROS accumulation) destroy membrane structure which consequently reduces CHL (Fang and Xiong, 2015). It The CHL (mainly chlorophyll a content) greatly declines with decrease in soil moisture content (Yoshida *et al*., 2014). However, chlorophyll a and b are both prone to depletion of soil water (Anjum *et al*., 2011; Yoshida *et al*., 2014). Accordingly, loss in chlorophyll contents (a + b) under drought is the main cause of photosynthesis decline (Anjum *et al*., 2011; Kumar *et al*., 2012). Most of the chlorophyll content loss is reported in mesophyll cells than in bundle sheath cells; emphasizing the efficiency of C4 plants that reserve CO2 in the bundle sheath cells (Juenger *et al*., 2005; McKenzie *et al*., 2009; Fang and Xiong, 2015). The CHL may be decreased or remain unchanged in certain crop species or varieties depending on severity and duration of water deficit stress (Juenger *et al*., 2005; Anjum *et al*., 2011). More light energy efficient crops which maintain high CHL amidst drought are considered drought tolerant. (Juenger *et al*., 2005; Hatamnia *et al*., 2013).

2.5.3.3 Changed plant-water relations

The most important attribute that describes plant-water relations is LRWC (Kumar *et al*., 2012). LRWC is regarded as a measure of the plant-water status (PWS) in form of physiological consequence of water deficit at cell level (Osakabe *et al*., 2014). Another useful measure of PWS is the leaf water potential (LWP). LWP deals with water transportation along soil-plant-atmosphere continuum; though LWP does not account for osmotic adjustment (OA) unlike LRWC (Dhanda *et al*., 2004; Tuberosa *et al*., 2007). The OA is useful in conserving cellular water status

under drought or water deficit stress (DS) (Dhanda *et al*., 2004). LRWC is therefore an proper measure of PWS in terms of cellular hydration under possible effect of both LWP and OA (Osakabe *et al*., 2014; Amelework *et al*., 2015). LRWC measures water deficit in leaf and it estimates prevailing water content of sampled leaf tissue relative to maximal water content it can hold at full turgidity (Blum, 2009; Kumar *et al*., 2012). LRWC tends to range from 98% in turgid (depending on crop species / genotype) and transpiring plant leaves to ~ 40% in dying leaves (under DS) (Kumar *et al*., 2012). A typical LRWC value for most crop species, though with exceptions, is ~ 60-70% at about wilting (Tuberosa *et al*., 2007; De Pascale *et al*., 2011; Fanaei *et al*., 2015). During day, as solar radiation and temperature varies, components of plant-water relations also change; with a smaller variation for not more than 2 hours at and after overhead sun light (at noon). Within a 2 hours range of solar noon thus represents recommended period for leaf sampling; unless a daily LRWC curve is a focus of study (Kumar *et al*., 2012; Osakabe *et al*., 2014).

LRWC is measured by sampling a top fully expanded leaf per plant for about 4-6 plants per genotype that constitutes a treatment; by means of a cork borer or a leaf disc cutter. Leaf samples at different parts of a plant; bottom, middle and top, could as well be obtained if the interest is to profile LRWC of the leaves of the plant (http://www.nrcresearchpress.com/doi/abs/10.1139/cjb-2015-0065#.V3jpxqK92eo). For large broad-leaves as the case is with *S. aethiopicum*, leaf discs that are large enough, at least 1.5 cm in diameter, of 5-10 cm^2 per sample are cut from leaves, avoiding the large veins (Kumar *et al*., 2012). In the case of small composite leaves such as in chickpeas and groundnuts, individual leaflets are used as the samples (www.neiker.net/neiker/.../LEAF%20RELATIVE%20WATER%20CONTENT.doc). The leaf samples are individually weighed to obtained fresh weight (FW), after which the samples are hydrated for four

(4) hours to full turgidity under room temperature and normal light and the turgid weight (TW) is taken (Osakabe *et al*., 2014). Samples are oven-dried at 70 – 80 ^{o}C for 24 hours, cooled, and re-wighed for dry weight (DW). LRWC is then calculated using the following formula: $LRWC = [(FW - DW)/(TW - DW)] * 100$; where FW, TW and DW is sample fresh weight, turgid weight and dry weight, respectively (Kesiime, 2014; Banik *et al*., 2016).

Plant water potential (PWP) measures plant water demand (Dhanda *et al*., 2004; ; Tuberosa *et al*., 2007; Osakabe *et al*., 2014). Like LRWC, PWP indicates the plant-water status from "plant's point of view" (Condon, 2004; Blum, 2009). High PWS (which also means plant moisture stress) causes many physiological disorders like slowed/ stopped photosynthetic activity (Amelework *et al*., 2015). The conditions producing high water potential such as drought stress result in reduced plant growth and eventual plant death at extreme stress levels (Kumar *et al*., 2012). Water potential in the plant can be measured in any one of three common methods namely LWP, stem water potential (SWP) and pre-dawn LWP (PDLWP). It is referred to as mid-day LWP if the LWP is measured at mid-day on a fully expanded leaf exposed to direct sun light (Blum, 2005). Small plastic bags are usually wrapped tightly around target leaf samples 90-120 minutes before cutting it/them off the stem for measurements as security against transpiration errors. Without bagging, data is considered imprecise as transpiration alters resultant pressure readings (Kesiime, 2014). The reading is taken immediately after cutting the petiole of covered leaf as close to the shoot as possible. The petiole is then placed into the pressure chamber and secured tightly using the chamber lid, with the bagged leaf blade placed inside the chamber, and the cut end of the petiole facing outside. The chamber is then sealed followed by slowly pressurizing it using liquid nitrogen gas. It is asserted that when positive pressure exerted on leaf in chamber equals negative pressure inside leaf, liquid in leaf blade begins to be forced out of cut edge of petiole (Blum, 2009; Osakabe *et al*., 2014). During

pressurization, cut edge is keenly watched for a first drop of water coming out. No sooner should the first drop appear at the cut edge of leaf than the pressure reading is taken at the chamber gauge (http://www.pmsinstrument.com/resources/pms-meaning-and-importance). The pressure reading taken is the LWP, recorded in negative (-) bars (1 Bar = 14.5 PSI). Mid-day LWP is subject to environmental pressures, and it is said to be variable (Blum, 2009; De Pascale *et al*., 2011; Kumar *et al*., 2012).

Mid-day SWP is considered more reliable than LWP since the stem is not as much vulnerable to changes in surrounding pressure than for the leaf (De Pascale *et al*., 2011). Because of possible shading effects on sample leaf aimly chosen by minimizing heat effects in addition to changes in handling of leaf samples, SWP enables detection of small differences among genotypes. Thus, SWP is this taken as more representative of actual drought stress levels in plants than LWP (Blum, 2009; Osakabe *et al*., 2014). Before measurement of mid-day SWP, target leaves are covered in black plastic bags which are then covered in aluminum foil for 90-120 minutes to prevent the leaf from overheating by the sun. The covering of leaf also stops transpiration from the sample leaf, and this allows LWP to come into equilibrium with SWP. At the end of the 90-120 minutes, the covered leaf is excised and SWP measurements are taken in the pressure chamber in the same manner as for LWP. Apart from mid-day LWP and mid-day SWP, leaf water potential can be measured as PDLWP (Banik *et al*., 2016). With PDLWP, the readings are taken on fully expanded leaves, beginning say at 3:30 am and ending just before sunrise. Although the timing before sunrise is difficult, PDLWP is considered a reliable measure/indicator of soil moisture potential (SWP) than mid-day LWP and SWP; basing on an assumption that before sunrise, PWP is in equilibrium with SWP (http://www.pmsinstrument.com/resources/pms-meaning-and-importance).

Pressure chambers are devices for exerting pressure to a leaf/shoot; with most of leaf inside chamber and cut end of petiole / stem kept outside chamber. It is understood that amount of pressure required to cause water to appear at cut end of petiole indicates how much tension a leaf is experiencing on its water supply. High pressure values mean high values of tension and high degree of DS. The water deficit stress levels in leaves vary among species and varieties of same species. Soil and atmospheric water saturation controls viability, growth potential, yield, and quality of crop (Jeanneau *et al*., 2002; Dhanda *et al*., 2004; Kumar *et al*., 2012; Fanaei *et al*., 2015).

2.5.4 Yield and morphological response

2.5.4.1 Reduced growth and yield

Drought (permanent or temporary) affects growth/development of crop plants, and consequently yield, more severely than other productivity factors (Anjum *et al*., 2011; Amelework *et al*., 2015). Angum *et al*. (2011) highlighted crop growth as a function of complex interplay between source-sink limitations of roots and shoots; the two main organs of the plant, which establishes functional equilibriums (Anjum *et al*., 2011). Growth and development is affected primarily through impairment of germination leading to poor plant stand, impaired mitosis, inhibition of cell elongation and expansion of higher plants (Kumar *et al*., 2012; Sagare and Mohanty, 2012). The ultimate effect is reduction in growth and yield related traits. Such traits, affected as a result of decreasing soil's water potential include PH, cob length in maize, LPP, LA (length x width) and leaf senescence (Amelework *et al*., 2015). Under water deficit stress, reduced PH is attributable to impaired cell enlargement and increased leaf senescence (Fang and Xiong, 2015; Pucholt *et al*., 2015). In their review, Angum *et al*. (2011) and Amelework *et al*. (2015) cite leaf size or leaf area growth through expansion being dependent on leaf turgor, temperature and assimilates supply. In Shum (a leafy vegetable), reduced fresh and dry biomass production or shoot yield is a direct

severe consequence of moisture deficit stress (Ssekabembe *et al*., 2003; Ssekabembe, 2007; Ssekabembe and Odong, 2008; Pucholt *et al*., 2015). The effect of drought differs at various developmental stages as cited by Amelework *et al*. (2015). In maize, imposing water deficit stress at silking, grain-filling, and grain maturity reduced total biomass production by 37%, 34% and 21%, respectively (Jeanneau *et al*., 2002; Anjum *et al*., 2011;). In grain crops, yield is a result of relationship among several plant growth components (Jeanneau *et al*., 2002; Golparvar, 2012; Said, 2014; Amelework *et al*., 2015) while in Shum group, yield is mainly a direct relation to leaf characteristics (Jeanneau *et al*., 2002; Ssekabembe and Odong, 2008; Kumar *et al*., 2012; Pucholt *et al*., 2015).

Apart from growth and development, and yield impairment which are morphological effects of plants to drought, a number of physiochemical responses occur (Yoshida *et al*., 2014; Amelework *et al*., 2015; Fang and Xiong, 2015). Physiologically, water deficit causes root-shoot modification through root signaling, impairs photosynthesis, affects chlorophyll content, interferes with plant-water relations, and leads to accumulation of osmolytes (Yoshida *et al*., 2014; Amelework *et al*., 2015). Biochemically, DS causes accumulation of ROS and antioxidant enzymes (Kumar *et al*., 2012; Kappachery *et al*., 2013; Pucholt *et al*., 2015).

2.5.4.2 Modified root-shoot ratio (RSR)

Although divergent evidence exists as per the root development behavior under water deficit stress, an extensive root system supports early plant growth and access to water / nutrients from deep in the soil (Anjum *et al*., 2011; Farshadfar *et al*., 2012). It is generally understood that the RSR increases under drought stress, for instance in *Cantharanthus roseus* (Kumar *et al*., 2012). In some crops like maize however, the root growth is reportedly not substantially affected (Jeanneau *et al*.,

2002). The general increase in RSR occurs under drought stress because roots are not as much responsive to growth impairment than shoots are at low soil water potential (De Pascale *et al*., 2011). When drought sets in, roots generate a signal through the xylem to the shoots leading to physiological changes for adaptation to the stress (McKenzie *et al*., 2009). A number of plant hormones such as cytokinins, malate, ethylene and ABA are implicated in the root-shoot signaling (Anjum *et al*., 2011; Yoshida *et al*., 2014). Stomata closure is important adaptation to DS in field (Yoshida *et al*., 2014), and it occurs as a result of water deficit-induced root-shoot signaling through transpiration stream (Fang and Xiong, 2015). ABA, a dominant growth and transpiration control signaling molecule, is also the most important signaling molecule associated with DS as it leads to efflux of potassium ions (K^+ ions) from guard cells (Kappachery *et al*., 2013; Pucholt *et al*., 2015). The consequence to K^+ ions efflux is reduced turgor pressure of guard cells, resulting in stomata closure (Juenger *et al*., 2005; De Pascale *et al*., 2011).

2.6 Water use efficiency

Generally, efficiency is given by output divided by input (Tuberosa *et al*., 2007). The WUE is then referred to either in financial or agronomic terms (Condon, 2004). Financial WUE refers to monetary return after spending money in establishing and operating a water supply/delivery system (Condon, 2004; De Pascale *et al*., 2011). However, costs/prices vary widely from place to place and year to year, making economic/financial WUE difficult to compare at a universal scale. Agronomically, WUE refers to extra yield arising from watering per unit of land area. It also refers to extra yield per unit amount of irrigation water applied (Condon, 2004; Blum, 2005, 2009). Principally, agronomic/crop WUE is the amount of vegetative dry matter produced per unit volume of water supplied from the soil (Blum, 2009). Up to 99% of water absorbed by plants is transpired; it is only a small proportion that is retained. That is to say, plant WUE is thus the reciprocal of

transpiration ratio (Jeanneau *et al*., 2002; Blum, 2005). Transpiration ratio is mass of water transpired per unit mass of dry matter produced (Blum, 2009). Apart from economic and agronomic criteria, there is also what is referred to as technical efficiency (or irrigation efficiency as referred to by irrigation engineers) (Tuberosa *et al*., 2007; AVRDC, 2010). Technical/irrigation efficiency is the net quantity of water added to root zone divided by quantity taken from a source. The technical efficiency is applicable to large/regional/individual farms/specific field(s); which reflects a loss incurred in conveyance and distribution (Blum, 2005; McKenzie *et al*., 2009; Kumar *et al*., 2012). It is estimated that irrigation efficiency in the farms allocated large flows is <50%; though there is always for improvement to irrigation efficiencies of 80-90% (Hillel, 1998; Codon, 2004; Blum, 2005, 2009). It is desired that water delivery techniques such as drip irrigation are implemented in *Solanum ethiopicum* Shum in order to maximize agronomic WUE.

2.7 Screening methods for drought tolerance

2.7.1 In the laboratory

According to Kappachery *et al*. (2013), *in vitro* methods involve growth of genotypes on tissue culture media supplemented with compounds such as polyethylene glycol (PEG) that reduce the osmotic potential of the medium such that water available for uptake by plantlets is reduced (Kappachery *et al*., 2013). PEG is a large molecular weight (MW) polymer (MW in range of 4000 to 8000) that does not significantly penetrate plants; and it modifies the osmotic potential of the culture medium thereby inducing plant water deficit in a controlled manner (Jeanneau *et al*., 2002; Blum, 2005). However, slow uptakes of PEG of high molecular weight at 1 mg/g fresh weight per week, or higher rates for damaged tissues in maize and beans can occur (Condon, 2004; Blum, 2005). In *S. tuberosum*, screening of genotypes under drought stress was reportedly successful in Murashige and Skoog (MS) medium (Farshadfar *et al*., 2012) containing 2.5 percent sucrose and

0.7 percent agar for three (3) weeks. Regenerated plantlets were then transferred to a liquid ½MS medium containing 0.5 percent sucrose and 25 percent PEG for seven (7) days, during which acclimatization was carried out by gradual opening of the culture bottles (Kappachery *et al*., 2013). PEG was used as the drought stress inducer; a manifestation of which included the wilting symptoms on plantlets in the PEG treated, and no wilting in the untreated culture bottles, after twenty four (24) hours (Arvin and Donnelly, 2010; Kappachery *et al*., 2013). In bread wheat (*Triticum aestivum* L.), Farshadfar *et al*. (2012) used PEG 6000 (in concentrations of 0, 5, 10, 15 and 20 percent) to assess twenty (20) genotypes for their reaction to drought stress by immature embryo culture followed by data collection on callus growth characteristics (Farshadfar *et al*., 2012). To identify the most drought tolerant genotypes, callus characteristics namely callus relative growth rate (CRGR), % of callus chlorosis (PCCH), % of callus water content (PCWC), and *in vitro* tolerance (INTOL) were measured (Farshadfar *et al*., 2012).

2.7.2 In the screen house and field

The use of water supply restriction on potted plants is the most common practice when evaluating for drought tolerance in the screen house, green house and field (Wang *et al*., 2012; Kesiime, 2014; Gong *et al*., 2015; Banik *et al*., 2016). The use of salt mainly chlorocholine chloride as a water restriction technique is also reported (Wang *et al*., 2012). The withholding and/or restricting of water supply were used in this study when applying water deficit stress to potted plants of Shum. In field however, assurance against rainfall during drought experiments should be provided through either off-season establishment or use of rain-out shelters (Ravi, 2013). In screen house and field, various morphological, physiological, biochemical and gene expression measurements are possible at seedling, vegetative and flowering stages. Morphologically, the commonly measured traits in the genus *Solanum* include plant height (PH), leaf blade length (LBL), leaf blade

width (LBW), leaf area (LA), leaf wilting score (LWS), leaf weight and fruit yield (Wang *et al*., 2012; Kesiime, 2014; Ramírez *et al*., 2014; Rolando *et al*., 2015; Banik *et al*., 2016; Kesiime *et al*., 2016). The commonly measured physiological parameter for drought tolerance assessment include stomatal conductance (Yoshida *et al*., 2014), transpiration rate, osmotic potential, LRWC, leaf temperature, gas exchange, photosynthetic rate and CHL (Kumar *et al*., 2012; Wang *et al*., 2012; Ramírez *et al*., 2014; Rolando *et al*., 2015; Banik *et al*., 2016; Kesiime *et al*., 2016). Biochemically, the commonly measured traits are taken on leaves. Such biochemical parameters include proline content, abscisic acid accumulation, glutathione content, electrolyte leakage rate, malondialdehyde content, cytokinin content, zeatin riboside content, dehydrogenase activity, carotenoids content, phenolics content, sodium concentration and potassium concentration (Wang *et al*., 2012; Gong *et al*., 2015; Banik *et al*., 2016). The mentioned biochemical compounds tend to as antioxidants which scavenger on reactive oxygen species in the plant as a result of prolonged exposure to water deficit stress.

CHAPTER THREE

STUDY 1: ASCERTAINING GENETIC DISTINCTIVENESS BETWEEN *S. aethiopicum* SHUM AND ITS PROGENITOR

3.1 Introduction

African indigenous vegetable species (AIVS) require genetic improvement in order to address constraints that curtail the crops' productivity and contribution to household income and food security in sub-Saharan Africa (Abukutsa-Onyango, 2014; Cernansky, 2015). *Solanum aethiopicum* and *S. anguivi* are some of the major AIVS that are research-neglected. The *S. aethiopicum* is morphologically diverse with four recognized groups, of which Shum is a leafy type (https://avrdc.org/african-eggplant-solanum-aethiopicum/). The *S. aethiopicum* evolved from SAN (Sękara *et al*., 2007; Ebert, 2014). Although the two species are domesticated, *S. anguivi* is grown for its fruits only. The SAN exists both in the wild and in farmers' fields; indicating its environmental robustness. The availability of germplasm of known diversity is important in variety improvement efforts (Gramazio *et al*., 2016). Morphological markers are cheap and can be used for diversity analyses (Kubie, 2013; Kouassi *et al*., 2014). However, the usefulness of morphological traits in accession discrimination for the Shum and *S. anguivi* has not yet been investigated.

The most commonly used morpho-agronomic traits for genetic diversity studies in *Solanum* spp. have been published (Adeniji *et al*., 2013; Gramazio *et al*., 2016). Multivariate statistical methods such as multidimensional scaling (MDS), linear (canonical) discriminant analysis (LDA), cluster and principal component analysis are suited for use in understanding genetic diversity (Harding and Payne, 2012). MDS is a form of non-linear dimensionality reduction for visualizing the level

of similarity of individual cases of a data set (information) that is contained in a distance matrix. Cluster analysis and PCA are the two most commonly used methods of genetic diversity analysis; the former handles both quantitative and qualitative variables while the latter is powerful and sensible with quantitative data sets (Zimisuhara *et al*., 2015). Clustering employs either the Ward's or "average" (Unweighted Pair Group Method with Arithmetic mean, UPGMA) method algorithms (Odong *et al*., 2011). Although both methods rely on coefficients such as cophenetic correlation coefficient (CPCC) to judge the strength (reliability) of subgroup differentiation, UPGMA is the most commonly used (Odong *et al*., 2011; Zimisuhara *et al*., 2015).

Further, diversity indices such as Richness, Shannon-Weaver, and Simpson can reveal groupings of accessions per level of qualitative variable (Altaye, 2015). A diversity index refers to a quantitative measure reflecting the number of different types (in this case the levels of qualitative variables) that there are in the dataset (i.e., community), and at same time takes into account the even distribution of basic entities (such as individuals) among those types (Zimisuhara *et al*., 2015). On the other hand, the PCA serves to identify how different variables work together to reduce dimensionality and redundancy; thus helping to reveal hidden structure (Zimisuhara *et al*., 2015; Coghlan, 2017). LDA is aimed at finding linear combinations of original variables that give the best possible separation among study groups (Harding and Payne, 2012; Coghlan, 2017). This study aimed to identify:

(i) hierarchical groups existing in the study accessions,

(ii) major drivers of variability in collected data set for the study accessions, and

(iii) variables that account for the greatest separation between the Shum and *S. anguivi* progenitors.

3.2 Materials and methods

3.2.1 Study location and germplasm

The study was carried out in a screen house at WACCI research farm, University of Ghana, Greater Accra-Ghana. During the experiment (October 2016 to April 2017), temperature ranged between 21-26^{o}C (morning), 31-43^{o}C (afternoon) and 29-35^{o}C (evening hours). Relative humidity ranged between 74-81% (morning), 57-75% (afternoon) and 62-69% (evening hours). The germplasm was obtained from UCU (http://ucu.ac.ug/academics/more-faculties/faculty-of-science-and-technology). The list of study germplasm is as shown (Table 2).

Table 2: Description of germplasm used for the distinctiveness study

Entry No	Code	Alternative code	Pedigree	Species
1	168G	E1	SAS168/G/2015	Shum
2	183G	E2	SAS183/G/2015	Shum
3	163	E3	SAS163/2015	Shum
4	163P	E4	SAS/163/P/2015	Shum
5	157P	E5	SAS/157/P/2015	Shum
6	160	E6	SAS160/2015	Shum
7	163G	E7	SAS163/G/2015	Shum
8	183P	E8	SAS183/P/2015	Shum
9	108	E9	SAS108/2015	Shum
10	157G	E10	SAS157/G/2015	Shum
11	148	E11	SAS/148/2015	Shum
12	145	E12	SAS145/2015	Shum
13	168P	E13	SAS/168/P/2015	Shum
14	184G	E14	SAS184/G/2015	Shum
15	137	E15	SAS137/2015	Shum
16	184P	E16	SAS184/P/2015	Shum
17	141	E17	SAS141/2015	Shum
18	108P	E18	SAS108/P/2015	Shum
19	185G	E19	SAS185/G/2017	Shum
20	185P	E20	SAS185/P/2015	Shum
21	146	E21	SAN146/2015	*SAN*
22	177	E22	SAN177/2015	*SAN*
23	163W	E23	SAN163/W/2015	*SAN*
24	163C	E24	SAN163/C/2015	*SAN*
25	114	E25	SAN114/2015	*SAN*

E, entry; Shum, *Solanum aethiopicum* Shum group; SAN, *Solanum anguivi* (progenitor for Shum)

3.2.2 Design

Twenty-five (25) accessions which comprised of 20 entries of Shum and five of SAN were studied using a completely randomized design. Three seeds of an accession were directly sown in individual pot on October 10, 2016 followed by thinning to one plant per pot at 4-leaf stage (seedling stage) on November 8, 2016. Twelve plants (one plant per pot; making twelve replications) of each accession were established in individual plastic pots of 5-litre size in a completely randomized block design. The potting soil was clay-loam. Optimum watering with uniform quantities of water daily (1.0, 2.0 and 3.0 litres per pot at seedling, vegetative and flowing stages, respectively; in response to increase in water requirement as a plant grows), appropriate fertilizer application with NPK 17:17:17 at 4 g per pot on a fort-nightly basis and preventive pesticide sprays using mancozeb and dimethoate once every 2 weeks was carried out.

3.2.3 Data collection

Data collection (qualitative and quantitative) was carried out at seedling and vegetative stages. At seedling stage, the following quantitative variables were measured; days to germination and emergence (DG), cotyledonous leaf blade length (CLBL), cotyledonous leaf blade width (CLBW), seedling leaf length (SLBL), seedling leaf width (SLBW), seedling fresh weight (SDFW) and seedling dry weight (SDDW) were recorded. The qualitative variables recorded at seedling stage were cotyledonous leaf color (CLC) and visual seedling vigor (VSV). At flowering stage, additional quantitative variables were measure namely date to first flower appearance (FLW), leaves per plant (LPP), plant branching (PB), plant height (PH), plant canopy width (PW), petiole length (PL), leaf blade length (LBL), leaf blade width (LBW), fresh shoot biomass (SBF) and fresh root weight (RWF). The qualitative variables recorded at flowering stage were spines on stem (SOS), plant growth habit (PGH), stem pubescence (SPU), stem color (SC), petiole color (PC),

leaf tip angle (LTA), leaf prickles on lower surface (LSL), leaf prickles on upper surface (LSU), petiole prickles (PP), leaf blade lobbing (LL), leaf blade color (LBC), leaf midrib color (LMC), leaf pubescence on upper surface (LHU), leaf pubescence on lower surface (LHL) and leaf vein pigmentation (LVP). All the variables were recorded based on a modified standard International Board on Plant Genetic Resources (IBPGR) *Solanum* species characterization manual (IBPGR, 1988) and as applied by Adeniji *et al*. (2012, 2013).

3.2.4 Cluster analysis

The raw data was summarized in Microsoft Excel to obtain means for different quantitative variables and cleaning up of the qualitative data followed by subsequent analysis in R (Everitt and Hothorn, 2014). A text delimited data frame of the mean values was imported into R followed by converting of traits to appropriate ordered (qualitative), nominal (qualitative) and numeric/integer (quantitative) variables. The target variables were then selected followed by installing and loading an R package cluster for cluster analysis. Because the data included both quantitative and qualitative variables, a function daisy() was used to group accessions based on a general coefficient of dissimilarity that combines and processes different types of variables according to their own mathematical type (Grum and Atieno, 2007; Zimisuhara *et al*., 2015). The hierarchical clustering was carried out using average (UPGMA) algorithm. A "re-ordered" dendrogram plot followed by Mantel testing for examining cluster distinctiveness at 1% error margin.

In order to ascertain that an optimum number of clusters was generated in the hierarchical tree, a Kelly-Gadner-Scutcliffe (1996) penalty function for pruning was calculated using a function kgs(). A function table() was then used to categorize the number of accessions in each cluster per level of qualitative variable. Diversity indices namely Richness, Shannon-Weaver and Simpson were

also calculated. The Richness index was calculated using a function specnumber() that is dependent on (contained in) packages permute, lattice and vegan. The Shannon-Weaver index (swi) was computed using a function diversity() whose default in R is set for the swi; otherwise an alternative index "simpson" that measures the evenness of group membership (Harding and Payne, 2012) was specified.

3.2.5 Principal component analysis (PCA)

The PCA complements cluster analysis in a way that PCA helps to interrogate the data so as to understand the contribution of each variable to the existing diversity among accessions (Zimisuhara *et al*., 2015). The PCA procedure was performed in R on quantitative traits of the data frame using a base function prcomp() (Everitt and Hothorn, 2014; Coghlan, 2017). By default, the function prcomp() centres the variable to have mean equals to zero. Thus, the standard deviations were also set to 1 with the parameter scale.=T in order to normalize the variables. The mean (center) and standard deviations (scale) of each variable, the principal component loadings (rotation) that constitute the rotation matrix which contains the principal component (PC) loading vector, and the matrix x that contains the PC score vectors were generated. A facility in the function prcomp() enables calculation of the standard deviation (sdev) of each PC (Coghlan, 2017). The variance (var) of each PC was then computed by squaring the sdev. The proportion of variance explained by each principal component (prop_varex) was calculated by dividing variance by total variance (i.e., prop_varex= var/sum(var). A biplot and scree plots were used to plot the first two PCs and proportion of explained variance.

3.2.6 Linear discriminant analysis

A MANOVA was carried out to identify variables that were significantly different between the two groups; SHUM and SAN at 1% significance level using GenStat Release 12.1 (VSN International Ltd). When running MANOVA, independent variables were considered the group (with levels as Shum, SAN) while dependent variables served as predictors (Li and Wang, 2014; Coghlan, 2017). However, in LDA, the independent variables are the predictors and the dependent variables are the groups. The LDA was performed on data variables with significantly different means between the groups. Canonical vector loadings of discriminant function, correlations between data variates, and the correlations between the variates and discriminant function were generated.

3.3 Results

3.3.1 Clusters

Hierarchical clustering of germplasm based on the "average" (UPGMA) method produced five clusters (Figure 1). The Mantel test revealed that the clusters were significantly distinct ($p < 0.01$) with a cophenetic correlation coefficient (CCC) of 0.73. On whether to prune the hierarchical cluster tree, a Kelly-Gardner Sutcliffe penalty function further showed that the optimum number of clusters was five (Figure 2). The first and fifth clusters contained one accession each. The second, third and fourth cluster had five, twelve and six members, respectively. A summary of the cluster groups and their members are shown in Table 3. Entry 17 which comprises the first cluster was the only accession with spines. Table 4 shows the way various qualitative variates are spread over clusters.

Table 3: List of accessions per cluster

Cluster name	Member (Entry)
1	17 (spiny accession)
2	22, 21, 24, 25 and 23 (all the SAN germplasm)
3	8, 20, 19, 15, 18, 19, 16, 13, 5, 10, 4 and 3
4	1, 2, 14, 11, 7 and 12
5	6 (large leaf size)

SAN, *Solanum anquivi* (progenitor for *S. aethiopicum* Shum)

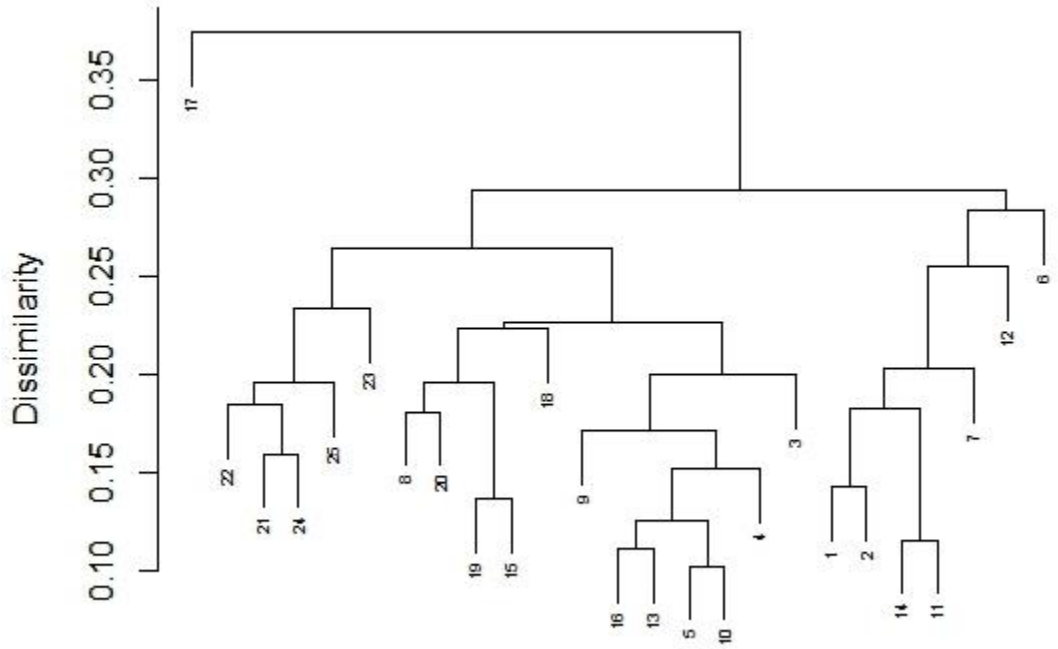


Figure 1: A re-ordered cluster dendrogram for the study accessions using UPGMA method of clustering

Labels are different study entries: 1-20 and 21-25 are the Shum (Shum) and S. anguivi (SAN) accessions, respectively.

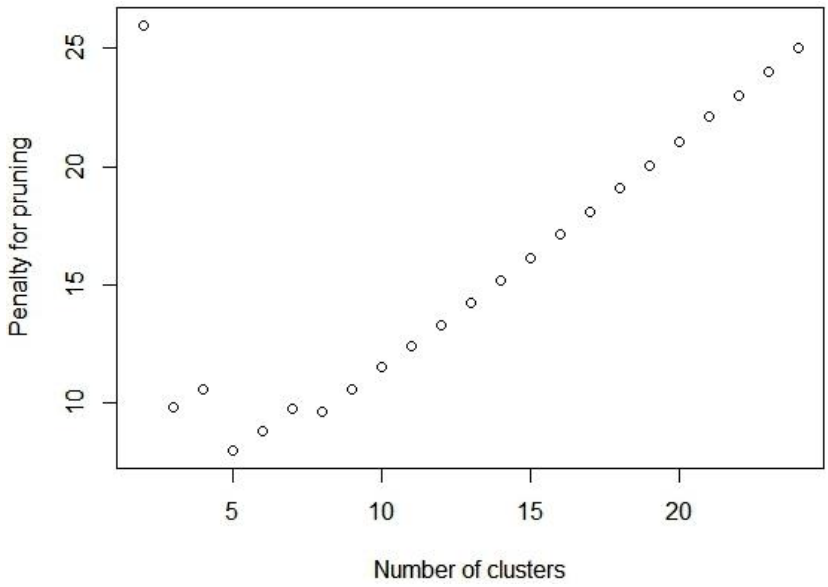


Figure 2: Kelley-Gardner-Sutcliffe penalty function for the cluster data showing that the optimum number of accessions is five.

Table 4: Number of members per cluster in different levels of qualitative variables

Levels per variable	No. of accessions per cluster					Levels per variable	No. of accessions per cluster				
	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5		Cluster1	Cluster2	Cluster3	Cluster4	Cluster5
CLC											
greenish white	1	5	12	6	1						
SOS						**PP**					
glabrous	0	5	12	6	1	glabrous	0	5	12	6	1
many	1	0	0	0	0	many	1	0	0	0	0
VSV						**LL**					
intermediate	0	2	4	2	0	weak	0	0	0	1	0
poor vigor	1	2	1	2	1	intermediate	0	0	6	3	0
very poor vigor	0	1	5	1	0	strong	1	5	6	2	0
very vigorous	0	0	1	1	0	very strong	0	0	0	0	1
vigorous	0	0	1	0	0						
PGH						**LBC**					
intermediate	0	3	5	1	1	green	0	0	0	6	1
prostrate	1	2	1	1	0	pale purple	0	5	11	0	0
upright	0	0	3	2	0	purple	1	0	1	0	0
very upright	0	0	3	2	0						
SPU						**LMC**					
glabrous	0	0	11	5	0	green	0	0	0	6	1
sparse	0	0	1	0	0	pale purple	0	5	12	0	0
medium	1	0	0	0	1	purple	1	0	0	0	0
dense	0	5	0	1	0						
SC						**LHU**					
green	0	0	0	6	0	glabrous	1	0	10	5	0
pale purple	0	4	9	0	1	sparse	0	1	2	0	1
purple	1	1	3	0	0	dense	0	4	0	1	0
PC						LHL					

green	0	0	0	6	0	dense	0	4	0	1	0
pale purple	0	5	10	0	1	glabrous	1	0	10	5	0
purple	1	0	2	0	0	sparse	0	1	2	0	1
LTA						**LVP**					
acute	1	4	7	4	1	green	0	0	0	6	1
intermediate	0	1	5	2	0	pale purple	1	5	12	0	0
LSL						**LSU**					
glabrous	0	5	12	6	1	glabrous	0	5	12	6	1
very many	1	0	0	0	0	very many	1	0	0	0	0

CLC, cotyledonous leaf color; SOS, spines on stem; VSV, visual seedling vigor; PGH, plant growth habit; SPU, stem pubescence; SC, stem color; PC, petiole color; LTA, leaf tip angle; LSL, leaf prickles on lower surface; LSU, leaf prickles on upper surface; LL, leaf blade lobbing; LBC, leaf blade color; LMC, leaf midrib color; LHU, leaf pubescence on upper surface; LHL, leaf pubescence on lower surface; LVP, leaf vein pigmentation

3.3.2 Diversity indices: Richness, Shannon-Weaver and Simpson

The Richness index (RI) was at maximum (at 5; the total number of clusters in the hierarchical tree) for greenish white cotyledonous leaf color, poor seedling vigor, and acute leaf tip angle. The lowest RI of 1 (which implies that all accessions belonged to only one of the 5 clusters) was observed for many spines on stem, sparse stem pubescence, green stem color, green petiole color, very many prickles on lower leaf surface, very many prickles on upper leaf surface, many petiole prickles, weak leaf lobbing, very strong leaf lobbing, and purple leaf mid-rib color. Shannon-Weaver index (swi), a measure of richness and evenness, all clusters contained greenish white accessions based on cotyledonous leaf color (i.e., swi is maximum at 1.27). The most diverse (in terms of number of clusters captured of out the total) and abundant (in terms of number of accessions represented) for spines on stem, seedling vigor, plant growth habit, stem pubescence, stem color, petiole color, leaf tip angle, leaf prickles was glabrous (swi=1.15), poor vigor (1.55), prostrate (1.33), medium (0.69), purple (0.95), acute (1.38), and glabrous (1.15). Generally, variable levels with high swi also had high values for the Simpson index (Table 5).

Table 5: Richness, Shannon-Weaver (swi) and Simpson indices for different variable levels

Variable / levels	Richness	swi	Simpson	Variable / levels	Richness	swi	Simpson
CLC							
greenish white	5	1.27	0.67				
SOS				**PP**			
glabrous	4	1.15	0.64	glabrous	4	1.15	0.64
many	1	0.00	0.00	many	1	0.00	0.00
VSV				**LL**			
intermediate	3	1.04	0.63	weak	1	0.00	0.00
poor vigor	5	1.55	0.78	intermediate	2	0.64	0.44
very poor vigor	3	0.80	0.45	strong	4	1.20	0.66
very vigorous	2	0.69	0.50	very strong	1	0.00	0.00
vigorous	1	0.00	0.00				
PGH				**LBC**			
intermediate	4	1.17	0.64	green	2	0.41	0.24
prostrate	4	1.33	0.72	pale purple	2	0.62	0.43
upright	2	0.67	0.48	purple	2	0.69	0.50
very upright	2	0.67	0.48				
SPU				**LMC**			
glabrous	2	0.62	0.43	green	2	0.41	0.24
sparse	1	0.00	0.00	pale purple	2	0.61	0.42
medium	2	0.69	0.50	purple	1	0.00	0.00
dense	2	0.45	0.28				
SC				**LHU**			
green	1	0.00	0.00	glabrous	3	0.83	0.51
pale purple	3	0.83	0.50	sparse	3	1.04	0.63
purple	3	0.95	0.56	dense	2	0.50	0.32
PC				**LHL**			
green	1	0.00	0.00	dense	2	0.50	0.32
pale purple	3	0.83	0.51	glabrous	3	0.83	0.51
purple	2	0.64	0.44	sparse	3	1.04	0.63
LTA				**LVP**			
acute	5	1.38	0.71	green	2	0.41	0.24
intermediate	3	0.90	0.53	pale purple	3	0.79	0.48
LSL				**LSU**			
glabrous	4	1.15	0.64	glabrous	4	1.15	0.64
very many	1	0.00	0.00	very many	1	0.00	0.00

CLC, cotyledonous leaf color; SOS, spines on stem; VSV, visual seedling vigor; PGH, plant growth habit; SPU, stem pubescence; SC, stem color; PC, petiole color; LTA, leaf tip angle; LSL, leaf prickles on lower surface; LSU, leaf prickles on upper surface; LL, leaf blade lobbing; LBC, leaf blade color; LMC, leaf midrib color; LHU, leaf pubescence on upper surface; LHL, leaf pubescence on lower surface; LVP, leaf vein pigmentation

3.3.3 Principal components (PCs)

Sixteen principal components (PCs) were generated; the first two and ten PCs accounting for up to 51.53% and 96.83% of variation, respectively (Table 8). The first PC that had higher loadings for DG, LBW, LPP, LBL and LBW than the rest of the variables, accounted for 28.46% of variation. The second PC (23.07%) had high loadings for CLBL, SDFW, SLBL, SLBW and FLW. When represented on a scaled biplot such that the longer the arrows the higher the contribution to variation, it was shown that CLBL, LPP, DG, LBW, FLW, SDFW, SLBW, LBL, and PW were shown to contribute to the highest variation among the study accessions (Figure 3). A scree plot showed that the first 10 PCs account for most of the variation at up to ~97% (Figure 4). Going by one variable per PC based on scores (loadings), the ten PCs that account for the greatest differences among the study accessions include DG, CLBL, CLBW, SBF, PH, PL, FLW, PW, PB and LPP.

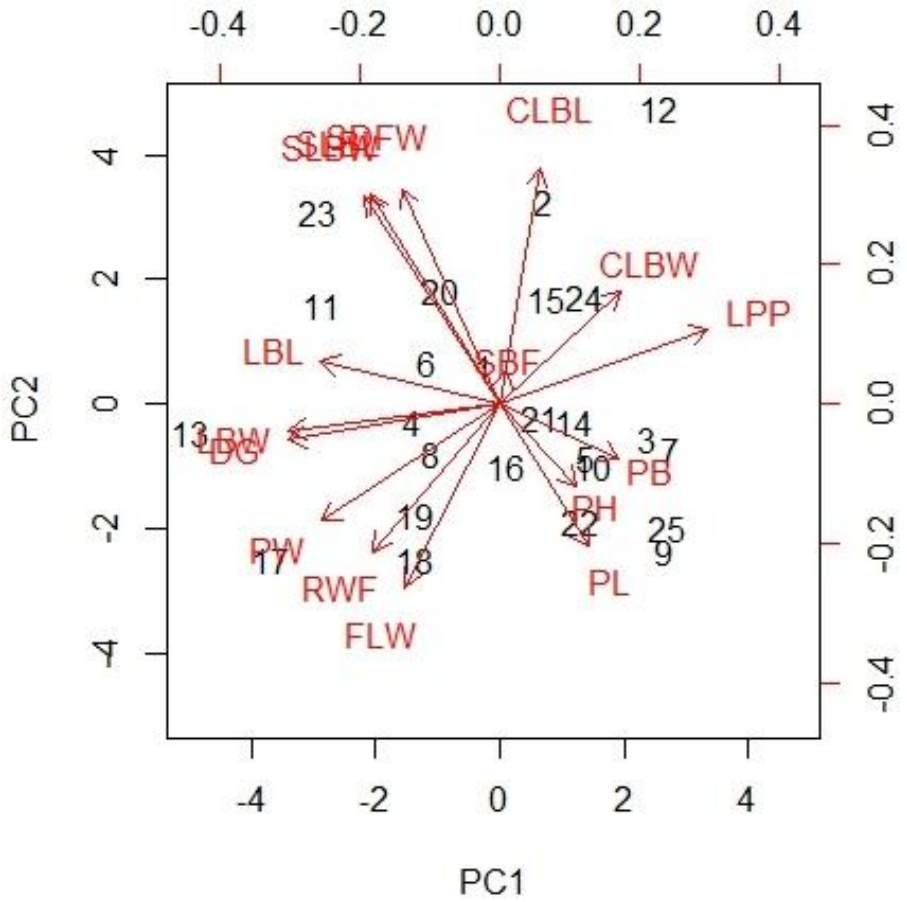


Figure 3: Biplot of first two PCs of variation scaled by loadings (arrow length) to show contribution to variation.

PC, principal component; DG, days to germination and emergence; CLBL, cotyledonous leaf blade length; CLBW, cotyledonous leaf blade width; SLBL, seedling leaf blade length; SLBW, seedling leaf blade width; SDFW, seedling fresh weight; LPP, number of leaves per plant; PH, plant height; PB, number of branches per plant; PW, plant canopy width; PL, petiole length; LBL, leaf blade length; LBW, leaf blade width; SBF, shoot fresh biomass; RWF, root fresh weight; FLW, days to first flower appearance.

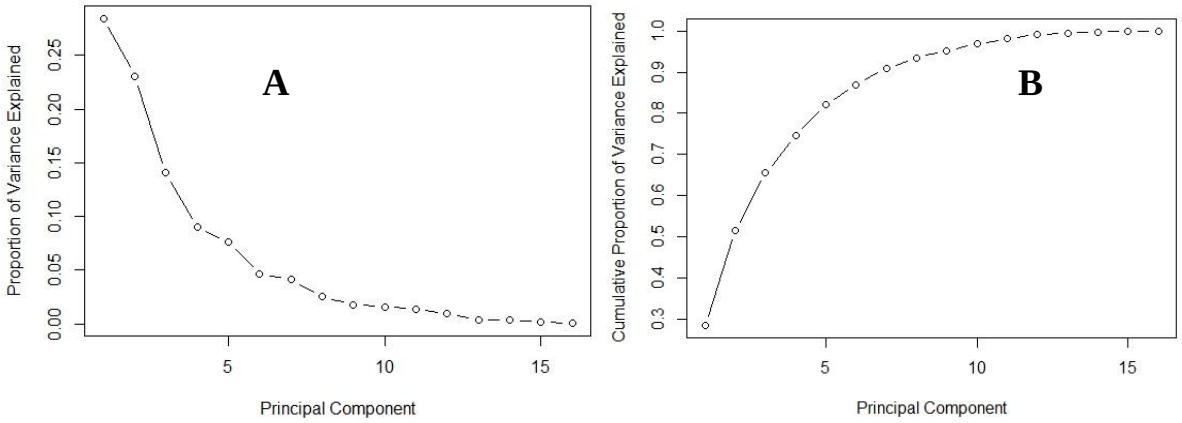


Figure 4: Scree plots for proportion (A) and cumulative proportion (B) of variance explained

3.3.4 Canonical variates

Significant differences ($p < 0.01$) between Shum, and *SAN* for CLBL, CLBW, DG, FLW, LPP, RWD, and SBD were obtained. Differences between groups were however, non-significant ($p > 0.01$) for LA, LBL, LBW, PB, PH, PL, PW, SDDW, SDFW, SLBL, SLBW and TBD (Table 6). Thus, subsequent linear discriminant analysis (LDA) was carried out on the seven significantly different variates between groups as predictors for the groups. The vector loadings (scores) that indicate unique contribution of each trait led to the following discriminant function (DF): $Group = 0.0586CLBL - 0.2609CLBW + 0.6321DG + 0.0727FLW + 0.0627LPP + 0.0310RWD + 0.0200SBD$. Further, variates with highest loadings (0.6321 and -0.2609) and highest correlations between variates and DF (0.7891 and -0.3491) were DG and CLBW, respectively (Table 7). The mean DG was 6 days and 13 days for Shum and SAN, respectively. The Mahalanobis' intergroup distance (D^2) between the two groups was estimated to be 24.51. The discriminant scores for the group means for SAN and Shum were 3.864 and -1.087, respectively (Figure 5).

Table 6: Mean squares and probability values for rejecting a hypothesis of no difference (α=1%) between *S. aethiopicum* Shum and *S. anguivi* accessions

Variate	Mean for SAN	Mean for SHUM	Between Groups MS	Within Groups MS	F.pr	Variate	Mean for SAN	Mean for SHUM	Between Groups MS	Within Groups MS	F.pr
CLBL	12.10	13.34	51.028	3.527	<0.001	PH	27.41	30.09	163.89	81.64	0.163
CLBW	4.10	5.36	42.447	0.7524	<0.001	PL	60.67	58.04	524.1	339.2	0.22
DG	13.00	6.25	989.908	3.104	<0.001	PW	51.51	47.84	204.67	33.95	0.018
FLW	42.33	36.65	849.97	43.18	<0.001	RWD	13.60	9.84	313.76	29.88	0.002
LA	358.94	303.17	37735	23466	0.211	SBD	23.07	19.14	491.20	43.05	0.001
LBL	21.90	20.71	8.38	19.17	0.511	SDDW	0.04	0.04	0.00076	0.00168	0.505
LBW	16.23	14.18	85.45	14.19	0.018	SLBL	30.60	25.63	341.4	113.1	0.088
LPP	34.25	41.20	839.8	110.7	0.008	SLBW	23.20	17.95	385.54	53.55	0.01
PB	10.07	10.21	2.846	2.864	0.324						

SAN, S. anguivi; Shum, S. aethiopicum. Range for days to germination and emergence (DG) was 4-11 and 12-15 for Shum and SAN, respectively. CLBL, cotyledonous leaf blade length (mm); CLBW, cotyledonous leaf blade width (mm); LPP, number of leaves per plant; PB, number of branches per plant; LBL, leaf blade length (cm); LBW, leaf blade width (cm); FLW, days to first flower appearance

Table 7: Discriminant functions (DF) and r for the correlations between variates and the DF

Variate	Scores for DF	Coefficients for correlations between variates and the DF (*r*)
CLBL	0.0586	-0.1251
CLBW	-0.2609	-0.3491
DG	0.6321	0.7891
FLW	0.0727	0.1971
LPP	0.0627	-0.1378
RWD	0.031	0.15
SBD	0.02	0.1561

DG, days to germination and emergence; CLBL, cotyledonous leaf blade length (mm); CLBW, cotyledonous leaf blade width (mm); LPP, number of leaves per plant; SBD, shoot dry biomass (g); RWD, root dry weight (g); FLW, days to first flower appearance.

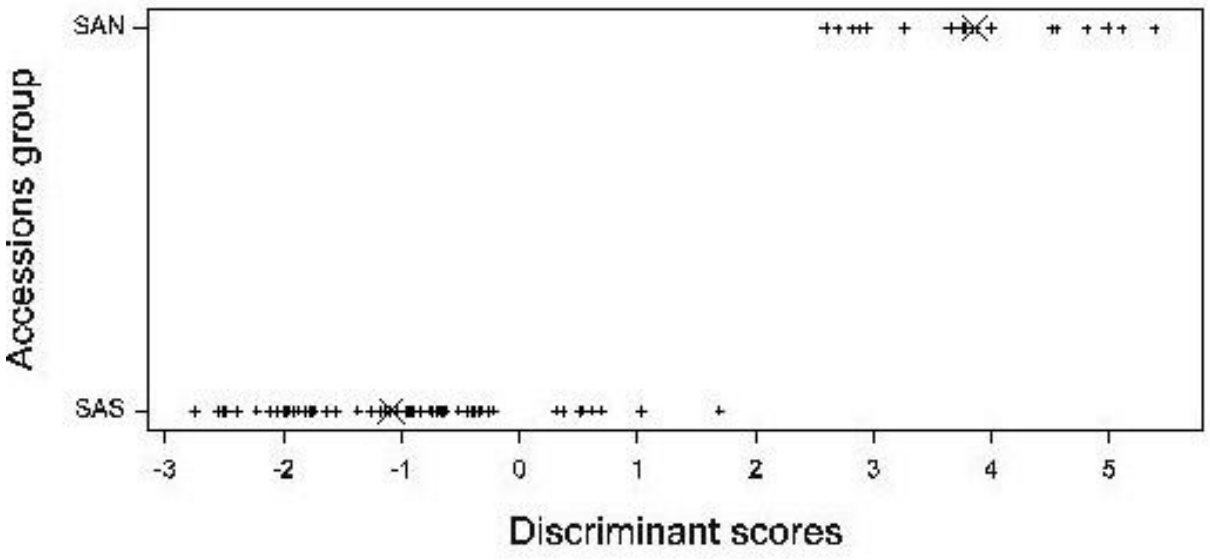


Figure 5: The discriminant scores for the two groups; SAS (*S. aethiopicum* Shum) and SAN (*S. anguivi*) germplasm

The position of a group mean score is marked with an 'X'.

Table 8: The loadings and proportion of variation explained by each of 16 PCs among study accessions

Variable	Mean	StDev	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12	PC 13	PC 14	PC 15	PC 16
DG	7.6	3.2	-0.38	-0.06	0.12	-0.03	-0.28	-0.31	0.04	-0.18	0.51	-0.03	0.38	0.25	-0.15	0.12	-0.20	-0.30
CLBL	13.1	1.7	0.07	0.42	-0.18	-0.19	-0.18	0.18	0.02	0.06	0.07	-0.50	-0.35	0.34	-0.35	0.24	0.05	-0.06
CLBW	5.1	0.9	0.22	0.20	-0.42	-0.23	0.17	0.02	-0.24	-0.10	0.47	0.07	-0.07	-0.14	0.14	-0.50	-0.14	-0.21
SLBL	26.6	10.3	-0.23	0.38	0.26	0.14	0.13	0.07	-0.03	-0.09	0.05	-0.03	0.09	-0.21	-0.03	-0.14	0.72	-0.31
SLBW	19.0	7.2	-0.24	0.37	0.27	0.13	0.07	0.04	0.04	-0.11	0.22	0.02	-0.02	0.06	-0.09	-0.30	-0.17	0.72
SDFW	0.4	0.3	-0.18	0.39	0.24	0.23	0.13	0.22	0.01	0.22	-0.03	0.08	-0.16	-0.12	0.32	0.21	-0.52	-0.36
LPP	39.8	9.2	0.37	0.13	0.22	0.03	-0.03	-0.08	-0.17	0.01	0.20	0.68	-0.22	0.33	-0.15	0.23	0.14	-0.02
PH	29.6	8.4	0.14	-0.15	0.30	-0.17	0.64	0.12	0.20	0.07	-0.06	-0.13	0.18	0.44	-0.17	-0.23	-0.09	-0.18
PB	10.2	1.4	0.21	-0.10	0.35	-0.44	-0.12	0.18	0.21	0.39	0.38	-0.06	0.09	-0.44	-0.05	0.15	0.04	0.11
PW	48.6	4.2	-0.32	-0.21	-0.01	0.07	-0.21	0.18	-0.39	0.66	0.00	0.07	-0.05	0.21	-0.12	-0.33	0.07	-0.03
PL	58.6	14.5	0.16	-0.25	0.24	0.16	-0.02	0.49	-0.58	-0.37	0.15	-0.25	0.09	-0.01	0.05	0.15	-0.03	0.04
LBL	21.0	2.8	-0.32	0.08	-0.21	-0.45	0.17	0.19	-0.09	0.02	0.02	0.14	0.18	0.27	0.50	0.32	0.20	0.20
LBW	14.6	2.4	-0.38	-0.05	-0.10	-0.32	0.17	0.22	-0.11	-0.23	-0.19	0.33	-0.09	-0.30	-0.57	0.09	-0.17	-0.06
SBF	137.8	32.8	0.01	0.06	0.40	-0.47	-0.43	-0.04	-0.04	-0.26	-0.35	0.02	-0.17	0.11	0.23	-0.35	-0.08	-0.14
RWF	51.3	12.2	-0.23	-0.26	0.19	-0.10	0.31	-0.46	-0.21	0.00	0.17	-0.21	-0.60	-0.08	0.11	0.12	0.07	0.03
FLW	37.8	6.4	-0.17	-0.33	-0.08	0.16	-0.12	0.44	0.52	-0.20	0.27	0.13	-0.40	0.12	0.12	-0.13	0.11	-0.07
Standard deviation			**2.13**	**1.92**	**1.50**	**1.20**	**1.10**	**0.86**	**0.81**	**0.63**	**0.53**	**0.50**	**0.46**	**0.38**	**0.24**	**0.23**	**0.18**	**0.08**
Variance			**4.55**	**3.69**	**2.25**	**1.44**	**1.22**	**0.74**	**0.66**	**0.40**	**0.28**	**0.25**	**0.22**	**0.15**	**0.06**	**0.05**	**0.03**	**0.01**
Proportion			**28.46**	**23.07**	**14.07**	**9.02**	**7.60**	**4.62**	**4.15**	**2.50**	**1.76**	**1.58**	**1.35**	**0.92**	**0.35**	**0.33**	**0.19**	**0.04**
Cumulative			**28.46**	**51.53**	**65.60**	**74.62**	**82.21**	**86.83**	**90.98**	**93.48**	**95.24**	**96.83**	**98.17**	**99.09**	**99.44**	**99.77**	**99.96**	**100**

PC, principal component; DG, days to germination and emergence; CLBL, cotyledonous leaf blade length; CLBW, cotyledonous leaf blade width; SLBL, seedling leaf blade length; SLBW, seedling leaf blade width; SDFW, seedling fresh weight; LPP, number of leaves per plant; PH, plant height; PB, number of branches per plant; PW, plant canopy width; PL, petiole length; LBL, leaf blade length; LBW, leaf blade width; SBF, shoot fresh biomass; RWF, root fresh weight; FLW, days to first flower appearance.

3.4 Discussion

A moderately high CCC obtained indicates presence of structure or strong group (subgroup) differentiation among the study accessions. It is notable however, that the higher the CPCC value up to over 0.9, the better the usefulness of dendrograms especially for taxonomic purposes (Odong *et al*., 2011; Coghlan, 2017). The clustering was unbalanced considering that one of the groups contained only one member compared to twelve in one of the four remaining groups. The dendrogram produced was the most appropriate with the data used considering that five clusters were shown; in concordance with optimum number read from the Kelley-Gardner-Sutcliffe penalty function (Kelley *et al*., 1996; Grum and Atieno, 2007). The UPGMA typically produces unbalanced dendrograms, leading to exposure of outliers (Grum and Atieno, 2007; Odong *et al*., 2011) like entry 17 that had leaf prickles. Leaf prickles are not a common attribute within the *S. aethiopicum* Shum and its progenitor *S. anquivi* (Adeniji *et al*., 2012, 2013). Because it is cultivated for its leaves, the Shum need not possess spines on leaves unless prickliness is a marker (for breeder's interest only) associated with a yield, quality or other desired attribute like tolerance to a major productivity constraint.

The Richness index of 1 for leaf prickles further indicates that only one cluster of the five clusters was represented; implying that apart from entry 17, the rest of the 24 accessions spread in the 2-5th cluster did not have spines. For the Shannon-Weaver index (swi), higher values correspond with higher diversity/abundance of a certain category of qualitative variable. It is thus suggested that poor VSV, acute LTA, prostrate PGH, greenish white CLC, strong LL, glabrous stem prickliness, glabrous petiole prickliness, sparse leaf pubescence, and glabrous leaf prickliness are the most diverse and abundant attributes among the study accessions. The poor VSV category had the highest Simpson index; suggesting a higher abundance of the accessions with poor vigor. The

other variable categories that were highly abundant include prostrate PGH, acute LTA, and greenish white CLC. The Simpson index is a measure of evenness of group membership or the "likelihood of two randomly selected accessions being different from each other" (Harding and Payne, 2012; Coghlan, 2017).

PCA indicated that by just selecting variables leading for loadings in first two PCs; at least half of the drivers of diversity among the accessions would be captured. To this end, it is suggested that CLBL, LPP, DG, LBW, FLW, SDFW, SLBW, LBL, and PW greatly contribute to variation captured by the first two PCs. Because the aim of conducting PCA is two pronged; reduce redundancy and retaining variables that explain as much variation as possible (Coghlan, 2017), a further scrutiny with help of the scree plots guided that the first 10 PCs explain up to ~97% of the diversity as revealed by hierarchical clustering. It is notable however, that the PCA was based on quantitative variables only. Therefore; DG, CLBL, CLBW, SBF, PH, PL, FLW, PW, PB and LPP, account for much of diversity (say ~97%) in the study accessions based on quantitative traits.

Based on group means for discriminant scores and Mahalanobis' intergroup distance (Harding and Payne, 2012), the morphological data clearly classified the Shum and *S. anguivi* accession groups as distinct. The two species can generally be distinguished based on variates; CLBL, CLBW, DG, FLW, LPP, RWD, and SBD in a discriminant function: $Group = 0.0586CLBL - 0.26CLBW + 0.63DG + 0.073FLW + 0.063LPP + 0.031RWD + 0.02SBD$. It is thus suggested that a clear distinction between the Shum and its progenitor can be made at seedling (CLBL, CLBW and DG), flowering (FLW) and harvest maturity (LPP, SBD and RWD). A correlation between variates and DF suggested that the DG, having a strong correlation (r = ~0.8), is a major canonical discriminant variate. The r obtained for DG was positive and strong; though a direction of correlation would

not affect interpretation in this case. It is thus suggested that a screening out of non-Shum genotypes can be done if DG exceeds eleven (min., 4; max., 11; mean, 6.25) days from the time of sowing; under conditions like those used in this study. Whereas morphological markers provide a good distinction between Shum and SAN accessions as reported earlier (Kouassi *et al*., 2014), a follow-up validation study with molecular markers could be complementary.

3.5 Conclusion

There is significant structure within the germplasm of Shum and SAN; with distinct clusters. Qualitative variables put aside, PCA revealed that diversity is mainly contributed by differences in DG, CLBL, CLBW, SBF, PH, PL, FLW, PW, PB, and LPP. It was further revealed that DG provides the greatest separation between SHUM and SAN; with the former emerging earlier than the latter. Other traits which were more favorable among Shum than SAN accessions include LPP, PB and PH. The information generated is useful in suggesting germplasm conservation and breeding approaches for development of improved varieties of Shum.

CHAPTER FOUR

STUDY 2: ASSESSING THE GENETIC DIVERSITY WITHIN *S. aethiopicum* SHUM GROUP

4.1 Introduction

The Shum, one of four morphological groups of *S. aethiopicum*, is a nutrient-rich leafy vegetable; a source of food and income in Uganda (Cernansky, 2015; Pincus, 2015) and other sub-Saharan Africa countries like Cameroon, Nigeria, Burkina-Faso and Côte d'Ivoire (Kouassi *et al*., 2014; Cernansky, 2015). Other groups such as the Gilo are also commercially produced in Latin America, Asia and across Africa (Plazas *et al*., 2014). The crop's cultivation is dominated by smallholder farmers who constitute the highest proportion of sub-Saharan Africa populations (Abukutsa-Onyango, 2014; Von Grebmer *et al*., 2015). Farmers, however, experience low yields and low leaf quality as a result of a number of constraints such as low soil fertility, drought stress, lack of quality seed, pests, diseases and postharvest deterioration (Abukutsa-Onyango, 2014; Pincus, 2015; Bisamaza and Banadda, 2017). Breeding for improved varieties to address the constraints is deemed the best strategy as it offers a lasting solution (Abukutsa-Onyango, 2014; Cernansky, 2015). However, the existence of within-group variability among the 'Shum' had not been investigated.

Lumped up accessions from the different cultivar groups are reported to exhibit diversity in morphological traits (Plazas *et al*., 2014). Conspicuously, however, the Shum were notably insufficiently represented when compared to the Gilo, for example (Adeniji *et al*., 2012; Plazas *et al*., 2014). The lumping up and limited representation for the Shum could have masked the

exposure of within-group structure (Plazas *et al*., 2014). Because Shum is a leafy type, but reproductively propagated crop, adequate inclusion of leaf traits at seedling and harvest-maturity (vegetative) stages, as well as reproductive attributes ought to be ensured in a comprehensive genetic diversity analysis based on morphology (World Vegetable Center, 2017). Statistical approaches like cluster analysis and linear discriminant analysis (LDA) are popular and adequate for the genetic diversity analysis (Asher *et al*., 2017). Cluster analysis in graphic user interface softwares like R is effective for large data sets for both quantitative and qualitative variables (Hornik and Böhm, 2017; Murtagh, 2017). The clustering can be based on a number of alogarithms such as complete linkage, Ward's and UPGMA method (Saraçli *et al.*, 2013). UPGMA is also referred to as the "average" method and it was applied in this study due to its popularity and acceptability. The strength of clustering can be evaluated by way of coefficients such as agglomerative coefficient and cophenetic correlation coefficient (CPCC, Saracli *et al*. (2013)). The CPCC is a common and reliable measure of dissimilarity among formed clusters (Saraçli *et al*., 2013; Silva and Dias, 2013; Murtagh, 2017; Nikolić *et al*., 2017).

In order to identify and quantify traits' contribution for best prediction of observed clusters in the dendrogram, LDA (also called canonical discriminant analysis) is a suited approach (Harding and Payne, 2012). Discriminant analysis refers to a linear combination of data variates (original variables) as loadings, which maximizes between-cluster variance and minimize within-cluster variance (Jombart and Collins, 2015). It relies on allocation of groups (observed or assigned) as dependent variables to be predicted by data (variates). LDA has been applied in medicine, animal (Arandas *et al*., 2017) and plant research. In plants, LDA has been applied in various studies such as taxonomic and germplasm characterization (Herklotz *et al*., 2017), phenotypic changes evaluation in plant species over time (Alberti *et al*., 2017) and crop diseases detection on remote

sensing generated data (Bajwa *et al*., 2017). In Shum, LDA was applied in this study to investigate the morphological diversity of available germplasm in order to ascertain its suitability for conservation and usefulness in genetic improvement of farmes' cultivars for desired traits through breeding. The aim was therefore to investigate existence and drivers of structure within Shum. The specific objectives were to: (i) identify hierarchical structure among a model set of Shum germplasm, and (ii) determine the contribution of different traits in cluster membership of the study accessions. We hypothesized that there is considerable variation within Shum, as a starting point towards selection for superior varieties and affordable germplasm characterization within the subspecies.

4.2 Materials and methods

4.2.1 Germplasm and research location

A pioneer set of 20 Shum accessions (some are shown in Picture 1) were obtained from secretariat of Africa Solanaceae Research Network (Afri-SOL, www.afri-sol.org/) at UCU. A screen house study was implemented at UCU between April 2016 and March 2017. The study accessions of Shum are described in Table 2.

Picture 1: Some of the accessions evaluated for morphological diversity

4.2.2 Design

The study was carried out in screen house between April - August 2016 (first experiment), and October 2016 – March 2017 (second experiment). The potting substrate was composed of thoroughly mixed topsoil (clay loams) and cowdung manure in a ratio of 5topsoil:2 manure. A fortnightly schedule for insecticide (profenofos and cypermethrin at 2ml/L of water), fungicide (metalaxyl and mancozeb at 2 g/L of water) and fertilizer (C.A.N at 4 g/pot) was observed. Watering was carefully carried out using tap water delivered through a hosepipe on a daily basis

to ensure availability of adequate moisture for nutrient uptake by the plants. A completely randomized design was used in which 24 plants (12 for data collection at seedling/vegetative stages, and remaining 12 for reproductive/fruiting stage) per accession were evaluated. Each pot was transplanted with two seedlings at four-leaf stage (four weeks after sowing in a nursery); followed by thinning to one plant per pot. The individual plant in a pot was the observational unit.

4.2.3 Data collection and analysis

4.2.3.1 Clustering

Hierrarchical clustering was based on 61 morpho-agronomic traits (in total) at cotyledonous/seedling, vegetative and reproductive/fruiting stages. This number of variables excludes those that did not significantly differ for at least two accessions at 5% level of significance. The vegetative stage at 6 weeks after planting (WAP) is the harvest stage (since SHUM is a leafy vegetable) at which leaf, shoot and root weights were taken. A summary of scale or units of measurement for the different traits is included in (Table 9), and most of these traits have been described earlier by Plazas *et al.* (2014), Adeniji *et al*. (2012, 2013), Kubie (2013) and IBPR (1988). The variables were summarised in Excel to obtain means. A table of means per trait for each accession was then imported into R statistical software for UPGMA reordered hierarchical cluster analysis. A mantel test (cophenetic correlation coefficient, CCC) for significance of clustering was also performed.

Table 9: Description of variables recorded during the within Shum diversity study

Variable	Unit /scale	Variable	Unit /scale	Variable	Unit /scale
DG	days	SBF	g	Flowers per inflorescence (FLI)	counts
CLBL	mm	Shoot dry biomass (SBD)	g	Stamen length (STL)	mm
CLBW	mm	Harvest fresh index (HIF)	ratio	Petal length (PEL)	mm
VSV	1-5	Harvest dry index (HID)	ratio	Sepal length (SEL)	mm
SLBL	mm	PGH	1-9	Relative style length (RSL)	mm
SLBW	mm	SOS	1-9	Pollen production (POL)	0-7
SDFW	mg	SPU	1-4	Fruit length (FRL)	mm
SDDW	mg	SC	1-3	Fruit breadth (FRB)	mm
LBL	cm	PC	1-3	Fruit pedicel length (FPL)	mm
LBW	cm	PP	1-9	Fruit pedicel thickness (FPT)	mm
Leaf area (LA)	cm^2	LL	1-9	Fruit color at physiological ripeness (FCP)	1-9
LPP	counts	LTA	1-9	Fruit position (FRP)	1-9
PB	counts	LBC	1-3	Fruit calyx length (FCL)	mm
PH	cm	LVP	1-3	Fruit cross section diameter (FCD)	mm
PW	cm	LMC	1-3	Locules per fruit (LPF)	counts
Leaf relative water content (LRWC)	%	LSU	1-9	Fruit fresh weight (FFW)	g/fruit
Leaf fresh weight (LFW)	mg	LSL	1-9	Fruits per inflorescence (FPI)	counts
Leaf dry weight (LDW)	mg	LHU	1-9	Fruits per plant (FPP)	counts
Leaf fresh yield (LFY)	g	LHL	1-9	Fruit fresh yield per plant (FFY)	g/plant
Leaf dry yield (LDY)	g	FLW	days	Seed color (SEC)	1-9
				Seeds per fruit (SPF)	counts

*LRWC (measured at vegetative/harvest maturity stage) = [(FW-DW)/(TW-DW)]*100; where FW=fresh weight of leaf sample disc, TW=turgid weight of leaf sample disc, and DW=dry weight of leaf sample disc*

4.2.3.2 Canonical discriminant analysis of clusters

After clustering, raw data file (in Excel) was updated with a column named 'cluster' to assign a group number to each accession (member) as determined from dendrogram. A MANOVA was carried out to identify traits that significantly ($p \leq 0.01$) contributed to observed clusters in the

dendrogram. The following two-way MANOVA model was analyzed in GenStat 12th Edition (VSN International Ltd):

$$y_{ij} = \mu + C_i + R_j + \varepsilon_{ij}$$

Where: y_{ij} is the observed trait measurement for a group of accessions in the i^{th} cluster (C) and j^{th} experiment (R); μ and ε_{ij} refer to overall cluster mean for a trait and random error, respectively.

The traits (variables) which did not significantly differ for at least two clusters at $\alpha = 1\%$ were excluded from discriminant analysis (DA). The DA in GenStat generates $c - 1$ number of discriminant functions (DFs, where $c = number\ of\ clusters$), correlations between data variates and DFs, discriminant scores for cluster means and Mahalanobis'(D^2) inter-cluster distances. Finally, a Pearson's correlation between canonical variates was carried out to identify variate (morphological marker) alternatives for strongly correlated traits.

4.3 Results

4.3.1 Hierarchical clusters

Observed clustering in the dendrogram (Figure 6) showed four distinct clusters with a CPCC of 0.87 at 99.99% level of confidence. Cluster three was the richest followed by cluster two. Cluster four and cluster one had only 1 and 2 members, respectively (Table 10). The mean performance of accessions also showed differences for different variables. Accessions 160 and 145, which constituted cluster four, had higher PEL, SEL, FRL, FRB, FCL, FCD, FFW and SPF than the rest of accessions. Accession 185P (Cluster 1) uniquely had the highest FPP and FFY.

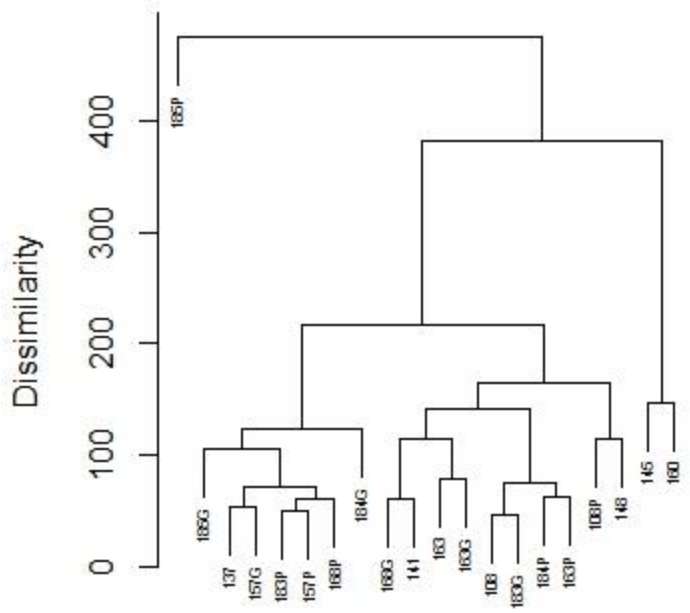


Figure 6: Reordered hierarchical clustering of study germplasm

Table 10: Cluster membership of Shum germplasm studied

No.	Entry code	Accession	Cluster	No.	Entry code	Accession	Cluster
1	E1	168G	three	11	E11	148	three
2	E2	183G	three	12	E12	145	four
3	E3	163	three	13	E13	168P	two
4	E4	163P	three	14	E14	184G	two
5	E5	157P	two	15	E15	137	two
6	E6	160	four	16	E16	184P	three
7	E7	163G	three	17	E17	141	three
8	E8	183P	two	18	E18	108P	three
9	E9	108	three	19	E19	185G	two
10	E10	157G	two	20	E20	185P	one

4.3.2 Canonical variates

The MANOVA eliminated 11 variables on the basis of non-significance in cluster discrimination at 99% level of confidence (Table 11). The eliminated variables include FPL, LBW, LL, LMC, LSL, LVP, PC, PP, SBD, SBF, and SOS. LDA on the remaining 50 variables (as factor variates) produced 3 DFs consisting of vector loadings or DF scores for each variate. Pearson's product-moment correlation coefficients for DF1, DF2 and DF3 were 0.9999, 0.9973 and 0.9727,

respectively. A biplot of the first two DF scores showed a clear separation among 4 clusters. The first DF separated Cluster four from the rest; while the second DF distinguished Cluster one, Cluster two and cluster three (Figure 7). Further, discriminant scores for cluster means under DF1, DF2 and DF3 were highest for clusters one and four, cluster one followed by cluster three and cluster one followed by cluster two, respectively. The D^2 (Mahalanobis' inter-cluster distances) scores were highest between cluster four and any of the remaining clusters followed by cluster one and cluster three ($D^2 = 2694$), and cluster one and cluster two ($D^2 = 1352$).

Cluster two and cluster three had the lowest score for inter-cluster distance at 438 (Table 12). Correlations between 50 data variates and 3 DFs produced SEC (-0.05), PEL (-0.03), SEL (-0.02), SPF (-0.02) and FCL (-0.02) as the canonical variates in the first DF. In the second DF, LFY and FFY had the highest scores, both at 0.06 and -0.06, respectively; followed by VSV and FPP both at 0.04 and -0.04, respectively. In the last DF, the following variates had high scores HID (0.13) followed by HIF (0.11), PGH (0.08), and LPP and LFY both at 0.07. Strong correlations between some variates were also observed. For instance, HIF and HID ($r = 0.52$), SEC and SEL ($r = 0.62$), and LFY and LPP ($r = 0.76$) are correlated traits.

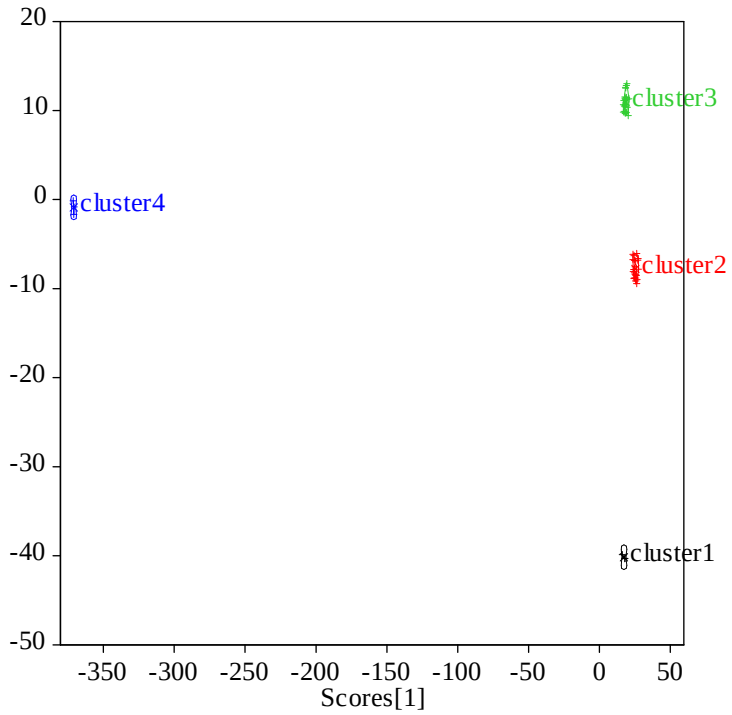


Figure 7: Biplot of first two DFs showing cluster dissimilarities

cluster1, cluster one; cluster2, cluster two; cluster3, cluster three; cluster4, cluster four; Scores[1], discriminant function (DF) 1; Scores[2], DF 2

Table 11: Mean squares of some of the variables and their significance in clustering of study accessions

Source	d.f	CLBL	CLBW	DG	FCD	FCL	FCP	FFW	FFY	FLW	FPI	FPL	FPP	FPT
Experiment	1	2515.8	539.1	1.008	113.2	4.123	4.453	345.37	168538.0	27319.4	45.72	19.97	30030.5	191.5
Cluster	3	418.8 ***	86.6 ***	102.5 ***	3154.5 ***	2040.5 ***	129.0 ***	3144.1 ***	1159353.0 ***	272.3 ***	32.0 ***	39.3 **	17918.5 ***	50.0 ***
Residual	48	7.418	1.348	2.259	11.28	3.276	2.902	15.16	19902.0	24.11	0.809	6.471	321.2	3.862

*, ** and *** stand for significance of cluster distinctiveness based on particular trait at α = 5, 1 and 0.1%, respectively.

CLBL, cotyledonous leaf blade length; CLBW, cotyledonous leaf blade width; DG, days to germination; FCD, fruit cross section diameter; FCL, fruit calyx length; FCP, fruit color at physiological ripeness; FFW, fruit fresh weight; FFY, fruit fresh yield; FLW, data to first flower appearance; FPI, fruits per inflorescence; FPL, fruit pedicel length; FPP, fruits per plant; FPT, fruit pedicel thickness

Table 12: Discriminant scores for cluster means and inter-cluster distances

Discriminant scores for cluster means			
	Scores [1]	Scores [2]	Scores [3]
Cluster one	17.35	-40.17	-11.31
Cluster two	25.42	-7.8	4.16
Cluster three	18.58	10.92	-2.2
Cluster four	-370.95	-0.88	0.57

Inter-cluster distances - Mahalanobis (D-squared)				
	Cluster one	Cluster two	Cluster three	Cluster four
Cluster one	0			
Cluster two	1352	0		
Cluster three	2694	438	0	
Cluster four	152466	157172	151879	0

4.4 Discussion

4.4.1 Hierarchical clusters

Existence of strong population structure within the Shum was observed, contrary to observations by Plazas *et al*. (2014) and Adeniji *et al*. (Adeniji *et al*., 2013, 2012). However, it is notable that the duo did not assess the structure based on Mantel test, which gives a CCC, and they compared all the four recognized morpho-types (Shum, Aculeatum, Kumba and Gilo) in 'one' analysis (Adeniji *et al*., 2013, 2012; Gramazio *et al*., 2016; Plazas *et al*., 2014). CCC is a reliable measure for genetic diversity applicable to both molecular and morpho-agronomic data variables (Murtagh, 2017; Nikolić *et al*., 2017). The higher the CCC closer to 1.0 at high confidence level, is the stronger the distinctiveness of observed dendrogram clusters and reliability of hierarchical clustering (Nikolić *et al*., 2017). After clustering, it was imperative to carryout MANOVA to eliminate data redundancy, which could have occurred as a result of introducing 'cluster' as a predictor variable for the response of different traits measured. After MANOVA, linear discriminant analysis (LDA) considers 'cluster' as a response variable as predicted by measured traits (Arandas *et al*., 2017).

4.4.2 Canonical discriminant variates

From LDA results, very high canonical correlation coefficients indicate that all three discriminant functions (DFs) generated were generally very important in dissecting variatal contribution to distinct clusters; in agreement with guidelines by Harding and Payne (2012) and Jombart and Collins (2015). According to Harding and Payne (2012) and Jombart and Collins (2015), the higher the correlation coefficients, the more clear-cut are the differences among clusters (Jombart and Collins, 2015). Specifically, the order of importance of the DFs in distinguishing among clusters was such that: DF1 was slightly superior to DF2, and the DF2 is slightly superior to DF3. The

biplot of DF2 scores and DF1 scores showed a visual display of four distinct clusters. The distinctness of clusters was supported by generally high values of both inter-cluster distances and discriminant scores for cluster means. Members of cluster4 (accessions 160 and 145) possessed uniquely higher mean values for PEL, SEL (or SEC), FCL and SPF than the rest of accessions. The SEL and SEC were moderate-strongly correlated. Specifically, accessions 160 and 145 had orange-yellow fruits. Further, the biplot showed that DF 1 largely served to separate cluster4 from the rest of clusters. The observation suggests that other than leaf area, reproductive traits are putatively good morphological markers in SHUM group. Similar views were made by Adeniji *et al.* (2012) and Plazas *et al*. (2014) when they studied world collections of *S. aethiopicum* groups namely Shum, Aculeatum, Gilo and Kumba for genetic diversity based on morphological attributes.

Judging from highest discriminant score for cluster one mean under DF2, supported by correlations between data variates and DF2, it implies that cluster one (composed of one member, accession 185P) was considered unique. This was due to extremely high FFY, the least LFW and relatively high FPP. The FFY and LFW were the most important predictors of cluster distinctiveness in DF2; the traits had the highest scores from the correlation between all data variates and the DF2. Reduced leaf size and high reproductive efficiency are known adaptive traits for survival under harsh environments (Fita *et al*., 2015; Basu *et al*., 2016; Mwale *et al*., 2017). Cluster one members showed the highest departure from cluster one and cluster three (basing on discriminant scores for cluster means and inter-cluster distances) under DF2. It implies that accession 185P is mainly different from cluster two and cluster three members for LFW and FFY. Although cluster two and cluster three had the shortest inter-cluster distance, their discriminant scores for cluster means were most extreme under DF2. Thus, traits that made cluster one unique are also important in

discriminating between cluster two and cluster three. DF3 and DF2 were important in separating cluster one from cluster two where LFY (or LPP; which are strongly correlated), FFY, VSV, FPP, HID (or HIF; HID and HIF being correlated traits), and PGH are responsible traits (Figure 7). Contrary to Plazas *et al*. (2014) and Adeniji *et al*. (2012) whose findings emphasized reproductive traits as major drivers of variability in *S. aethiopicum*, results from this study indicated an equal importance of both vegetative and reproductive traits in distinguishing among the Shum accessions. Additionally, the visual seedling vigor offers opportunity for early-stage discrimination among Shum germplasm.

4.5 Conclusion

There is significant genetic diversity within Shum, which can support breeding interventions to develop improved varieties, as a way of addressing the crop's productivity constraints. Thirteen (13) canonical variates which best explained the structure by way of high loadings in each discriminant function, are potential morphological markers in the Shum. The canonical variates include VSV for early-stage separation among accessions or individuals of a breeding population. At vegetative (harvest maturity of 8 WAP); petiole length, leaf fresh weight, leaves per plant, fresh harvest index, dry harvest index and plant growth habit are the major discriminating variables. Varieties with favorable scores of the said traits (harvest index and leaf fresh weight, in particular) at vegetative stage are of direct importance to consumers and farmers. To breeders and seed companies, the possession of reproductive fitness traits is key to sustainability. As such; sepal length, seed color, fruit calyx length, number of seeds per fruit, fresh fruit yield and number of fruits per plant which were among the best predictors of diversity; offer opportunities for improving crop productivity through crossbreeding research and seed supply to farmers.

CHAPTER FIVE

STUDY 3: INDENTIFYING DROUGHT TOLERANT PARENTAL MATERIAL IN *S. aethiopicum* SHUM GROUP

5.1 Introduction

Cultivation of Shum under irrigation for continuous production is the norm (Bahadur *et al*., 2009). Yet, water supply is becoming both inadequate and unreliable as climate change (CC) is experienced (Bahadur *et al*., 2009; A&N Technical Serives, 2015). In order to sustain production amidst CC indicators (particularly drought); there is need to identify superior varieties which are stable in yield irrespective of fluctuations in water availability (Sękara *et al*., 2007; Kumar *et al*., 2012). Shum is largely a neglected and under-utilized crop (NUC) species (Padulosi *et al*., 2013), although some molecular tools are available for the *Solanum aethiopicum* complex and its close relatives like *S. melongena* (Prohens *et al*., 2013; Plazas *et al*., 2014; Acquadro *et al*., 2017, 2016; Gramazio *et al*., 2017). No Shum germplasm had been screened for response to drought (Blum, 2009; Abukutsa-Onyango *et al*., 2010; Kumar *et al*., 2012). Water deficit stress directly affects productivity of Shum (a leafy vegetable) and quality of its leaves (Ssekabembe and Odong, 2008; Kumar *et al*., 2012).

Commonly, a drought stress signal is generated in roots as increase in ABA consequently modifying root architecture, leaf temperature, LRWC, gas exchange, CHL and photosynthetic rate (Yoshida *et al*., 2014; Pucholt *et al*., 2015). Mechanisms of drought tolerance (DT) are classified as recovery, tolerance, avoidance and escape; and these are exhibited at morpho-physio-biochemical and cellular/molecular levels (Yoshida *et al*., 2014; Singh *et al*., 2015; Amelework *et al*., 2015). Physiologically, plants tend to undergo OA through accumulation of various osmolytes

for adaption towards maintaining osmotic pressure under abiotic stresses (Singh *et al*., 2015). OA guards plants against consequences of ROS (Kumar *et al*., 2012; Yoshida *et al*., 2014; Singh *et al*., 2015). Measurements like SWP/LWP, LRWC and morpho-agronomic attributes are useful indicators for PWS and variety response to water drought (Blum, 2005, 2009; Kumar *et al*., 2012; Kesiime, 2014; Amelework *et al*., 2015; Mwale *et al*., 2017). LRWC is the most applied traits as a measure of PWS (Anjum *et al*., 2011; Kumar *et al*., 2012; Kesiime, 2014; Fang and Xiong, 2015). Usually, high LRWC of ≥80 percent implies that a variety is drought tolerant (Banik *et al*., 2016; Kesiime *et al*., 2016;). Specific variables for reliable drought screening in Shum have not been documented.

Genotype performance across season and/or location environments can be understood by way of calculating coefficients for stability superiority (Oliveira *et al*., 2014; Mendes de Paula *et al*., 2014). Such coefficients have severally been used in identification of superiorly stable genotypes (Eberhart and Russell, 1966; Kamidi, 2001; Altaye, 2015). Additive main effects and multiplicative interaction (AMMI) and genotype x genotype x environment interaction (GGE) do similarly rank varieties (Mendes de Paula *et al*., 2014); and their concurrent use only helps to validate output (Kamidi, 2001; Oliveira *et al*., 2014; Mendes de Paula *et al*., 2014). As a matter of emphasis, AMMI / GGE interaction analyses utilize stability coefficients in different ways to rank varieties and/or environments for multi-location and/or multi-season data (Oliveira *et al*., 2014); the same coefficients were applied in this study. Water deficit variation was applied as test environments so as to implement the study. The aim was to evaluate Shum genotypes for identification of those accessions which excel across moisture variations. The specific objectives were to:

i. determine effect of moisture deficit on LRWC, LBL, LBW, LPP and PH in Shum,

ii. identify stable and superior genotypes under moisture deficit stress, and

iii. identify applicable traits for breeding of drought tolerant varieties in Shum.

Findings from this study are useful guide when designing breeding programmes for developing drought tolerant/farmer-preferred varieties.

5.2 Materials and methods

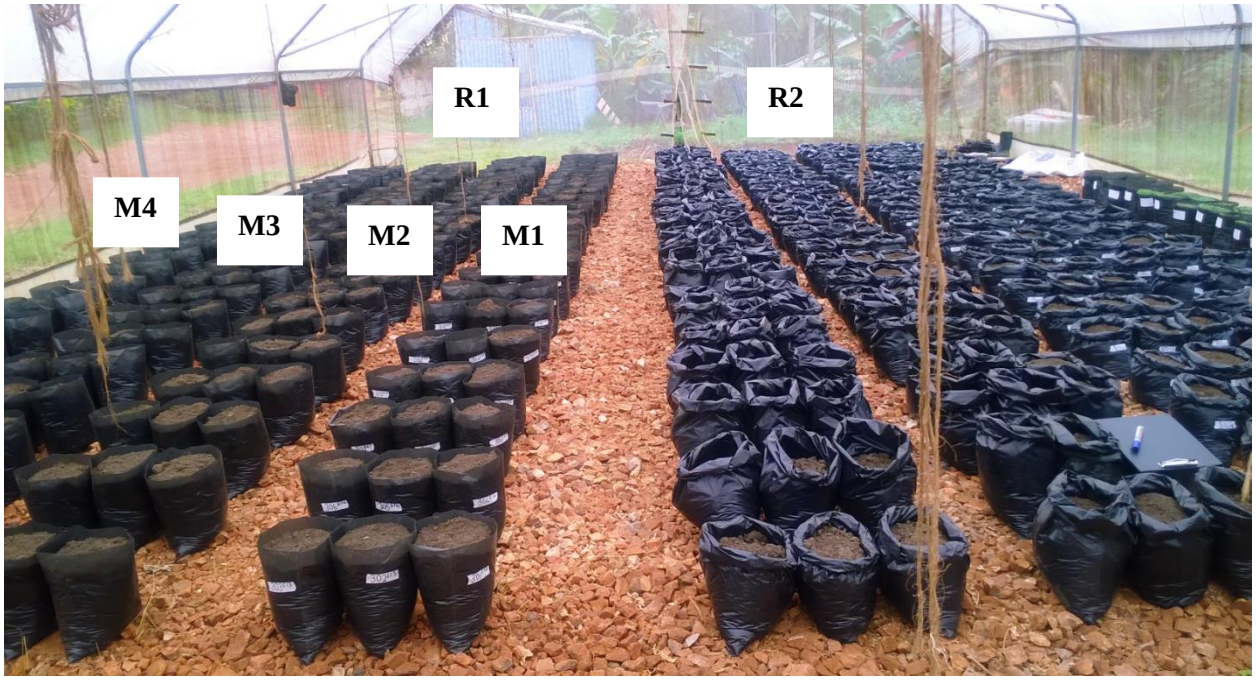
5.2.1 Plant material and study location

Twenty Shum accessions obtained from UCU were self-pollinated for two seasons for purification and seed increase purposes. The purification was carried out under screen house and plastic mesh bagging (Picture 2) in order to ensure pollinator exclusion. The germplasm (Table 2) differed in morphological attributes such as SC, PH and PGH (Sseremba *et al*., 2017b).

Picture 2: Seed increase and purification by self-pollination of accessions in screen house

5.2.2 Design

Four moisture levels (WLs) as whole plots and 20 Shum genotypes as subplots were organized in a split-plot arrangement with two replications over two seasons in screen house (Picture 3). Three pots per subplot were used. Sowing followed by transplanting in 10-kg substrate pots in April-July 2016 and November 2016 – February 2017 seasons in screen house was carried out. The WLs were generated based on field capacity (FC) (Kesiime, 2014) of the potting substrate. The estimation of FC, calculation of moisture treatments (100, 75, 50 and 25 percent of FC) as well as detailed management of the experimental pots is as detailed in Sseremba *et al*. (2018a). The screen house was consisted of reflective aluminized polypropylene shading net with over 75% illumination rate compared to outdoor daylight intensity (Ferreira *et al*., 2014).

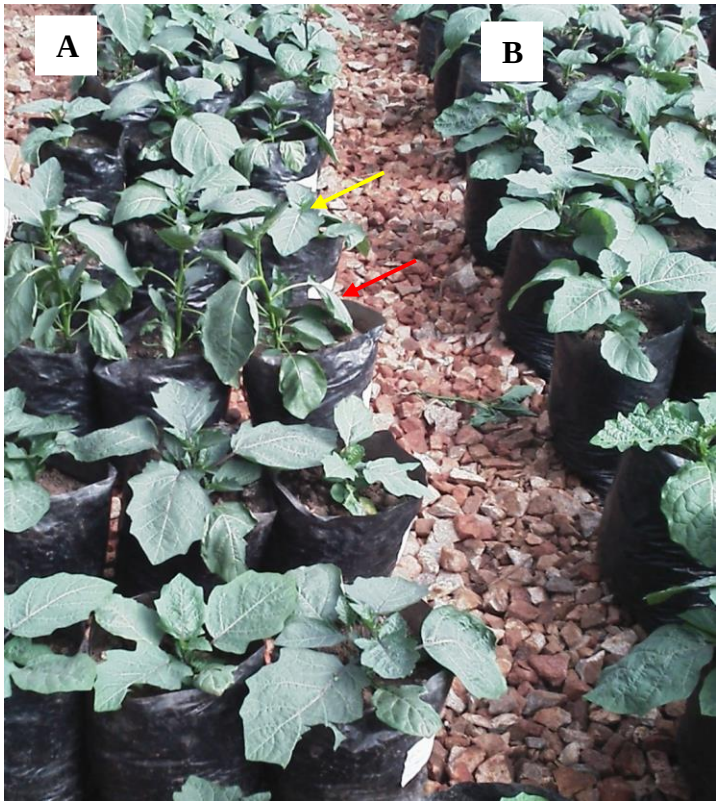


Picture 3: Layout of pots for drought screening of accessions in 2016B

Three pots (each for 1 plant) were allocated per accession within a main plot. Data was collected on individual potted plants. R1, first replication; R2; second replication; M1-M4, main plots (for moisture levels) each containing 20 subplots (for accessions)

5.2.3 Data collection

At five weeks after transplanting (WAT), data was collected on each of individually potted plants (Picture 4). LRWC (percentage) was measured from one most fully open leaf from top of each plant; and the following formula was used: $\%LRWC = \frac{FW-DW}{TW-DW} * 100$; where FW, TW and DW stand for fresh weight, turgid weight and dry weight of leaf sample, respectively (Banik *et al*., 2016; Kesiime *et al*., 2016). The following morphological variables were measured: LBL (cm), LBW (cm), LPP and PH (cm) (Amelework *et al*., 2015; Kesiime, 2014; Mwale *et al*., 2017). The same leaf sample per plant was used for measuring LRWC, LBW and LBW.



Picture 4: Potted plants showing differences in response to drought stress at 4 WAT

The red and yellow arrows show wilted and non-wilted plants belonging to different accessions, respectively. A, drought stress plants; B, well-watered controls

5.2.4 Statistical analysis

5.2.4.1 Influence of DS on LRWC and morphological variables

The following generalized linear model (GLM) was analyzed using GenStat 12th edition:

$y_{ijkl} = \mu + R_i + W_j + W * G_k + G_l + \varepsilon_{ijkl}$; where μ and y_{ijkl} stand for grand mean, and measured observation, respectively, due to the i^{th} replication (R), j^{th} drought stress level (W), k^{th} drought stress x genotype interaction (W*G), l^{th} genotype (G) effects, and random error (ε_{ijkl}).

5.2.4.2 δ components and GCV estimates

For each of first (2016A) and second (2016B) screen house experiments (Table 13) and parameter measured, variance (δ) components, obtained from ANOVA, were partitioned out into expected mean squares (mean squares method) so as to estimate H^2 and GCV as guided by Falconer and Mackay (1996).

Table 13: Expectation of mean squares for each experiment

Source of variation	Degrees of freedom	Mean squares (MS)	EMS	F-test
Block (R)	r - 1	MSR	$wg\sigma_r^2 + g\sigma_{rw}^2 + \sigma_\varepsilon^2$	$\frac{MSR}{MSM}$
Moisture (W)	W - 1	MSW	$rg\sigma_w^2 + g\sigma_{rw}^2 + r\sigma_{wg}^2 + \sigma_\varepsilon^2$	$\frac{MSW + MSE}{MSM + MSS}$
Whole plot error (M)	(r - 1)(w - 1)	MSM	$g\sigma_{rw}^2 + \sigma_\varepsilon^2$	$\frac{MSM}{MSE}$
Genotype (G)	g - 1	MSG	$rw\sigma_g^2 + r\sigma_{wg}^2 + \sigma_\varepsilon^2$	$\frac{MSG}{MSS}$
M x G interaction	(rw-1)(g - 1)	MSS	$r\sigma_{wg}^2 + \sigma_\varepsilon^2$	$\frac{MSS}{MSE}$
Error (E)	(rwg-1)(p-1)	MSE	σ_ε^2	
Total	rwgp-1			

EMS, expected mean squares

Using Table 13, genotypic variance (V_G), H^2, GCV and expected genetic variance (GA) were estimated as follows:

V_G: $$V_G = \frac{(rw\sigma_g^2 + r\sigma_{wg}^2 + \sigma_\varepsilon^2) - (r\sigma_{wg}^2 + \sigma_\varepsilon^2)}{r * w}$$

Broad sense heritability (H^2): $$H^2 = \frac{\sigma_g^2}{\delta_P^2}$$

Phenotypic variance (V_P): $$V_P = \delta_g^2 + \frac{\delta_{wg}^2}{w} + \frac{\delta_\varepsilon^2}{rw}$$

Genotype by water deficit stress interaction (V_{WG}): $$V_{WG} = \frac{\left(r\delta_{wg}^2 + \delta_\varepsilon^2\right) - \delta_\varepsilon^2}{r}$$

Genetic coefficient of variation ($GCV\%$): $$GCV\% = \frac{\sqrt{\delta_g^2}}{\mu} * 100$$

GA $$K * H^2\sqrt{\delta_P^2}$$

GA as percentage of μ ($GA\%$) $$\frac{GA}{\mu} * 100$$

r, w, g, ε, μ stand for replications, moisture levels, accessions, random error and grand mean, respectively. K=1.3 (i.e., selection intensity) at 20 percent selection proportion.

5.2.4.3 Stability superiority coefficients estimation

A model by Eberhart and Russell (1966) and modified by Linn and Binns (1988) was applied using GenStat 12th edition: $P_i = \sum_{j=1}^{n}(X_{ij} - M_j)^2/(2w)$ where X_{ij}, M_j and w refer to observed measurement of i^{th} genotype at j^{th} moisture level, maximum value among all the genotypes at j^{th} moisture level, and number of moisture levels, respectively. The model has widely been used for variety selection (Kamidi, 2001; Oliveira *et al*., 2014; Mendes de Paula *et al*., 2014).

5.2.4.4 Identifying water stress deficit selection variables

Variables where significant differences for WL x G interaction at 1 % significance level were suggested as prospective breeding traits when selecting genotypes for resistance to water deficit

stress in SHUM. Potential traits for DS screening were additionally inspected for individuality by Pearson's correlation analysis (CA) so as to remove any redundancy. It was only the LRWC which was selected based on its significant WL x G interaction (rather than additional scrutinizing through CA); on the account on the wide acceptance of the LRWC as a drought tolerance (DT) screening variable in well studied crops like tomato (Zhu *et al*., 2014), potato (Banik *et al*., 2016) and cassava (Turyagyenda *et al*., 2013).

5.3 Results

5.3.1 Influence of DS and genotype on LRWC and morphological traits

Highly significant differences ($p<0.01$) were observed among WLs and G for all the measured variables in each season. For both seasons (experiments), LRWC, as well as means morphological traits; LBL, LBW, LPP (Picture 5) and PH, reduced progressively with rising DS from W1 (100 %) to W4 (25 % FC) (Figure 8). DS levels (75, 50, 25), compared to 100 % FC, enlarged data spread of leaf relative water content (Figure 8), but not for LBL, LBW and LPP; which showed a reduced spread in 2nd season's data for 50 % FC and 25 % FC (Figure 9). Both seasons generated alike trends (Figures 8, 9); prompting decision to base on data from 1st season only for subsequent analyses. Analysis of variance for 1st season's data is presented (Table 14). H^2 was very high for all traits (ranged from 89 to 97 %) and genetic coefficient of variation is higher for PH than for LRWC, LBL, LBW and LPP (Table 16).

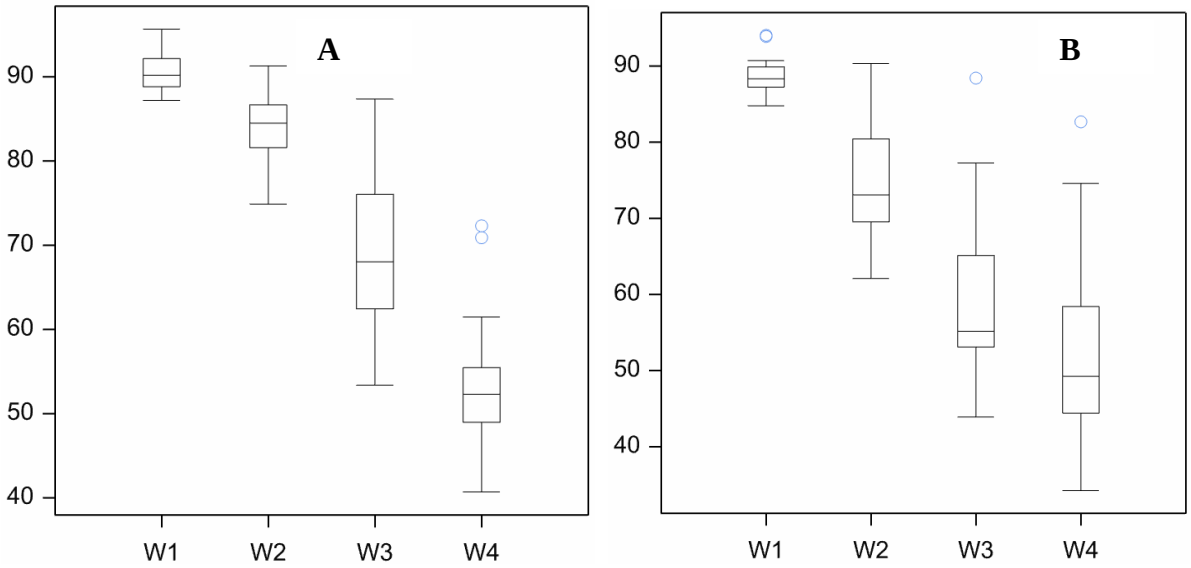


Figure 8: Box plot for LRWC at rising drought stress (100 – 25 % FC)

A, first season; B, second season; LRWC, leaf relative water content; FC, field capacity

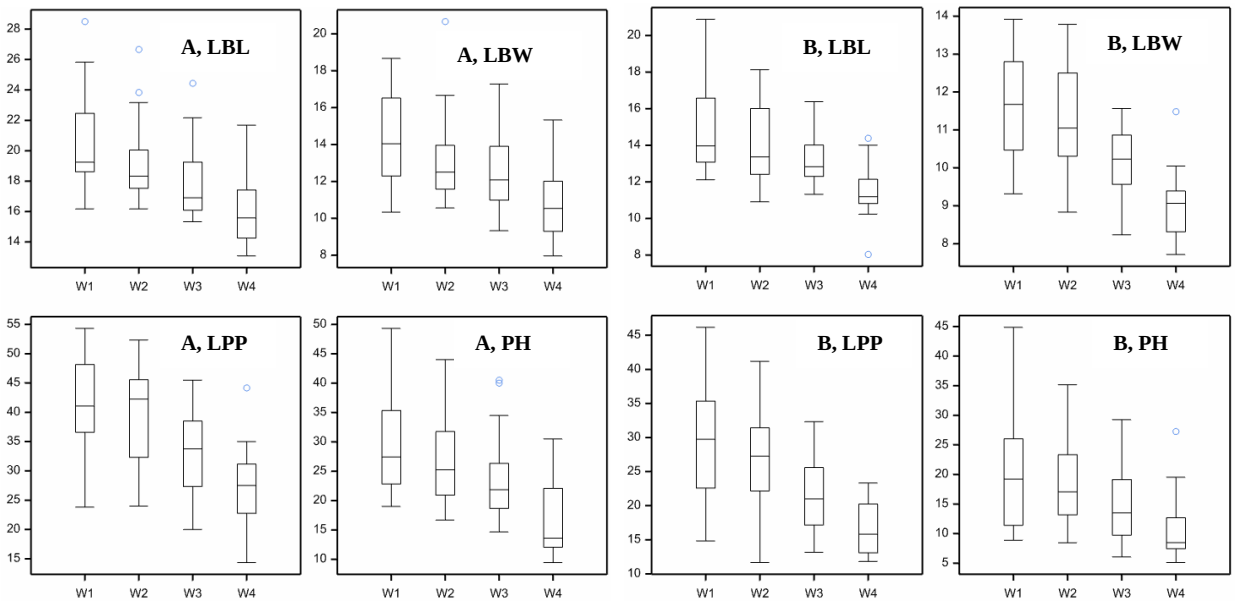


Figure 9: Box plot for LBL, LBW, LPP and PH for each of two seasons

A, first season; B, second season; PH, plant height; LPP, leaves per plant; LBW, leaf blade width; LBL, leaf blade length

Table 14: Mean squares, δ components, GCV and GA across drought stress regimes

Source of variation	d.f	LRWC	LBL	LBW	LPP	PH
Replications (R)	1	9.60	1.51	0.28	20.83	15.05
Watering regime (W)	3	33059.10***	494.96**	244.18*	5255.39***	4193.97***
Main plot error (M)	3	0.97	9.18	7.06	28.90	23.17
Accessions (G)	19	764.43***	135.34***	92.26***	1195.84***	1453.96***
W x G interaction	57	112.60***	12.76	12.07	89.84***	48.95***
Subplot error (S)	76	13.15	10.31	9.07	18.03	14.97
Error (E)	320	12.17	3.78	4.07	11.10	9.95
Variance components, broad sense heritability and genotypic coefficient of variation						
V_G		88.58	15.50	10.24	143.38	178.05
V_{WG}		21.80	3.79	3.14	18.85	9.79
V_P		95.55	16.92	11.53	149.48	181.75
H^2 (%)		92.70	91.61	88.78	95.92	97.97
$GCV\%$		12.67	21.29	25.11	34.02	54.71
GA		11.78	4.90	3.92	15.25	17.17
$GA\%$		16.86	26.49	30.73	43.30	70.38

, **, * stand for significance at α=5, 1 and 0.1 % error margin, respectively; GA, genetic advance; GCV, genotypic coefficient of variation; δ, variance; PH, plant height; LPP, leaves per plant; LBW, leaf blade width; LBL, leaf blade length; LRWC, leaf relative water content; d.f., degrees of freedom*

5.3.2 Coefficients for stability of genotypes and traits for DT breeding

5.3.2.1 Estimates of genotype stability

Genotypes having highest and most stable leaf relative water content (LRWC) across WLs include E6, E12, E5 and E18 while lowest and least stable LRWC was observed for E7, E17, E3 and E9 (Table 15). Further, four genotypes; E6, E12, E11 and E18 had largest and most stable LBL and

LBW across WLs though the four genotypes had very low LPP. Genotypes with highest and most stable number of green leaves per plant (LPP) include E20 followed by E19, E4 and E10. Genotypes E4, E7, E1 and E3 remained tallest while E15, E16, E2 and E18 were shortest across WLs.

5.3.2.2 DT selection variables

Very highly significant ($p < 0.001$) moisture level by genotype interactions were obtained for leaf relative water content (LRWC), number of green leaves per plant (LPP) and plant height (PH). Such variation allowed for separation of stable/superior from unstable/inferior accessions especially basing on, leaf relative water content (Figure 10) and plant height (Figure 11). Most superiorly stable accessions retained high LRWC (as well as for other traits) across WLs but least stable/inferior accessions had a very high reduction in values as WL declined for most of measured variables.

On the other hand, non-significant ($p > 0.05$) moisture level by genotype interactions for leaf blade length and leaf blade width was obtained. Similarly, very strong and very highly significant correlation between leaf blade length and leaf blade width ($r = 0.8$, $p < 0.001$, two-sided *t*-test) were observed. Therefore, leaf blade length and leaf blade width are similar and inferior parameters when compared with leaf relative water content, leaves per plant and plant height for use selection for stable and superior varieties from a given set. Notably further, correlations between leaf relative water content and each of the other variables in this study are significant; leaf relative water content with leaf blade length ($r = 0.5$, $p < 0.001$), leaf relative water content with leaf blade width ($r = 0.4$, $p < 0.001$), leaf relative water content with leaves per plant ($r = 0.4$, $p < 0.001$) and leaf relative water content with plant height ($r = 0.2$, $p < 0.001$). Still, weak but significant correlation was obtained for number of green leaves per plant and plant height ($r = 0.2$, $p < 0.001$).

Table 15: Coefficients for stability superiority and means for each variable across WLs

Genotype		LRWC		LBL		LBW		LPP		PH	
		CSS	Mean	CSS	Mean	CSS	Mean	CSS	Mean	CSS	Mean
E6	160	0.0	86.6	0.0	25.2	0.2	17.7	375.4	21.8	324.8	15.8
E12	145	0.5	85.9	2.5	23.4	2.7	16.4	259.6	26.5	178.9	22.2
E15	137	37.9	78.8	36.2	16.8	21.3	11.6	45.4	41.6	344.8	15.0
E18	108P	42.0	78.6	13.7	20.2	3.7	15.5	236.6	27.6	290.1	17.1
E14	184G	65.7	78.0	34.4	17.2	18.6	12.4	43.4	40.2	211.8	21.2
E5	157P	70.2	77.2	37.1	17.2	20.9	11.9	71.6	37.5	246.1	19.0
E10	157G	74.2	76.0	28.2	18.1	22.9	11.4	36.5	43.3	206.0	20.9
E4	163P	89.9	75.5	44.1	16.2	22.3	11.6	24.8	42.6	4.5	39.6
E1	168G	116.8	72.6	37.6	16.8	17.3	12.4	81.1	37.3	11.9	37.1
E11	148	118.3	73.8	11.2	20.7	8.2	14.4	347.0	22.8	131.1	25.5
E13	168P	122.3	73.1	21.3	19.1	16.9	12.4	124.2	33.5	55.6	31.3
E2	183G	125.1	72.0	39.8	16.6	15.7	12.7	110.3	34.5	297.6	17.0
E20	185P	127.2	73.0	36.9	16.8	36.1	9.6	20.9	44.4	181.3	22.3
E8	183P	127.4	72.3	31.6	17.4	20.6	11.7	127.8	33.2	174.5	22.4
E16	184P	144.4	72.1	37.3	16.7	24.2	11.1	149.4	31.9	238.7	19.6
E19	185G	145.3	71.7	26.9	18.1	19.6	12.1	24.4	44.7	150.2	23.8
E9	108	155.0	71.0	23.8	18.7	17.8	12.7	60.8	40.3	157.5	23.4
E3	163	212.0	67.8	26.4	18.1	20.3	12.3	95.0	35.9	13.1	37.0
E17	141	294.2	65.3	36.2	16.9	24.3	11.1	75.1	37.3	224.8	20.0
E7	163G	296.4	64.5	16.5	19.7	8.6	14.1	237.8	27.3	9.0	37.7

***Genotypes with smaller values are more stable across WLs. Genotypes are ranked by CSS for leaf relative water content (LRWC). CSS, stability superiority coefficient; LPP, leaves per plant; PH, plant height; LBW, leaf blade width; LBL, leaf blade length; WLs, moisture deficit stress levels*

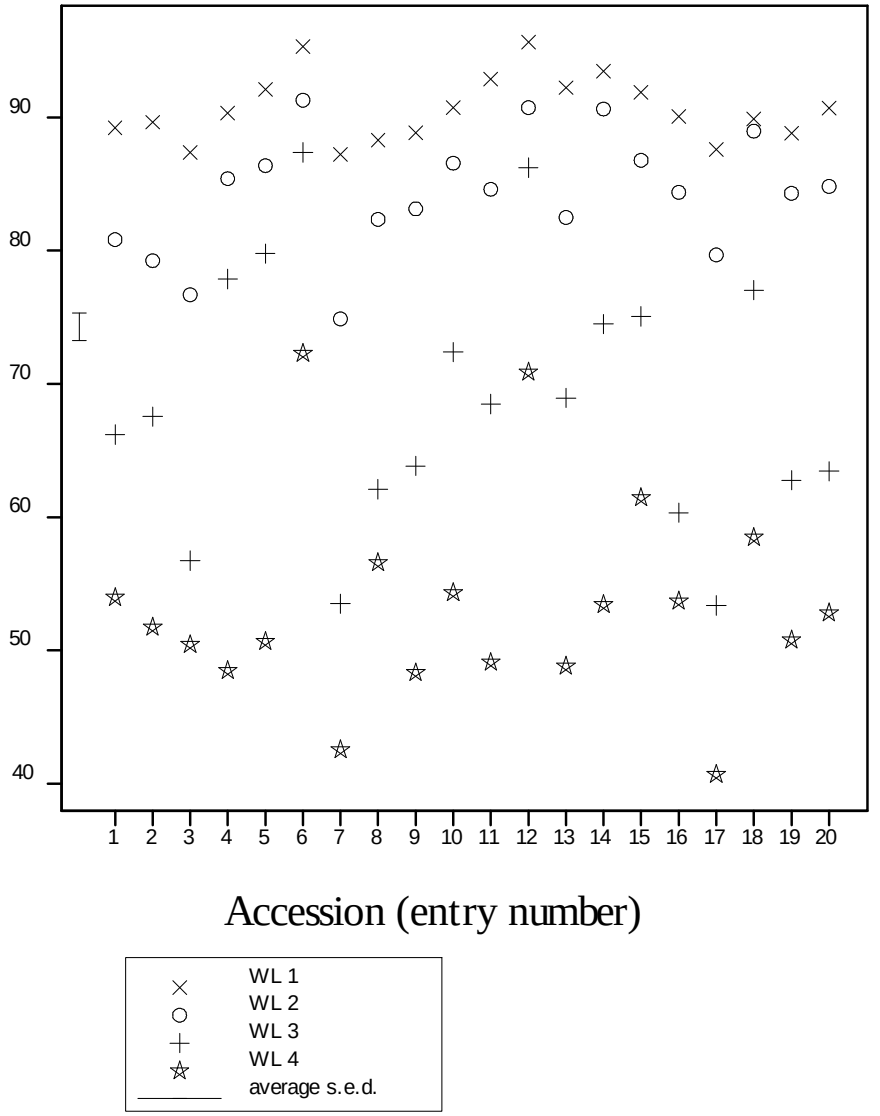


Figure 10: Leaf relative water content of genotypes at different WLs

1 to 20 on X-axis, genotypes; 1, E1(168G); 2, E2(183G); 3, E3(163); 4, E4(163P); 5, E5(157P); 6, E6(160); 7, E7(163G); 8, E8(183P); 9, E9(108); 10, E10(157G); 11, E11(148); 12, E12(145); 13, E13(168P); 14, E14(184G); 15, E15(137); 16, E16(184P); 17, E17(141); 18, E18(108P); 19, E19(185G); 20, E20(185P). WLs, water deficit stress levels; WL 1, 100 % FC; W 2, 75 % FC, WL 3, 50 % FC, WL 4, 25 % FC; FC, field capacity of potting substrate; LRWC, leaf relative water content

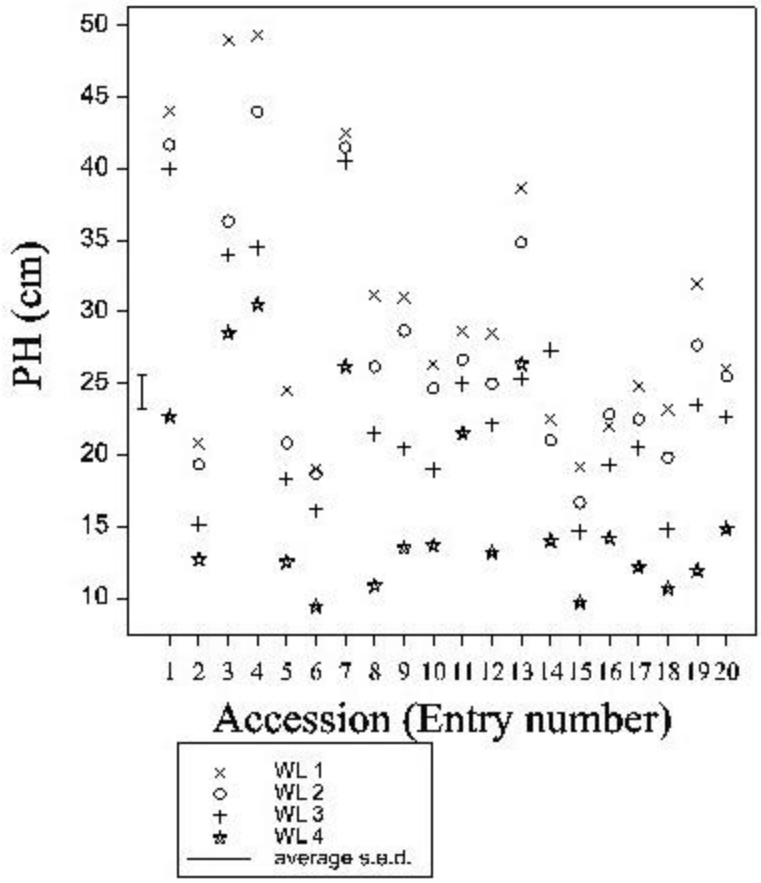


Figure 11: Plant height of genotypes at different WLs

1 to 20 on X-axis, genotypes; 1, E1(168G); 2, E2(183G); 3, E3(163); 4, E4(163P); 5, E5(157P); 6, E6(160); 7, E7(163G); 8, E8(183P); 9, E9(108); 10, E10(157G); 11, E11(148); 12, E12(145); 13, E13(168P); 14, E14(184G); 15, E15(137); 16, E16(184P); 17, E17(141); 18, E18(108P); 19, E19(185G); 20, E20(185P). WLs, water deficit stress levels; WL 1, 100 % FC; W 2, 75 % FC, WL 3, 50 % FC, WL 4, 25 % FC; FC, field capacity of potting substrate; PH, plant height

Picture 5: Number of green leaves per plant varied depending on drought tolerance of an accession

5.4 Discussion

Highly significant moisture deficit stress level by genotype interactions for leaf relative water content, number of green leaves per plant and plant height indicate that the parameters are important in selection of genotypes for stability superiority under drought stress (DS). On the contrary, leaf blade length and leaf blade width (correlated but non-significant moisture level by genotype interaction) are considered as non-vital in deciding on genotype superiority and stability. Further, bigger spread in mean performance of accessions was obtained for leaf relative water content than for other study parameters under DS; which suggests that Shum varieties are best discerned from one another under DS using leaf relative water content. The observations emphasize importance of leaf relative water content as a DT selection variable, as already used in well studied crops such as *Manihot esculenta* (Turyagyenda *et al*., 2013); *S. tuberosum* (Banik *et al*., 2016) and *S. lycopersicum* (Zhu *et al*., 2014).

Also, existence of correlation between leaf relative water content (LRWC) and each of leaf blade length, leaf blade width and number of green leaves per plant suggests that targeting LRWC alone (for selection of superiorly stable genotypes) comes along with improvement in the traits related to the LRWC. That said, weak correlation between LRWC and plant height was observed; deeming it necessary to select genotypes with favorable coefficients of superiority stability for both leaf relative water content and plant height. Though leaf relative water content is the most important indicator of plant-water status (PWS) in most crops (as related to drought tolerance stability in this study), stability in plant height should also be considered as well for possibility of increasing number of branching nodes hence better leaf yield (as number of leaves per plant can increase). In Shum (a leafy vegetable), stably superior genotypes under DS are desired for leaf yield assurance/guarantee (Kumar *et al*., 2012; Yoshida *et al*., 2014).

However, when data was subjected to additional inquiry, it was observed that very tall genotypes such as E4, E7, E1 and E3 maintained their tallness across WLs yet many of them suffered significant reductions in leaf relative water content. It is only genotype E4 which remained relatively superiorly stable for both plant height and leaf relative water content. This is to suggest thus, that prioritizing choice of drought tolerance selection variables in Shum should take interest in leaf relative water content as first choice, plant height as second choice and number of green leaves per plant as third option. The recommended variables/traits are strategic in that leaf relative water content can directly influence postharvest deterioration and market value of Shum because the higher the leaf relative water content the higher the quality of produce (Kumar *et al*., 2012; Ssekabembe and Odong, 2008). Plant height and number of green leaves per plant tend to affect leaf yield (Kumar *et al*., 2012), that is to say, tall and leafy varieties can lead to high leaf yield potential in Shum as well as other leafy vegetables.

The heritability of each of desired traits; leaf relative water content, plant height and number of green leaves per plant was greater than 0.90 which implies that the observed genetic variance (V_G) is highly heritable (Ogunniyan and Olakojo, 2014). Genotypic coefficient of variation as well as expected genetic advance (GA) was also favorable particularly for plant height and number of green leaves per plant; emphasizing prospect for a good response to selection in subsequent generations (Falconer and Mackay, 1996; Roychowdhury and Randrianotahina, 2011). Thus, germplasm used for this study is adequate for selection of superiorly stable genotypes across WLs using plant height (very high likelihood), then; number of green leaves per plant, leaf blade length/leaf blade width and leaf relative water content (lowest likelihood). Leaf relative water content had moderate genetic coefficient of variation and GA which makes it largely reliable as a drought tolerance breeding trait though plant height and number of green leaves per plant had better values (Al-Tabbal and Al-Fraihat, 2011; Roychowdhury and Randrianotahina, 2011; Ogunniyan and Olakojo, 2014; Ahsan *et al*., 2015). Nonetheless, basing on positive and significantly correlated leaf relative water content (LRWC) and number of green leaves per plant (LPP), superiorly stable genotypes for LRWC can be indirectly selected for using LPP (Falconer and Mackay, 1996).

It is suggested thus, that the order of preference as breeding traits (selection criteria) for stability superiority of a genotype across WLs is as follows: leaf relative water content is better than plant height is better than number of green leaves per plant. Also, considering that leaf relative water content (LRWC) determines quality of leaves (and their market value) under DS (Ssekabembe and Odong, 2008; Kumar *et al*., 2012), LRWC has been applied to select for superiorly stable genotypes. Therefore, E6 is better than E12 is better than E15 is better than E18 is better than E14.

Similarly, E7 is least superiorly stable genotype followed by E17, E3 and E9. Notably, the number of accessions evaluated was only 20 which is quite a small number compared drought screening studies in other vegetable crops such as potato and tomato (Kesiime *et al*., 2016; Basu *et al*., 2016). However, the Shum accessions are a pioneer set available in Uganda at the time of this study; and it has provided insight into existence of germplasm for use in drought tolerance breeding.

5.5 Conclusion

Whereas water deficit stress impacted negatively on all measured traits of Shum germplasm, the observed differences allowed for selection of drought tolerant genotypes. Among all traits, it was understood that leaf relative water content, plant height and number of green leaves per plant provide the best opportunity in selecting for superiorly stable varieties across WLs. It thus, goes that when breeding for drought tolerance in Shum, leaf relative water content is better than plant height is better than number of green leaves per plant as selection criteria. Basing on leaf relative water content, the following genotypes are superiorly stable in performance under water deficit stress; E6, E12, E15, E18 and E14. On a lower extreme; E7 is worse than E17 is worse than E3 is worse than E9 in terms of susceptibility to drought. High H^2, GCV and GV values obtained for most of measured traits also gives positive prospect for improvement of drought tolerance in Shum over generations.

CHAPTER SIX

STUDY 4: DETERMINING THE COMBINING ABILITY FOR DROUGHT TOLERANCE IN *S. aethiopicum* SHUM GROUP

6.1 Introduction

Water deficit stress threatens production per unit area for all crops. Commonly, plants react to water deficiency by escaping, avoiding, tolerating and/ or recovering from the stress after exposure (Kumar *et al*., 2012; Yoshida *et al*., 2014; Amelework *et al*., 2015; Beyene *et al*., 2015; Kamanga *et al*., 2018). For instance, may flower early by having a short vegetative phase (VP) so as to complete a life cycle before onset of drought stress (DS) as a drought escape (DE) strategy (Turyagyenda *et al*., 2013; Basu *et al*., 2016; Pucholt *et al*., 2015). Crops which employ DE mechanism undergo enhanced metabolic rates with water use efficiency (WUE) (Shavrukov *et al*., 2017; Kamanga *et al*., 2018). Drought avoidance (DA) strategies like high WUE involves slowed growth of plants and this is associated with small or closed stomata, reducing photosynthetic rate which prepares plants for a possible water deficit stress period (Shavrukov *et al*., 2017; Kamanga *et al*., 2018).

DS stimuli are sensed in roots resulting in biosynthesis of ABA (Yoshida *et al*., 2014). This ABA - dependent homeoregulation of osmotic stress (OS) is facilitated by Ca^{2+} kinase and phosphatase proteins and components that traffic signals across cell membranes (Fita *et al*., 2015; Kamanga *et al*., 2018). ABA non-dependent control of ion channels in guard cells as result of OS also reportedly occurs (Yoshida *et al*., 2014; Fita *et al*., 2015; Basu *et al*., 2016). DS sensing by either ABA or calcium ions motivate closure of in-ward and opening of out-ward pores for K+ ions

transfer out of guard cells with a consequence of stomata closure (Parry *et al*., 2014; Ramírez *et al*., 2014; Kröber *et al*., 2015; Basu *et al*., 2016;). When stomata close gas exchange (conductance for CO_2 and O_2) is reduced (Galmés *et al*., 2013; Yoshida *et al*., 2014; Fang and Xiong, 2015). As a result, internal carbon dioxide and oxygen concentrations reduce and increase, respectively, favoring photorespiration to the detriment of photosynthesis. The scenario leads to accumulation of reactive oxygen species (ROS) (Anjum *et al*., 2011; Amelework *et al*., 2015; Beyene *et al*., 2015; Banik *et al*., 2016). ROS cause damage to thylakoids (chlorophyll membranes); which are sites for light stages of photosynthesis (Kesiime, 2014; Yoshida *et al*., 2014; Fita *et al*., 2015). Morphological attributes get impaired namely plant height (PH), leaf area (leaf blade length, LBL; and leaf blade width, LBW), fresh leaf yield (LYF) (and dry leaf yield, LYD) as highlighted by Nakanwagi *et al*. (2018) and Sseremba *et al*. (2018a) among other yield/agronomic traits. This research focused on Shum; and the following variables were studied: leaf wilting score (LWS), number of green leaves per plant (LPP), LYF/LYD, leaf area (LA), leaf relative water content (LRWC), leaf mass area (LMA) and chlorophyll content (CHL) (Sseremba *et al*., 2018b).

In Shum (like any other leafy vegetable), the desire is to prolong vegetative growth phase if productivity is to be maximized with optimum water use efficiency (WUE) (Kumar *et al*., 2012). This provides vegetable breeders with options of breeding for drought avoidance (DA), drought tolerance (DT), drought recovery (DR) or a combination of strategies. It is desirable that genotypes tolerate water deficit stress to enable attainment of substantial yield by growers (Galmés *et al*., 2013; Kamanga *et al*., 2018). Further, quality attributes of leafy vegetables such as LRWC (Ramírez *et al*., 2014; Banik *et al*., 2016; Sseremba *et al*., 2018a), LMA and CHL (Galmés *et al*., 2013; Yoshida *et al*., 2014; Shavrukov *et al*., 2017; Sseremba et al., 2018b) should remain favorable under drought. Under field conditions, drought can strike, and rainfall resumes

afterwards, leaving plants with possibility to recover (drought recovery, DR) (Kamanga *et al*., 2018). Different varieties have varied potential to resume into productivity after a DS episode (Fita *et al*., 2015).

Breeding methods like hybridization are useful in supporting enhancement DT in crop plants. Hybridization exploits heterosis and this has been fully explored in *Zea maydis* (Sprague, 1936; Pioneer Hi-Bred, n.d.). In other crops like sorghum, hybrid vigor has also been utilized for improvement in yield, disease and pest tolerance (Ben-Israel *et al*., 2012; Mindaye *et al*., 2016). Tomato (*S. lycopersicum*) and cabbage varieties are vegetables reported with yield improvement in hybrids (Kibar *et al*., 2015; Sharma *et al*., 2015; Saeki *et al*., 2016). On the contrary, hybrid vigor has not been sufficiently investigated in Shum (Lester and Thitai, 1989; Meier, 2011).

On top of the benefits of hybrid vigor in hybrids of good specific combining ability (SCA), managing crosses is important in introgressing of desired attributes into farmers' varieties by aid of parent material of known general combining ability (GCA) considering that some of phenotypic variation is not heritable (Falconer and Mackay, 1996; Ahsan *et al*., 2015). Appropriate mating designs such as diallel (full or partial) and North Carolina (NCI, NCII and NCIII) can be used to organize crosses using sets of females and male parents in order to obtain variance components as one of the ways through which heritability can be estimated (Stuber, 1980; Nduwumuremyi *et al*., 2013; Bu *et al*., 2015; Murtadha *et al*., 2016).

Successfully produced hybrids are reported in Gilo (one of the morphological groups of *S. aethiopicum*) (Lester and Thitai, 1989; Meier, 2011). However, hybridization possibilities in Shum

are yet to be studied. This study was aimed at determining GCA and SCA of Shum under varied watering regimes; with specific objectives as:

i. Determining heritability of drought tolerance in Shum,
ii. identifying appropriate traits for selection of parents and hybrids with good combining ability under varied watering regimes, and
iii. identifying parents with good GCA and hybrids with good SCA in Shum under varied water deficit stress regimes.

6.2 Materials and methods

6.2.1 Germplasm and study location

Thirteen (13) accessions obtained from Uganda Christian University (UCU) were self-pollinated for three seasons (2015B, 2016A and 2016B) for purification and seed increase purposes. The purification was carried out under screen house and plastic mesh bagging (Picture 2) in order to ensure pollinator exclusion. From an earlier study (Sseremba *et al*., 2018a); contrasting parents for drought tolerance (DT) were identified (four DT and nine as drought susceptible). Crosses were then made in a 9 x 4 NC II mating design (9 females being susceptible to drought and four being DT) to produce 24 F1 hybrids (Table 16) out of a potential 36 (67 % success). The 24 hybrids were then evaluated in screen house in 2017 at UCU. The screen house conditions at the time of the experiment are as elaborated in Sseremba *et al*. (2018b) (Figure 12).

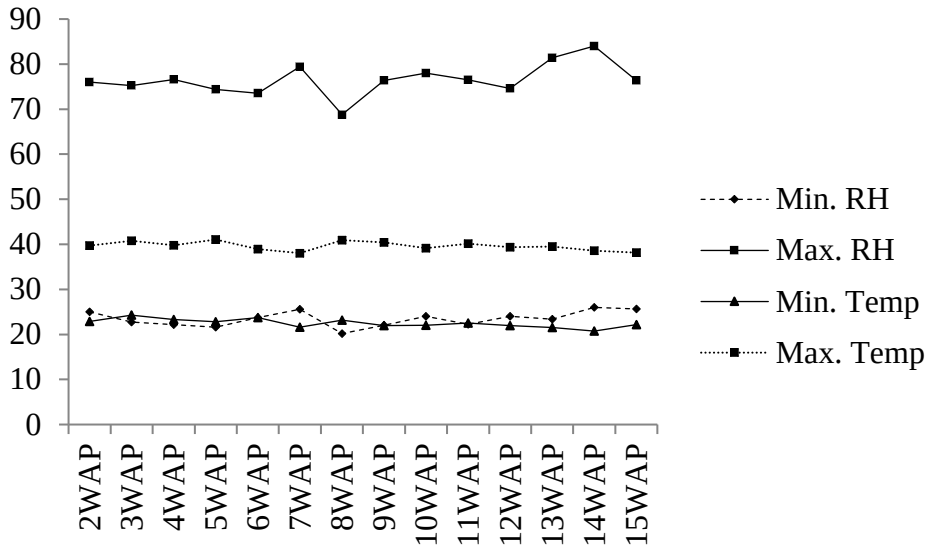


Figure 12: Stability of RH and temperature within screen house during experiment

WAP, weeks after planting; RH, relative humidity; Min. RH, minimum weekly relative humidity; Max. RH, maximum weekly relative humidity; Min. Temp, Minimum weekly temperature; Max. Temp, maximum weekly temperature

Table 16: List of F1 hybrids used for the combining ability analysis

No	Hybrid	Female	Male	No	Hybrid	Female	Male
1	E1 x E4	E1	E4	13	E3H x E15	E3H	E15
2	E3S x E4	E3S	E4	14	E7S x E15	E7S	E15
3	E7H x E4	E7H	E4	15	E7H x E15	E7H	E15
4	E10 x E4	E10	E4	16	E10 x E15	E10	E15
5	E11 x E4	E11	E4	17	E11 x E15	E11	E15
6	E13 x E4	E13	E4	18	E13 x E15	E13	E15
7	E1 x E6	E1	E6	19	E1 x E20	E1	E20
8	E3S x E6	E3S	E6	20	E3S x E20	E3S	E20
9	E7H x E6	E7H	E6	21	E7S x E20	E7S	E20
10	E13 x E6	E13	E6	22	E10 x E20	E10	E20
11	E1 x E15	E1	E15	23	E11 x E20	E11	E20
12	E2 x E15	E2	E15	24	E13 x E20	E13	E20

E3S, smooth leaf surface selection from E3; E3H, hairy leaf surface selection from E3; E7S, smooth leaf surface selection from E7; E7H, hairy leaf surface selection from E7

6.2.2 Design

Twenty-four F_1 hybrids (subplot) under different watering regimes (main plot) was evaluated in a split-plot randomized complete block design (RCBD) in two blocks. The watering regimes include well watering (WW), drought stress (DS) and re-watering after drought (DR). Sunshine was the blocking factor. Detailed potting and potted plants' routine management practices have been published and the same were applied in this study (Sseremba *et al*., 2018b). Three plants per genotype per replication per watering regime were maintained (one plant per pot).

Under WW treatment, plants were watered to field capacity until eight weeks after planting (8 WAP). Thereafter, DS was applied for two weeks on experimental plants by withholding watering. This makes ten WAP (10 WAP) of plant maturity. At 10 WAP (including two weeks of DS), watering to field capacity was resumed for two weeks. Thus, by end of the two weeks of re-watering, the potted plants were 12 weeks of age (12 WAP). Meanwhile control plants were maintained under normal watering throughout the experiment. Thus by factoring in both age of plant and moisture treatments, five environments were generated and considered namely: (i) well-watered at 8 WAP (WW 8), (ii) well-watered / control at 10 WAP (DT – CT10), (iii) drought stressed at 10 WAP (DT - DS10), (iv) well-watered / control at 12 WAP (DR - CT12), and (v) re-watered / drought recovery at 12 WAP (DR - RE12).

6.2.3 Data collection

6.2.3.1 Timing for data collection

At eight weeks after planting (WAP), data was collected for the WW experiment. At ten WAP, data was collected for the DS experiment. After wards, at 12 WAP, data was collected for the DR experiment. The same parameters were measured per during each of the three experimental stages.

6.2.3.2 Leaf morphological traits

Data on LWS (scale of 0-5), LPP, LA (cm^2), LYF (g/plant) and LYD (g/plant) was collected. LYF and LYD was measured on green leaves per plant and oven-dried leaves, respectively. LWS was recorded as described by Banik *et al*. (2016) while LA was measured using a leaf area meter, model AM350 as detailed in Sseremba *et al*. (2018b). At each of 8, 10 and 12 WAP, all three plants per moisture regime per replication were individually observed for all the morphological variables.

6.2.3.3 Leaf physiological traits

Traits measured include LMA (mg/cm^2), LRWC and CHL on the most fully open leaf from top of individual potted plants. Each genotype contained three plants per replication per moisture level. Two leaf samples were used from each of the plants per moisture regime per replication. On each plant, a first and second most fully open leaves from a plant top were sampled. The first leaf was used for CHL and LRWC while the second leaf was used to measure leaf mass area. Measurement for LMA, LRWC and CHL were measured as described in Ramírez *et al*. (2014), Banik *et al*. (2016), Galmes *et al*. (2013), Parry *et al*. (2014) and summarized in Sseremba *et al*. (2018b). At each of 8, 10 and 12 WAP, all three plants per moisture regime per replication were individually observed for all the three physiological variables.

6.2.4 Statistical analysis

6.2.4.1 Influence of watering regime on combining ability

A multivariate linear mixed model general form $y = X\beta + Zu + \varepsilon$) provided in R sommer package (Covarrubias-Pazaran, 2016) was used. This enabled estimation of variance components for general combining ability (GCA) and specific combining ability (SCA) in the NC II design with

missing values. The model provides y (measured phenotype), X (incidence matrix for fixed effects like grand mean [μ]), Z (incidence matrix for random effects like GCA and SCA), β (vector for BLUEs), u (vector for BLUPs), and ε (residuals, that is to say, environmental variance, V_E) (Bu *et al*., 2015; Covarrubias-Pazaran, 2016). The BLUEs and BLUPs refer to best linear unbiased estimates of fixed effects and best linear unbiased predictions of random effects, respectively.

Any phenotype taken on an individual potted plant is thus predictable as follows: $y = X\beta + Zu_{GCA_{males}} + Zu_{GCA_{females}} + Zu_{SCA} + \varepsilon$. y, X and Z is observed phenotype (any of LWS, LPP, LA, LYF, LYD, LRWC, CHL and LMA), matrix for fixed effects (μ, block and watering regime) and incidence matrix for random effects (GCA_{males}, $GCA_{females}$ and SCA), respectively. Under WW experiment (at 8 WAP), fixed effects include μand block. Under DS (and DR experiments), fixed effects are μ, block and watering regime. Across watering environments, fixed effects included μ and moisture regime. Decision on significance of GCA and SCA effects was made at 5 % error margin and subsequent estimates for variance components were carried out as guided by Falconer and Mackay (1996), Kang (2002), and then summarized in Sseremba *et al*. (2018b).

For elaboration purposes, the decision on significance of combining ability effects was made basing on critical values of Z-ratio; 1.64, 2.33 and 3.09 (Bu *et al*., 2015; Covarrubias-Pazaran, 2016) for probability of difference by chance (α) being set at 0.05, 0.01 and 0.001, respectively. The GCA and SCA variance estimates were generated and used to calculate additive (V_A) and dominance genetic variance (V_D), respectively as follows: $V_A = 4 * V_{GCA}$ and $V_D = 4 * V_{SCA}$ where $V_{GCA} = V_{GCA_{males}} + V_{GCA_{females}}$. Genotypic variance ($V_G$) was thus calculated as follows: $V_G = V_A + V_D$. The measured or phenotypic variance (V_P) was then estimated as: $V_P = V_A + V_D + {}^{V_E}/_{n}$; on the assumption of negligible epistatic effects (Falconer & Mackay, 1996; Kang, 2002), in order

to enable calculation of study traits heritability. For across watering environments, n =5; for within environment analysis, n = number of blocks = 2. Traits that produced non-significant effects (GCA or SCA) were eliminated from calculations of values for each study genotype.

6.2.4.2 Combining ability effects of parents and crosses

General combining ability of parents and crosses (Falconer and Mackay, 1996; Kang, 2002) were calculated by considering missing crosses as missing data using Excel. The following formulae were applied in Excel.

i. $GCA_{males} = Mean\ performance\ of\ progeny\ of\ a\ given\ male - Mean\ of\ progeny\ from\ all\ males$

ii. $GCA_{females} = Mean\ performance\ of\ progeny\ of\ a\ given\ female - Mean\ of\ progeny\ from\ all\ females$

iii. $SCA\ effect = Observed\ mean\ of\ a\ cross - Expected\ mean\ of\ the\ cross$

iv. $Expected\ mean\ of\ a\ cross = Overall\ mean\ (\mu) + GCA\ for\ a\ given\ male + GCA\ for\ a\ given\ female$

6.2.4.3 Heritability of drought resistance

The broad- (H^2) and narrow-sense (h^2) heritability was also consequently calculated: $H^2 = \frac{V_G}{V_P}$ and $h^2 = \frac{V_A}{V_P}$. To estimate heritability, 5 environments namely well-watered at 8 WAP, well-watered at 10 WAP, drought-stressed at 10 WAP, well-watered at 12 WAP and re-watered at 12 WAP) were used in the model.

6.2.4.4 Identifying variables for separating genotypes for combining ability

F-test and observation of spread in box plots (of means performance) were used. This enable suggesting for suitability of certain traits for discriminating genotypes for GCA (parents) and SCA (crosses) effects. Box plots provide visual dispersion in data; and it was on this basis that some variables were considered eligible for discriminating the combining ability effects under each watering regime (WW, DS and DR).

6.3 Results

6.3.1 Influence of watering regime on combining ability

Across watering regimes

General combing ability effects were significant among females ($p < 0.05$) for chlorophyll content but non-significant for rest of variables (Table 18). Based on chlorophyll content, parent E11 had highest and positive (best) general combining ability effect followed by E1 and E7S. General combining ability effects of E11 were also positive for all other measured traits though a positive effect for leaf wilting score is not desired (Table 19). However, E3H had the lowest negative general combining ability effect for chlorophyll content. General combining ability effect of E3H was also negative for rest of variables. Males with favorable general combining ability effects for leaf wilting score, leaves per plant, leaf yield (fresh leaf yield and dry leaf yield), leaf area, leaf relative water content, chlorophyll content and leaf mass area were E6 / E15, E20 / E15, E6, E6, E6 / E20, E4 / E20 / E15, and E4 / E6, respectively. Among females, parents with favorable combining ability effects for leaf wilting score, leaves per plant, leaf yield, leaf area, leaf relative water content, chlorophyll content and leaf mass area were E3H / E7S / E1, E13 / E1 / E2, E2 / E13 / E3H, E10 / E7H / E11 / E2, E10 / E13 / E1 / E2, E11 / E1 / E7S / E2 / E10, and E11 / E10 / E1, respectively. The '/' is read as 'followed by'.

Specific combining ability effects were significant for leaf wilting score, leaves per plant, fresh leaf yield, dry leaf yield, leaf mass area, leaf relative water content and chlorophyll content; and highly significant ($p < 0.01$) for leaf area. Cross E7H x E6 had the best (negative) specific combining ability effect for leaf wilting score followed by E11 x E15 and E7S x E20 (Table 20) while E3H x E15 had the highest positive (worst) effect. For leaves per plant, E3H x E15 had the best specific combining ability effect followed by E13 x E20 while E10 x E20 had the worst effect. Specific combining ability effects for E3H x E15 were also positive for fresh leaf yield (dry leaf yield), leaf area, leaf mass area, leaf relative water content and chlorophyll content.

Well-watered

There were non-significant general combining ability effects ($p > 0.05$) for all measured traits (Table 18). Specific combining ability effects were also non-significant for leaf wilting score. On the other hand, specific combining ability effects were significant ($p < 0.05$) for leaves per plant, fresh leaf yield, dry leaf yield, leaf mass area and chlorophyll content; and highly significant ($p < 0.01$) for leaf area and leaf relative water content.

Drought tolerance

Across and within watering treatments, general combining ability effects were non-significant for all the variables measured (Table 18). Specific combining ability effects across watering treatments were significant for leaf wilting score, leaf mass area, chlorophyll content and leaf relative water content and highly significant for leaf area. Within the control, specific combining ability effects were significant for leaves per plant, fresh leaf yield, dry leaf yield, leaf area, leaf mass area and chlorophyll content; and highly significant for leaf relative water content. Within

drought-stressed plants, specific combining ability effects were significant for leaves per plant, fresh leaf yield, leaf mass area and leaf relative water content; and highly significant for leaf wilting score, dry leaf yield, leaf area and chlorophyll content.

Drought recovery

Non-significant general combining ability effects were exhibited for all measured traits across and within watering treatments (Table 18). Across treatments, specific combining ability effects were significant ($p < 0.05$) for leaf wilting score, leaf area, leaf mass area and leaf relative water content but non-significant ($p > 0.05$) for leaves per plant, fresh leaf yield, dry leaf yield and chlorophyll content. Within control, specific combining ability effects were significant for leaves per plant, dry leaf yield, leaf area, leaf mass area, leaf relative water content and chlorophyll content. Within re-watered plants, specific combining ability effects were significant for all measured variables and highly significant for leaf mass area.

6.3.2 Broad and narrow sense heritability of drought tolerance

6.3.2.1 Leaf morphological variables

Each of the variables (LWS, LPP, LYF and LYD) had broad sense heritability value of above 80 percent. Leaves per plant had the highest broad sense heritability at 92 percent while leaf wilting score had the lowest at 81 percent (Table 17). Narrow sense heritability was highest for leaves per plant (79 percent) followed by dry leaf yield, and leaf wilting score had the lowest h^2 (6 percent).

6.3.2.2 Leaf physiological variables

Broad sense heritability was highest for chlorophyll content and leaf relative water content (each at 80 percent) and lowest for leaf mass area at 73 percent. Narrow sense heritability was highest

for chlorophyll content at 68 percent. Narrow sense heritability was relatively low for leaf relative water content and leaf mass area at 15 and 14 percent, respectively.

Table 17: Heritability estimates of the traits across watering regimes

Variable	Additive variance	Dominance variance	Environmental variance	H^2	h^2
Leaves per plant (LPP)	411.23	66.97	205.15	0.92	0.79
Chlorophyll content (CHL)	57.50	10.27	86.04	0.80	0.68
Leaf dry yield (LYD)	49.16	20.05	66.65	0.84	0.60
Leaf fresh yield (LYF)	5,248.33	2,642.60	8,231.39	0.83	0.55
Leaf area (LA)	1,626.07	6,869.11	4,872.00	0.90	0.17
Leaf relative water content (LRWC)	8.62	36.72	58.13	0.80	0.15
Leaf mass area (LMA)	30.60	124.07	282.11	0.73	0.14
Leaf wilting score (LWS)	0.01	0.14	0.18	0.81	0.06

h^2, narrow sense heritability; H^2, broad sense heritability. The traits are ranked by narrow sense heritability.

Table 18: Mean squares for GCA and SCA effects for measured traits under WW, DS and DR experiments

Trait	Source	WW experiment	Drought tolerance (DT) experiment			Drought recovery (DR) experiment			Across experiments
			DT-AT	DT-CT	DT-DS	DR-AT	DR-CT	DR-RE	
Leaf wilting score (LWS)	GCA_{Males}	0.000	0.007	0.000	0.026	0.013	0.000	0.051	0.002
	$GCA_{Females}$	0.000	0.000	0.000	0.000	0.020	0.000	0.079	0.000
	SCA	0.000	0.087*	0.000	0.384**	0.062*	0.000	0.284*	0.036*
	Residual	0.000	0.222***	0.000	0.233***	0.260***	0.000	0.313***	0.182***
Leaves per plant (LPP)	GCA_{Males}	72.314	81.220	389.100	5.345	73.080	389.100	1.508	76.880
	$GCA_{Females}$	9.582	40.900	142.800	0.101	38.390	142.800	3.880	25.920
	SCA	11.586*	11.750	112.000*	3.034*	15.320	112.000*	18.342*	16.740*
	Residual	48.479***	275.640***	270.800***	2.609***	289.640***	270.800***	15.039***	205.150***
Leaf fresh yield (LYF)	GCA_{Males}	3534.925	314.500	1176.000	53.537	208.750	859.500	184.443	744.700
	$GCA_{Females}$	1.891	897.100	3638.000	0.194	705.930	2388.600	5.691	567.400
	SCA	1998.698*	1789.700	8514.000*	24.757*	91.010	1014.600	59.118*	660.700*
	Residual	4159.612***	11724.900***	12197.000***	18.723***	3509.900***	4724.100***	118.285***	8231.400***
Leaf dry yield (LYD)	GCA_{Males}	49.129	5.452	24.880	1.845	0.285	1.992	2.201	5.893
	$GCA_{Females}$	5.317	8.372	34.280	0.000	3.641	9.884	0.121	6.397
	SCA	27.122*	9.449	41.930*	0.976**	1.404	10.653*	1.087*	5.013*
	Residual	57.276***	73.164***	95.720***	0.546***	23.551***	32.141***	1.265***	66.647***
Leaf area (LA)	GCA_{Males}	3378.000	0.000	0.000	0.000	2.559	2.242	3.078	406.500
	$GCA_{Females}$	0.000	0.000	0.000	0.000	0.772	2.916	0.000	0.000
	SCA	6418.000**	20180.000**	1649.000*	2741.000**	0.937*	1.953*	1.010*	1717.300**

	Residual	7553.000***	2555.000***	3071.000***	1686.000***	3.999***	3.794***	1.660***	4872.000***
Leaf mass area (LMA)	GCA_{Males}	0.136	0.147	0.020	0.261	63.730	0.686	238.400	7.650
	$GCA_{Females}$	0.010	0.000	0.036	0.000	0.000	0.000	0.000	0.000
	SCA	0.103*	0.223*	0.353*	0.630*	297.300*	4.030*	1341.000**	31.020*
	Residual	0.245***	0.760***	0.478***	0.498.000***	614.150***	6.143***	405.800***	282.110***
Leaf relative water content (LRWC)	GCA_{Males}	2.370	24.014	4.706	158.064	0.160	0.000	0.510	0.000
	$GCA_{Females}$	5.386	1.871	0.000	2.526	0.000	0.739	0.000	2.155
	SCA	31.640**	21.895*	21.234**	80.957*	4.530*	2.797*	20.685*	9.181*
	Residual	14.077***	88.506	11.316***	64.436***	23.533***	5.426***	26.144***	58.133***
Chlorophyll content (CHL)	GCA_{Males}	3.516	0.000	5.106	0.000	5.582	2.263	9.372	0.376
	$GCA_{Females}$	8.633	7.385	22.660	0.000	11.230	6.775	4.867	13.998*
	SCA	7.748*	32.157*	26.535*	110.140**	12.545	42.169*	53.762*	2.568
	Residual	34.108***	106.695***	42.519***	73.080***	79.552***	61.484***	37.606***	86.040***

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

WW, well-watered; AT, across treatments; CT, control; DS, drought stressed; RE, rewatered; GCA_{Males}*, general combining ability for males;* $GCA_{Females}$*, combining ability for females; SCA, specific combining ability; DT, drought tolerance; DR, drought recovery*

Table 19: General combining ability of parents for the variables measured across watering regimes

Parent (genotype)	Role played	Leaf wilting score (0+5-)	Leaves per plant (#)	Fresh leaf yield (g/plant)	Dry leaf yield (g/plant)	Leaf area (cm^2)	Leaf relative water content (%)	Chlorophyll content (CCI)	Leaf mass area (mg/cm^2)
E11	Female	0.2	0.2	50.3	4.5	18.3	2.0	6.8	8.2
E1	Female	0.0	-0.8	2.5	-0.7	7.0	6.6	5.7	0.9
E7S	Female	-0.1	-1.1	-10.5	-1.4	-7.5	3.9	4.0	-1.0
E2	Female	0.2	2.1	49.3	5.1	14.6	4.0	3.9	-2.6
E10	Female	0.0	7.9	-23.4	-3.2	27.6	6.7	3.8	3.2
E4	Male	0.1	-2.7	-35.2	-2.7	-23.2	-1.4	1.3	5.0
E20	Male	0.1	9.7	0.7	-0.5	-3.9	0.1	1.2	-2.7
E15	Male	0.0	3.2	-1.0	-0.3	-16.5	-0.8	0.7	-2.6
E13	Female	0.0	10.7	31.3	3.0	-2.3	6.7	-0.1	-1.1
E7H	Female	0.0	-1.6	8.9	2.7	24.1	3.2	-0.6	-0.4
E6	Male	-0.2	-10.2	35.4	3.6	43.5	2.1	-3.2	0.3
E3S	Female	0.3	-4.5	-27.5	-1.8	-13.6	3.2	-5.0	-1.9
E3H	Female	-0.4	-12.9	-80.8	-8.3	-68.2	-36.4	-18.4	-5.2

Parental performance is ranked by chlorophyll content. CCI, chlorophyll content index; #, number

Table 20: Specific combining ability of crosses for measured traits across watering regimes

Cross (F1 hybrid)	Leaf wilting score (0+5-)	Leaves per plant (#)	Fresh leaf yield (g/plant)	Dry leaf yield (g/plant)	Leaf area (cm^2)	Leaf relative water content (%)	Chlorophyll content (CCI)	Leaf mass area (mg/cm^2)
E3H x E15	0.3	18.5	73.7	7.7	60.6	35.5	13.1	6.2
E11 x E20	0.1	-3.4	47.2	4.3	-7.3	-4.2	-4.3	-8.0
E7H x E4	0.0	2.3	36.8	3.0	11.9	-5.6	-0.5	-6.5
E13 x E6	0.0	-4.5	23.3	1.3	13.2	-6.0	0.2	0.3
E10 x E4	0.0	0.6	19.1	2.1	-49.0	-4.3	-2.8	3.3
E10 x E15	-0.1	-3.0	17.7	0.9	-52.1	-4.1	-1.9	-2.8
E7S x E20	-0.2	-6.1	16.3	2.1	-9.2	-2.6	-2.8	2.2
E1 x E6	0.0	-3.3	11.4	0.3	37.8	-2.6	-0.6	4.5
E1 x E15	0.1	-4.1	0.1	-0.1	-5.8	-4.1	-1.9	0.3
E3S x E20	-0.1	-1.8	-0.6	-1.1	-24.9	-5.2	-2.4	2.5
E11 x E15	-0.2	-4.4	-1.4	-0.1	13.4	-1.8	-3.3	-8.4
E2 x E15	-0.1	-5.5	-6.8	-0.4	9.4	-4.0	-2.7	1.8
E13 x E15	-0.2	-4.4	-14.5	-0.2	4.9	-3.1	-2.0	1.4
E3S x E6	0.2	0.3	-18.6	-1.9	-28.4	-8.8	-2.7	-2.2
E7H x E6	-0.4	-0.8	-19.1	-1.6	-17.8	-1.5	1.8	1.0
E13 x E20	0.1	5.9	-19.6	-0.9	-35.3	-6.2	-3.8	0.3
E1 x E4	0.0	-3.2	-20.0	-2.1	-14.4	-6.4	-5.8	-7.2
E13 x E4	-0.1	-6.4	-20.5	-3.1	-11.2	-3.6	-2.1	-5.4
E1 x E20	-0.1	1.1	-22.8	-1.1	-46.0	-5.8	0.5	-1.1
E10 x E20	-0.2	-14.9	-24.8	-1.7	123.3	-3.7	-4.2	-2.7
E7S x E15	0.0	-11.5	-31.7	-2.7	15.3	-6.2	-2.9	1.5
E11 x E4	-0.2	-9.4	-33.8	-2.8	16.1	-6.2	-1.4	14.2
E7H x E15	0.3	1.1	-40.4	-4.1	-19.3	-7.0	-6.0	0.2
E3S x E4	-0.1	-9.1	-82.2	-6.5	-12.8	-1.8	-8.8	-4.9

Hybrid performance is ranked by fresh leaf yield. CCI, chlorophyll content index; #, number

6.3.3 Characters for discriminating F1 hybrids for specific combining ability effects

Leaves per plant, fresh leaf yield, dry leaf yield, leaf wilting score and leaf relative water content were identified to be correlated ($p < 0.001$) (Table 21). Dry leaf yield was also positively correlated with leaf area. Thus, leaf area, leaf relative water content, chlorophyll content and leaf mass area were used for choosing best F1 hybrids. In addition, box plots revealed that widest dispersion in leaf area was under WW experiment at 8 WAP (WW 8) and DS treatment under DT experiment at 10 WAP (DT - DS10) followed by control treatment under DT experiment at 10 WAP (DT -

CT10), control treatment under DR experiment at 12 WAP (DR - CT12) and the narrowest spread was observed with RE treatment under DR experiment (DR - RE12) (Figure 13). Also, wider spread in leaf relative water content for DT - DS10 and DT - CT10 than for DR - CT12 and DR - RE12 was observed. Leaf mass area was very wide for DR - RE12 but it was very narrow for rest of treatments. Basing on chlorophyll content, dispersion among F1 hybrids was widest for both DT - DS10 and DR - RE12 followed by DR - CT12, DT - CT10 and WW 8.

Therefore, under optimum growth conditions (WW 8), hybrids were separated for specific combining ability effects using leaf area. Ranking of hybrids' specific combining ability effects for DT was done based on leaf area, leaf relative water content and chlorophyll content. Further, ranking of hybrids' specific combining ability effects for DR was based on leaf mass area and chlorophyll content.

That is to say that under WW 8, E10 x E20 had the best specific combining ability effects on leaf area followed by E3H x E15 while E10 x E4 had the worst effect (Table 22). Basing on leaf area, E10 x E20 had the best specific combining ability effects under DS followed by E1 x E6 while E11 x E4 had the worst effect. Leaf relative water content under DS produced E3H x E15 with the best specific combining ability effects followed by E11 x E15 while E11 x E4 had the worst effect. Basing on chlorophyll content, the best specific combining ability effects under DS were obtained for E7H x E6 followed by E1 x E20 while E11 x E4 performed the worst. When plants were rewatered (resumption of water supply to field capacity, RE) after two weeks of DS exposure, specific combining ability effects for leaf mass area were highest on E11 x E4 followed by E1 x E6 while E11 x E20 had the lowest effect. Basing on chlorophyll content, specific combining

ability (SCA) effects for drought recovery were most favorable for E3H x E15 followed by E7H x E4 while E3S x E20 had the worst SCA effect.

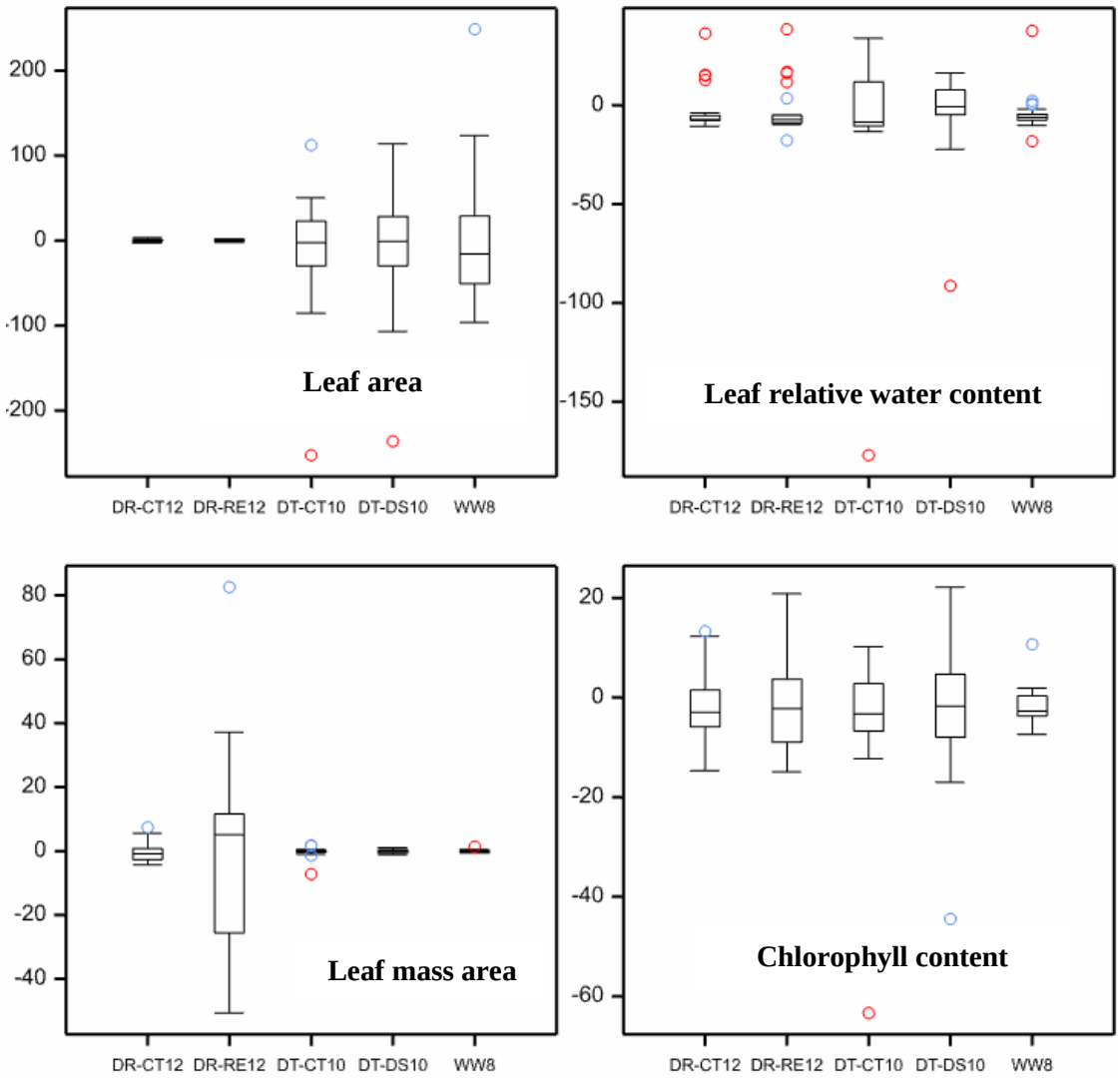


Figure 13: Dispersion in specific combining ability effects of crosses for selected traits at different watering regimes

DR-CT12, control experiment at 12 weeks after planting (WAP); DR-RE12, re-watered experiment at 12 WAP; DT-CT10, control experiment at 10 WAP; DT-DS, drought stressed experiment at 10 WAP; WW8, well-watered experiment at 8 WAP

Table 21: Significance of correlation between different study variables

Trait (variable)		1	2	3	4	5	6	7	8
Leaf wilting score	1	-	<0.001	<0.001	<0.001	<0.001	0.8208	<0.001	<0.001
Leaves per plant	2	-0.4495	-	<0.001	<0.001	0.1426	0.5295	<0.001	<0.001
Fresh leaf yield	3	-0.4263	0.5652	-	<0.001	<0.001	<0.001	<0.001	0.3183
Dry leaf yield	4	-0.4164	0.5116	0.886	-	<0.001	<0.001	<0.001	0.0103
Leaf area	5	-0.1427	-0.0349	0.3966	0.4899	-	<0.001	0.043	<0.001
Leaf mass area	6	-0.0054	0.015	-0.2096	-0.1658	-0.3555	-	<0.001	<0.001
Leaf relative water content	7	-0.4985	0.5417	0.4483	0.4201	0.0481	0.3772	-	<0.001
Chlorophyll content	8	-0.097	0.114	-0.0238	-0.061	-0.2831	0.1827	0.3772	-
		1	2	3	4	5		7	8

Correlation coefficients and probability of correlation-by-chance values are on the bottom left and top right sides of the table, respectively

Table 22: Specific combining ability effects of crosses under WW, DS and RE treatments

Cross (FI hybrid)	LA		LRWC	CHL		LMA
	WW 8	DT - DS10	DT - DS10	DT - DS10	DR - RE12	DR - RE12
E3H x E15	123.6	47.2	16.4	7.3	20.9	22.2
E11 x E15	26.7	-8.1	13.1	6.1	-14.1	-49.6
E1 x E4	-39.9	1.3	11.3	5.2	-12.3	-33.8
E13 x E15	10.5	-18.7	9.0	4.2	-9.4	5.3
E1 x E6	72.8	80.0	8.7	-2.3	-11.8	37.2
E7S x E20	-14.1	-27.0	8.4	-3.0	-14.8	11.6
E7H x E6	-61.7	33.4	7.5	22.2	3.7	7.4
E10 x E20	248.6	113.9	7.2	4.1	-8.2	-20.3
E7H x E4	31.0	26.0	0.4	3.0	14.9	-30.9
E7H x E15	-35.7	16.0	0.3	-17.0	10.0	10.7
E13 x E4	-24.8	-11.0	-0.2	-7.2	-7.5	-34.9
E2 x E15	25.8	-4.8	-0.2	-4.0	-4.0	9.3
E1 x E15	-9.0	3.3	-1.1	-6.4	-4.8	11.7
E10 x E4	-96.3	-67.1	-1.2	-10.5	0.3	21.4
E11 x E20	-18.9	-2.9	-1.4	-13.7	3.7	-50.7
E1 x E20	-84.2	-32.7	-2.2	15.0	0.6	4.4
E10 x E15	-89.4	-60.0	-2.6	3.8	-8.5	-18.8
E3S x E6	-30.8	-107.1	-4.2	-11.3	6.3	-10.3
E3S x E4	-17.3	51.7	-5.1	8.1	-0.5	-34.9
E7S x E15	46.0	6.9	-7.8	-1.2	1.8	6.3
E13 x E20	-62.0	-54.6	-10.7	-8.7	0.1	4.2
E3S x E20	-62.0	6.6	-14.7	-4.2	-14.9	15.4
E13 x E6	15.9	30.8	-22.3	0.3	3.8	4.9
E11 x E4	55.0	-236.1	-91.4	-44.5	-6.0	82.6

Hybrid SCA effects are ranked by LRWC. CHL, chlorophyll content in CCI (chlorophyll content index); LMA, leaf mass area (mg/cm^2); LA, leaf area (cm^2); LRWC, leaf relative water content (percentage, %); DR - RE1; rewatered plants at 12 WAP; DT - DS10, drought-stressed plants at 10 WAP; WW 8, well-watered plants at 8 WAP

6.4 Discussion

Significance of general combining ability effects for chlorophyll content among females rather than males indicate maternal inheritance (Lester and Thitai, 1989; Meier, 2011; Day, n.d.). After all, chloroplast organelles are found in cytoplasm, and chloroplasts contain deoxyribonucleic acid (Ohmiya *et al*., 2014). North Carolina II mating designs do not involve reciprocals (Stuber, 1980; Nduwumuremyi *et al*., 2013). As a follow-up, a diallel analysis of drought resistance in Shum is necessary in order to explore the veracity of maternal effects for chlorophyll content (CHL) as well as other traits recommends a diallel analysis (Griffing, 1956; Stuber, 1980). Nevertheless, genotypes having high CHL are healthier than those with low CHL. E11 (female parent) had the highest and positive general combining ability effects, which implies that the genotype to bring about improved crop heath and photosynthetic efficiency (Parry *et al*., 2014). On the other hand, E3H (female parent) had the worst general combining ability effects for chlorophyll content. Besides leaf wilting score (LWS), positive general combining ability effects for leaves per plant, fresh leaf yield / dry leaf yield, leaf area, leaf relative water content, chlorophyll content and leaf mass area indicate that such parental material are useful in improving for particular traits. Males which reduced LWS across watering regimes were E6 and E15. For female parents, E3H, E7S, E1, E10, E13 and E7H reduced the leaf wilting in hybrids.

Generally, specific combining ability effects were significant while general combining ability effects were mainly not significant for most variables measured. The results point to prevalence of dominance rather than additive gene actions (Falconer and Mackay, 1996; Golparvar, 2012) whilst influencing phenotypic expressions in Shum. As a matter of emphasis, broad sense heritability was favorable and above 80% for all but leaf mass area. In spite of high narrow sense heritability at

over 50% for leaves per plant, chlorophyll content and leaf yield; supportive of use of breeding method(s) for a self-pollinated crop (Kang, 2002), a principal approach should ideally be cross breeding for high gains in drought tolerance (DT) and DT improvement objectives (Blum, 2005; Sharma *et al*., 2015; Amelework *et al*., 2015; Banik *et al*., 2016). Across watering environments, best performing F1 hybrid on basis of positive specific combining ability effects for fresh leaf yield was E3H x E15 whereas the worst was E3S x E4. It is necessary to study produce ability of F1 hybrids of Shum for the benefit of profit-making seed companies and ultimately for smallholder farmers.

Highly correlated traits offer prospect to decide on genotypes basing on their combining ability effects under particular watering regimes. For example, since leaves per plant (number of green leaves per plant), fresh leaf yield, dry leaf yield, leaf wilting score and leaf relative water content are correlated to one other in a favorable way, only leaf relative water content was chosen. Expressly, F1 hybrids with high number of green leaves per plant, fresh leaf yield, dry leaf yield and low leaf wilting score (low leaf wilting score means slight wilting or no wilting at all), also had high leaf relative water content. Leaf relative water content is a known indicator for DT in Shum (Sseremba *et al*., 2018a). It was also known that dry leaf yield is positively correlated with leaf area. Chlorophyll content and leaf mass area did not demonstrate strong correlation with other variables. In order to explore diversity for specific combining ability effects under DS in Shum, this study recommends using leaf area, leaf relative water content, chlorophyll content and leaf mass area as selection criteria. The traits have been demonstrated to be reliable in selecting for DT in Shum (Sseremba *et al*., 2018a) and other *Solanum* spp. like tomato and potato (Tuberosa *et al*., 2007; Galmés *et al*., 2013; Ramírez *et al*., 2014; Banik *et al*., 2016).

Observed spread in data for each candidate/prospective trait among watering environments implies that such trait(s) is/are suited in specific ways when discriminating specific combining ability effects of F1 hybrids (crosses) under drought, as similarly reported by Sseremba *et al*. (2018a) when selecting for superiorly stable accessions under water deficit stress (Sseremba *et al*., 2018a). Leaf area is appropriate under both WW and DS environments. Leaf relative water content and leaf mass area are rather appropriate for DS and DR screening, respectively; when analyzing for specific combining ability effects. However, chlorophyll content is appropriate for both drought stress (DS) and drought recovery (DR) screening. Thus, basing on leaf area under well-watered, leaf relative water content under drought stress, chlorophyll content under recovery (re-watering after DS episode) and leaf mass area under drought recovery, the best performing F1 hybrids (crosses) as far as specific combining ability effects in studied Shum germplasm are concerned were E10 x E20, E3H x E15, E3H x E15 and E11 x E4, respectively. Worst performing hybrids for leaf area under well-watered, leaf relative water content under drought stress and chlorophyll content under drought recovery were E10 x E4, E11 x E4 and E3S x E20, respectively.

6.5 Conclusion

Across watering environments, all the study variables (traits) had high H^2 though it was only number of green leaves per plant (LPP), chlorophyll content (CHL) and leaf yield (LYF/LYD) with high h^2. Aside general combining ability effects of females for chlorophyll content (CHL), principal combining ability in Shum was identified to be the specific combining ability. Predominance of specific combining ability other than general combining ability effects indicate potential for hybrid vigor in Shum both under normal and drought stress conditions of crop growth; this offers opportunity to improve the crop's productivity. Moreso, variables/traits that are considered appropriate for selecting of F1 hybrids (crosses) basing on specific combining ability

effects under given water supply levels include leaf area (for WW and DS), leaf relative water content (for DS), leaf mass area (for DR) and chlorophyll content (for both DS and DR).

Female E11 had the best general combining ability for chlorophyll content. Males E6 / E15, E20 / E15, E6, E6, E6 / E20, E4 / E20 / E15, and E4 / E6 also had favorable GCA effects for LWS, LPP, leaf yield (LYF and LYD), LA, LRWC, CHL, and LMA, respectively. Females with favorable GCA effects were identified as E3H / E7S / E1 / E10, E13 / E10 / E2 / E11, E2 / E13 / E7H, E10 / E7H / E11 / E2, E10 / E13 / E1 / E2, E11 / E1 / E7S / E2 / E10, and E11 / E10 / E1 for LPP, leaf yield (LYF and LYD), LA, LRWC, CHL, and LMA, respectively. Crosses with best specific combining ability effects were identified as E10 x E20 (for leaf area under well watering), E3H x E15 (for fresh leaf yield across watering regimes, for leaf relative water content under DS and for chlorophyll content under DR) and E11 x E4 (for leaf mass area under DR). Undoubtedly, water deficit stress had direct negative consequences on productivity of Shum (as other leafy vegetables) but the findings from this study present remedial breeding options for sustainable production amidst fluctuations in soil moisture.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

This research provided vital information for fostering an improvement of drought tolerance in Shum group for increased crop productivity. The scientific hypotheses set out at the thesis research inception were answered by the findings in the affirmative as follows:

i. The Shum cannot be confused with its progenitor (*Solanum anguivi*, SAN) based on morphological attributes as a guarantee to proper identification. A null hypothesis (H_o) that Shum and SAN are morphologically indistinct is rejected. It implies that morphological markers are useful in germplasm discrimination of such research-neglected crop species. It was revealed that days to germination and emergence (DG), cotyledonous leaf blade length (CLBL), cotyledonous leaf blade width (CLBW), dry shoot biomass (SBD), dry root weight (RWD), plant height (PH), petiole length (PEL), date to first flower appearance (FLW), plant canopy width (PW), plant branching (PB), and number of leaves per plant (LPP) are the major drivers of variability in the study accessions. The discriminant function: $Group = 0.0586CLBL - 0.26CLBW + 0.63DG + 0.073FLW + 0.063LPP + 0.031RWD + 0.02SBD$ can be relied on to discern between Shum and SAN. For any two accessions being discerned, the value from the equation is higher for SAN than for Shum. Further, the mean values for flowering time, LPP, PB and PH were more favorable for Shum than its wild progenitor accessions; suggesting a positive evolution for desired traits.

ii. There exists within-group selectable variability among Shum germplasm. The H_o that there exists no selectable variation among Shum germplasm is rejected. It implies that the measured

morphological variables during the study can be relied on to discern among genotypes. Such variables as petiole length (PEL), sepal length (SEL) (or seed color, SC), fruit calyx length (FCL), number of seeds per fruit (SPF), leaf fresh weight (LWF) (or number of leaves per plant, LPP), fresh leaf yield (LYF), visual seedling vigor (VSV), number of fruits per plant (FPP), fresh harvest index (HIF) (or dry harvest index, HID) and plant growth habit (PGH) were polymorphic among the study accessions. The differences among Shum germplasm can be detected at various stages; seedling, vegetative and reproductive. Such diversity will boost chances of effective selection for desired traits, while the discriminant traits are potential morphological markers within Shum for a low-cost germplasm characterization approach.

iii. Accessions of Shum group differ in drought tolerance. The H_o that there is no difference in reaction to drought stress among Shum accessions is rejected. At least two of the study accessions differed in response to drought stress. The most important parameter in separating accessions for drought tolerance was identified as leaf relative water content (LRWC) followed by plant height (PH) and number of green leaves per plant (LPP). Based on LRWC, accessions having relatively high tolerance to drought were found to be E6, E12, E15, E18 and E14. Conversely, drought susceptible accessions were identified as E7, E17, E3 and E9. The broad sense heritability of drought tolerance for the measured traits was also very high at ≥90% with potential response to selection of up to 70%. Overall, it is concluded that although drought stress negatively affects performance of *S. aethiopicum* Shum, superior genotypes can be selected from the observed variation.

iv. Shum genotypes vary in their combining ability for drought tolerance. The H_o that Shum accessions do not vary in their combining ability for drought tolerance is rejected. Dominance gene action was exhibited more than additive gene action in Shum. Nonetheless, GCA effects

were significant for chlorophyll content (CHL). Basing on CHL, female E11 was the best combiner (for additive gene action) which suggests such accession is useful when breeding for increased plant health and photosynthetic efficiency; while E3H was the worst. Other parents with relatively good GCA effects for leaf wilting score (LWS), LPP, leaf yield, leaf area (LA), LRWC, CHL and leaf mass area (LMA) were identified as E3H, E13, E11, E6, E10, E11 and E11, respectively. Second options as parents (still based on GCA effects) for improvement in LWS, LPP, leaf yield, LA, LRWC, CHL and LMA include E6, E20, E2, E10, E13, E1 and E4, respectively. For SCA effects, the following conclusions are made:

- Leaf area (LA) showed suitability as selection criterion for best combiners under well-watered (WW) and drought stress (DS) treatments.
- Leaf relative water content (LRWC) served best in separating SCA effects of crosses under DS.
- Chlorophyll content (CHL) produced clear separations of specific combining ability (SCA) effects under both drought stress (DS) and drought recovery (DR).
- Basing on leaf area (LA) under WW, leaf relative water content under DS and chlorophyll content under DR, the best performing F1 hybrids (crosses) for specific combining ability effects were E10 x E20, E3H x E15 and E3H x E15, respectively.
- The worst performers for leaf area under well-watered (WW), leaf relative water content under DS and chlorophyll content under DR were E10 x E4, E11 x E4 and E3S x E20, respectively.

7.2 Recommendations

Follow-up field studies to corroborate screen house findings are necessary. Specifically, the following recommendations are made from this thesis research (organized per study conducted):

Study 1: Ascertaining the genetic distinctiveness between Shum and its progenitor (*S. anquivi*);

- For any two accessions being discerned, the value from the discriminant function (hereafter provided) is higher for *S. anguivi* than for Shum.
- The discriminant function: $Group = 0.0586CLBL - 0.26CLBW + 0.63DG + 0.073FLW + 0.063LPP + 0.031RWD + 0.02SBD$ can be relied on to discern between SHUM and SAN; where CLBL, CBLW, DG, FLW, LPP, RWD and SBD refer to means for cotyledonous leaf blade length, cotyledonous leaf blade width, days to germination and emergence, days to first flower appearance, number of leaves per plant, dry root biomass and dry shoot biomass, respectively.
- Days to germination and emergence should be applied in distinguishing between *S. aethiopicum* Shum group from its progenitor, *S. anguivi*.
- Whereas morphological markers provide a good distinction between the Shum and *S. anguivi* accessions, a follow-up validation study with molecular markers could be complementary.

Study 2: Assessing the genetic diversity within *S. aethiopicum* Shum group;

- *Solanum aethiopicum* Shum being a research-neglected subspecies with scantly available genomic resources, use of morphological markers (at both vegetative and reproductive stages) can suffice to enable kick-starting varietal improvement programs for the crop.
- A follow-up molecular diversity analysis is necessary to support the findings from this morphological analysis.

Study 3: Identifying parental material for development of drought tolerant Shum varieties;

- Leaf relative water content (LRWC), plant height (PH) and number of leaves per plant (LPP) are recommended as variables for use when selecting for drought in Shum. The highest preference should be on use of LRWC followed by PH and then LPP. Root traits were not considered in this study; and a follow-up detailed research where leaf, shoot and root attributes (gene expression, biochemical, physiological and morphological parameters) are measured is recommended.
- Accessions 160 (E6), 145 (E12), 137 (E15), 108P (E18) and 184G (E14) are recommended as sources of drought tolerance (and accessions 163G (E7), 141 (E17), 163 (E3) and 108 (E9) are drought susceptible) though a follow-up gene expression analysis can help in corroboration of the findings.

Study 4: determining the combining ability of selected Shum group germplasm for drought tolerance;

- It is recommended to exploit possibilities for hybrid variety development since dominance gene action was the overriding source of variation among crosses.
- Studies into heterosis for leaf yield, seed yield and profitability of hybrid seed production and use by farmers should be conducted.
- Parent E11 is recommend for use in developing healthy plants with large leaf size.
- Hybrids E3H x E15 and E10 x E20 are potential hybrids in terms of vigor for leaf size, leaf turgidity and general plant health under normal as well as drought stress situations.

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