CONDITIONS FOR OPTIMUM GERMINATION OF SPRAWLING BAUHINIA SEED (*Tylosema fassoglense)* (Kotschy ex Schweinf.) Torre & Hillc

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DECLARATION

I certify that this thesis is my original work and has not been submitted for award of a degree or certificate in any other university.

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DEDICATION

This work is dedicated to my family, friends and colleagues whose support and encouragement ensured its success.

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List of Acronyms

AEZ	Agro-ecological Zones
ANOVA	Analysis of Variance
ASALs	Semi-Arid and Arid Lands
DNA	Deoxyribonucleic Acid
eRH	Equilibrium Relative Humidity
FAO	Food and Agriculture Organisation of the United Nations
IPCC	Inter-governmental Panel on Climate Change
IPGRI	International Plant Genetic Research Institute
ISTA	International Seed Testing Association
KEFRI	Kenya Forestry Research Institute
MC	Moisture Content
MSB-P	Millennium Seed Bank Partnership
NUS	Neglected and Underutilised Species
RH	Relative Humidity
RBG Kew	Royal Botanic Gardens, Kew
SDGs	Sustainable Development Goals
SSA	Sub Saharan Africa
UPP	Useful Plants Project
UN-DESA	United Nations Department of Economic and Social Affairs
UN	United Nations

GENERAL ABSTRACT

Understanding seed biology and germination ecology of plants is critical for domestication of neglected and underutilized species (NUS). However, this information is not readily available for *Tylosema fassoglense* [Family: Fabaceae]; despite its potential as a future crop. This research is primarily intended to benefit small-holder farmers of Kenya, by improving nutrition and enhancing sustainable agriculture, while contributing to good health and well-being. The main objective of this study was to evaluate the seed germination requirements of *T. fassoglense* by determining dormancy type, the effects of light, temperature and water potential on germination of scarified seeds as well as assessing the correlations among seed functional traits. Seed lots of *T. fassoglense* were collected from Busia, Migori and Siaya counties in Kenya. Seed dormancy was determined by water imbibition rates and germination tests on scarified and non-scarified. Scarified seeds were germinated under 12/12 plus 0/24 hour photoperiod as well as over a range of temperatures from 10 to 45 °C with intervals of 5 °C and at varied water potentials; 0, -0.25, -0.5, -1.0, -1.5 Megapascal (MPa). Water imbibition rates (g), germination percentages as well seed mass, oil and morphology were measured. Percentage data were normalized by arcsine transformation, subjected to analysis of variance (ANOVA) and the means separated with Tukey's HSD ($p<0.05$) using SAS Version 2002-2003 statistical software. Scarification significantly ($p<0.05$) improved water uptake by 4 to 22 times as well as germination percentage from 30% to 90% (Migori seed lot) and from 70% to ≥90% (both Busia and Siaya seed lots). Seeds germinated well under light/dark or dark photoperiods while relative light germination index (RLG) ranged from 0.46 to 0.57. Seed germination was significantly ($p<0.05$) reduced at 10 °C and 40 °C whilst germination was zero at 45 °C in all the seed lots. The calculated base, optimum and ceiling temperatures (T_b, T_o and T_c; 50th percentile) ranges were 4.05-8.0 °C, 33.61-35.75 °C and 46.54-47.24 °C respectively while thermal time ($\theta_{T(50)}$) suboptimal ranged from 76.19 to 89.02 degree Celsius days (°C d). Low water potential of -0.5 MPa significantly ($p<0.05$) reduced final germination to less than 50% in all seed lots while germination was zero at both -1.0 and -1.5 MPa. The base water potential (Ψ_b) and hydro time ($\theta_{H(20)}$) ranges was -0.92 to -0.97 MPa and 3.95-

4.11 Megapascal days (MPa d) respectively. Siaya seed lot were significantly ($p<0.05$) heavier than the other lots. Negatively significant ($p<0.05$) correlations were observed between T_b and θ_T; Ψ_b and θ_T while non-significant correlations were observed between germination and physical traits. Scarification improved water imbibition as well as final germination percentage and seeds of *T. fassoglense* are probably neutral photoblastic. The optimum temperature range for germination was 30-35 °C while seed germination was tolerant to low water potential up to -0.5 MPa. There were interrelationships among seed functional traits of *T. fassoglense*. The findings of this study will be useful in future research contributing towards the domestication of *T. fassoglense* as a future crop to enhance a sustainable agriculture and nutrition in Kenya and potentially worldwide.

Keywords: dormancy; germination; light; potential crop; physical traits; temperature; *Tylosema fassoglense;* water potential

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The current food security is threatened by rising population, coupled with climate change impacts such as droughts, heat waves and floods (Lal, 2013; Wheeler and Von Braun, 2013; Rosenzweig *et al.,* 2014; Valin *et al.,* 2014; UN DESA, 2015). Nowhere is this challenge more formidable than in the sub-Saharan Africa (SSA) countries where population growth rates are among the highest in the world while basic infrastructure is inadequate and fertilisers are extremely costly (Cordell *et al.,* 2009). To address food security and mitigate climate change effects, there is need to adopt climate-resilient, low-input crops for SSA countries.

Adapting mainstream crops to climate change phenomena involves conventional crossing and selection approaches, sometimes aided by biotechnological as well as genomic means, or through crossing with old landraces and crop wild relatives (CWRs) (Lopes *et al.,* 2015; Castañeda-Álvarez *et al*., 2016; Kissoudis *et al.,* 2016). However, mainstream crops are adapted to relatively favourable moisture and nutrient conditions, which can be improved incrementally by breeding but not to the extent required to be productive in harsh, infertile environments in the marginal production areas (Figure 1.1). Therefore, adoption of neglected and underutilised legume crops such as *Tylosema fassoglense, Vigna subterranea, V. unguculata,* is a more promising approach to sustainable agriculture, since they are nutritionally rich, highly adapted to severe climates and capable of growing in infertile soils (Padulosi *et al.,* 2013).

The family Fabaceae to which cultivated legumes belong, exhibits great diversity in terms of distribution, life form (liana, shrub, tree) and morphological variation in seed size, shape as well as dormancy (Baskin and Baskin, 2014). Despite cultivated grain legumes being second to cereals as major plant-protein sources on a world scale, their production does not meet the ever increasing demand (FAO Statistics, 2016). *Tylosema fassoglense* is a nutritionally rich neglected and underutilized grain legume for Africa. Its drought-tolerance (Brink, 2006) and excellent nutrient content comparable to commercially cultivated crops (soybean, peanut, common bean), has the potential to provide sustainable food and feed in the future (Otieno *et al.,* 2015). The seeds of *T. fassoglense* are nutritionally rich in protein (30-40%), lipids (24-40%), minerals and starch (Dubois *et al.,* 1995; Okumu, 2011; Otieno *et al.,* 2015), therefore can contribute towards the country's protein demand partially or completely substitute other animal proteins in the household’s diet. Most legume plants also fix atmospheric nitrogen allowing them to colonise diverse habitats of poor soils and when intercropped with food crops provides reprieve to farmers on nitrogen fertilizer application (Graham and Vance, 2003). Wild plants also display diverse adaptive traits to their habitat that permit seed germination and seedling establishment under unpredictable conditions unlike crops that are adapted to favourable environments (Dürr *et al*., 2015; Castillo-Lorenzo *et* al., 2019b).

Despite, a number of plants including legumes having been domesticated several decades ago, a large proportion with great socioeconomic importance still remain in the wild with unrealized potential (Ulian *et al.*, 2017). The lack of sufficient knowledge on how to propagate these wild species also hampers their utilisation in livelihood and conservation programmes (Rodríguez-Arévalo *et al.,* 2017). Seed collecting and tuber extraction of *T. fassoglense* from the wild is not only unsustainable but also hampers natural regeneration and negatively impacts on natural populations. The understanding of seed functional traits of this neglected and underutilised grain legume is important for its domestication, conservation and sustainable utilization through propagation for cultivation and reintroduction to their natural habitat. Furthermore, the wide distribution of *T. fassoglense* provides an opportunity for diversity due to genetics, environment and interactions between these two factors resulting in variation of seed germination, genetic and seed composition traits which are essential for selecting suitable germplasm for future breeding and promotion. However, despite the immense potential for adoption and cultivation in marginal production areas, *T. fassoglense* is still in the wild. Therefore, there is a need for intensive agronomic research to bring it into cultivation and to utilize its potential as a cash crop.

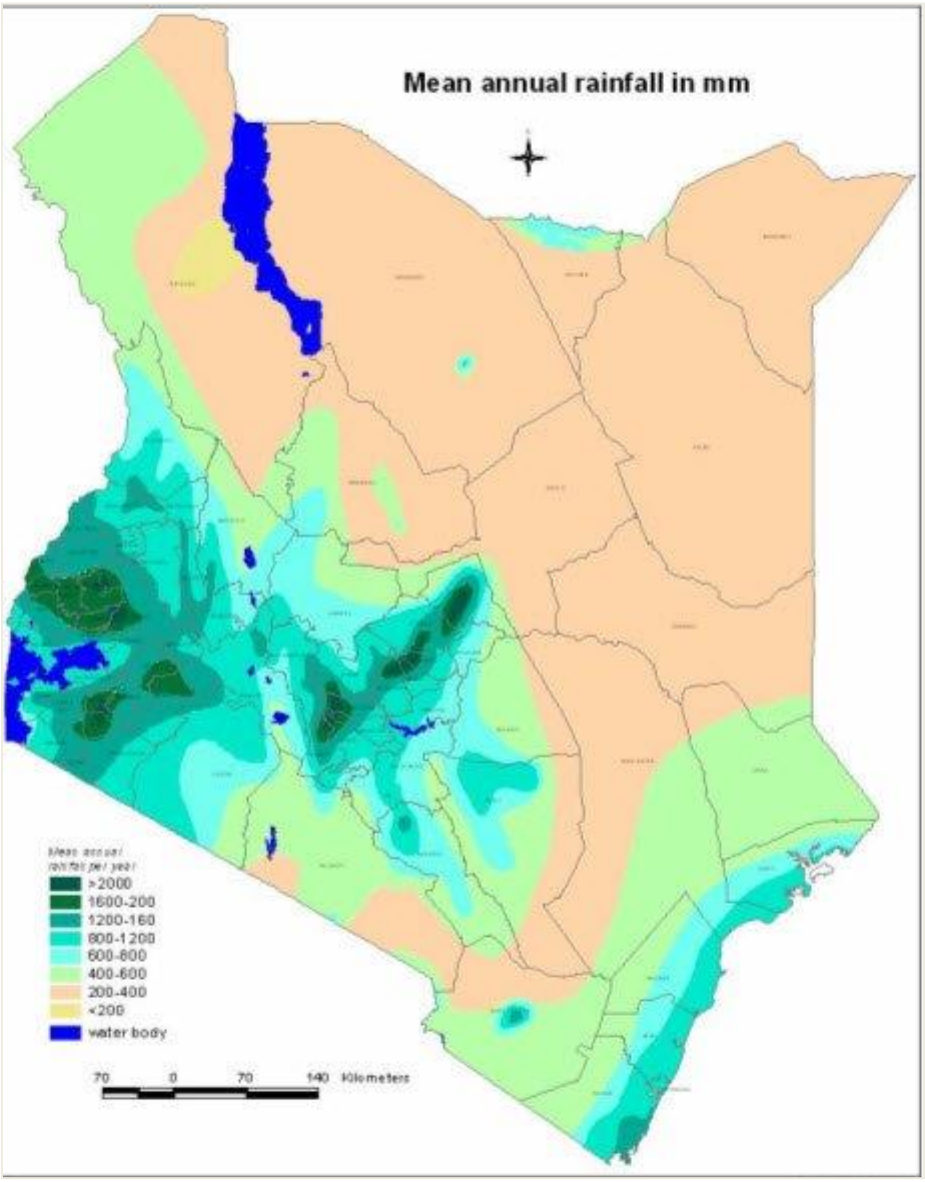

Figure 1:1: Map of agroecological zones, AEZ of Kenya (Source: FAO System: Infonet Biovision Home)

1.2 Problem Statement

Research on *T. fassoglense* has focussed on its economic importance and utilisation with little emphasis on its propagation. The protein content in *T. fassoglense* seed is comparable to soybean while oil content is more than that in peanut (Dubois *et al.*, 1995; Okumu, 2011; Otieno *et al.*, 2015). The species is adapted to drylands of sub-Saharan Africa, SSA (Castro *et al.*, 2005; Sinou *et al.*, 2009). However, information on seed germination and seedling establishment of *T. fassoglense* that is important in its regeneration and propagation has not been generated. Therefore, understanding seed biology and germination ecology of *T. fassoglense* is crucial in its production for consumption and commercialisation. Furthermore, "true seed" is the most preferred planting material under crop production over vegetative material due to ease of storage and as a commodity for trade.

Physical dormancy, PY which is simply the presence of water impermeable seed/fruit coat is an adaptive trait found in 18 angiosperm plant families including Fabaceae (Baskin and Baskin, 2014). Breeding (selection) has eliminated dormancy in cultivated grain legumes, however it still remains a challenge in propagating wild species (Bewley and Black, 1994; Cheng and Bradford 1999). While PY has been reported in *T. esculentum* (Fabaceae) (Travlos *et al.*, 2007) seed dormancy and germination of the other four species in this genus including *T. fassoglense* seeds has not been documented. It has therefore been difficult to promote this nutritious grain legume species for adoption by small-holder farmers in Kenya.

Seed physical traits (mass and shape) have been used in predicting light requirements for germination, soil seed bank formation and dispersal (Baskin and Baskin, 2014). Seed sowing depth is often related to crop emergence, however light filtering through canopy and burial under leaf litter or cracks in the soil affects species regeneration ability in their natural habitat (Fenner, 1991). Baskin and Baskin, (2014) proposes three groups of plants depending on their light requirement for germination: positive photoblastic (those that require light), negative photoblastic (those that don't require light) and neutral photoblastic (those insensitive to light). Under cultivation crop seeds are buried in the soil, however germination ecology of *T. fassoglense* in relation to light requirement remains unknown despite its potential as a future crop.

Tylosema fassoglense a NUS growing in the wild have adaptive mechanisms displayed by varied seed functional traits that interact with the environmental factors to ensure regeneration and survival within a habitat (Dürr *et al.*, 2015). Therefore, knowledge of germination traits (hydro and thermal thresholds) is important for predicting its environmental limits (Ghaderi-Far *et al.*, 2010) and formulating adoption strategies (Hardegree *et al.*, 2016). Seed vigour, as a descriptor of regeneration potential (ISTA, 2017) can also be inferred from the germination traits; that is germination rate, thermal as well as hydro times quantified by characterisation of the germination-physiological processes. This important information to guide the domestication and conservation of *T. fassoglense* has not been generated.

Seed maternal environment has been shown to influence seed physical traits (i.e. mass, size, composition) which are often correlated to germination parameters (i.e. hydro and thermal thresholds) (Norden *et al.,* 2009; Dürr *et al.*, 2015; Gardarin *et al.,* 2016; Castillo-Lorenzo *et al*., 2019a, b). The understanding of correlations between seed physical and germination traits and interrelationships among germination traits is not only important for germplasm sourcing and future breeding but also the environmental range for adoption (Ghaderi-Far *et al.,* 2010; Dürr *et al*., 2015). However, literature on interrelationships amongst seed functional traits for *T. fassoglense* has not been generated. The current research aimed at determining the type of seed dormancy and effects of light, temperature and water potential on germination as well as assessing the interrelationships among seed traits of *T. fassoglense*.

1.3 Justification

The major challenge to the exponential human population growth is malnutrition which has created two spectra of populations, calorie deficient (hungry) and calorie excess (obese). For example the undernourished population in Kenya is estimated at 10 million (IFAD FAO and UNICEF, 2017). The need to address food insecurity through conversion of forest land to increase conventional crop production has resulted in biodiversity loss. Furthermore, about two thirds of Kenya's landmass is unarable and support wild life or livestock production. Therefore, there is need to produce nutritious foods sustainably while protecting the environment.

During plant domestication (7,000-12,000 years ago) several species were cultivated, however less than 200 have been extensively cultivated leading to narrow genetic diversity (Padulosi *et al.,* 2013). Currently, rice, wheat and maize provide about 40% of the world's calorie intake (Ulian *et al.,* 2020). Intensification of agriculture during the "Green Revolution of 1960s to 1980s" resulted in unforeseen challenges such as land degradation, environmental pollution, increased pests and diseases as well as decreased dietary diversity and neglect of traditional food crops (Webb and Eiselen, 2009 in Ulian *et al.,* 2020). However, there is currently increased recognition of neglected and underutilized species of plants to improve agrobiodiversity, livelihoods as well as sustainable agriculture and food security (Ulian *et al*., 2020).

Tylosema fassoglense is a neglected drought-tolerant grain legume native to ASALs of sub-Saharan Africa (Castro *et al*., 2005; Brink, 2006). It produces seeds rich in protein (30-45%) and oil (24-40%) as well as carbohydrate-rich tubers with potential as a source of food and feed (Dubois *et al.,* 1995; Okumu, 2011; Otieno *et al.,* 2015). Its' root tubers are highly valued for medicinal properties (Adongo *et al.,* 2012; Maundu *et al.,* 1999), which threatens its natural population. Therefore adopting *T. fassoglense* into mainstream agriculture has the potential to contribute towards agrobiodiversity, food security, ensure sustainable agriculture and reduce exposure to climate change impacts which supports the Government of Kenya, Big 4 Agenda and United Nations Sustainable Development Goals, (UN SDGs) of 2015. In addition, its cultivation will increase seed production, provide an opportunity to understand harvest maturity of its starch-rich tuber which will also support conservation and sustainable utilization of germplasm resources according to the Convention on Biological Diversity **(CBD)**.

There is need to create awareness on the potential of *T. fassoglense* to public institutions, farmers, non-governmental organisations (NGOs) as well as other organisations engaged in crop development and germplasm conservation, however there is no literature on seed biology and germination ecology to guide its propagation. To contribute to promotion and adoption of *T. fassoglense* as a climate-resilient, low input grain legume by resource-poor farmers in Kenya, the study envisaged to generate information on the species domestication and conservation through determination of seed dormancy type, light requirement for germination; to characterise seed germination response to temperature and water potential as well as to examine the interrelationships among seed functional traits of *T. fassoglense* with a view to determine conditions for optimal seed germination. The knowledge generated in this study will help Government institutions, NGOs, farmers and genebank managers in domestication, conservation and germplasm testing of *T. fassoglense*.

1.5 Main Objective

To determine optimal germination conditions of *Tylosema fassoglense* for enhanced adoption as a climate-resilient, low input grain legume by small-holder farmers in Kenya.

1.5.1 Specific objectives

This study was designed with the following specific objectives:

1. To evaluate seed dormancy condition and role of light on germination of scarified seeds of *T. fassoglense*
2. To determine the effects of temperature and water potential on germination of scarified *T. fassoglense* seed
3. To assess interrelationships among seed functional (physical and germination) traits of *T. fassoglense*

1.5.2 Hypotheses

1. *Tylosema fassoglense* seeds are dormant while germination is not influenced by light
2. The optimum temperature for germination of *T. fassoglense* seeds is between 25°C and 30°C while germination process have a high tolerance to low water potentials
3. There are correlations amongst seed functional traits of *T. fassoglense*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.1 Taxonomy and botany of *Tylosema fassoglense*

The family Fabaceae is the third largest of flowering plants, with three subfamilies; Caesalpiniodeae, Mimosoideae and Papillionoideae (cultivated legumes), 800 genera and about 20,000 species (Baskin and Baskin, 2014). The Fabaceae family has a characteristic flower structure that produce fruit pods which are consumed with the seed when immature and the seeds are also consumed after maturation when they are dry (Maundu *et al.,* 1999). *Tylosema fassoglense* (Kotschy ex Schweinf.) Torre & Hillc. (sprawling bauhinia) is a wild perennial tuberous vine belonging to the tribe Cercidea in the subfamily Caesalpiniodeae. *Tylosema* was included in the genus *Bauhinia* before a taxonomic review was conducted (Castro *et al*., 2005). The advancement in molecular genetics has enabled reorganisation of the Cercideae tribe and recognition of *Tylosema* as a distinct genus (Hao *et al.,* 2003; Wunderlin, 2010). The other four species in this genus are *T. angolense. T. argenteum T. esculentum,* and *T. humifusum. Tylosema fassoglense* is locally known as Ivole (Kamba), Wanga, Imbasa (Luhya), Kumuchayu, Kamachayu, Chingayu (Luhya-Tachoni), Chingaayu (Luhya-Maragoli), Ombasa (Luo), Esinkarua (Maasai), Bassac (Somali), Dalamboi (Samburu). *Tylosema fassoglense* and *T. esculentum* have been extensively researched on their potential economic uses.

Tylosema fassoglense is a liana and sometimes a creeping plant depending on the habitat with several stems that grow in every direction up to 6 m long (Castro *et al.,* 2005; Brink, 2006). The plant produces large tubers which can weigh up to 70 kg as shown in Figure 2:1 (Brink, 2006) and therefore requires wider spacing for cultivation than most of other legumes. The tuber acts as a water reservoir for the plant during prolonged drought and can store up to 90% water during the rainy season (Brink, 2006). *Tylosema spp.* are heterostylous, therefore self-incompatible due to existence of pin/thrum stigma and anther (Hartley *et al.,* 2002). The existence of heterostyly and distribution of the different morphs in a population may hinder its propagation and production. The chromosome count for this species is 2n=52 (Goldblatt and Davidse, 1977). There is no phenological and cultivation data currently on *T. fassoglense,* however successful cultivation of *T. esculentum* was conducted in Australia, Israel, Kenya and South Africa (Brink, 2006). Information on yield is not readily available, while each pod bears one or two large seeds.

Figure 2:1: ***Tylosema fassoglense*** **root tuber partially excavated (Photo: V. Otieno)**

Figure 2:2: ***Tylosema fassoglense*** **flowers (Left) and seeds (Right) (Photo: V. Otieno)**

2.1.2 Ecological distribution

Tylosema fassoglense is endemic to the ASALs of Sub Saharan Africa (Castro *et al.,* 2005; Sinou *et al.,* 2009) and has a wide distribution across East Africa from Ethiopia to Mozambique, and in Angola (Lebrun and Stork, 2008). In Kenya, it is common in dry hot areas such as Mtito Andei, Busia, Homabay and Siaya, especially in dry Acacia, Adansonia, Commiphora or scattered-tree bushland. The species prefers sandy to deep loam or coastal soils as well as loose red clay-loam soil (Maundu *et al.,* 1999).

2.1.3 Traditional uses

Tylosema fassoglense has been wild-harvested by indigenous communities of Kenya, South Africa, Zambia, Zaire, and Congo for edible seeds and medicine for centuries (Maundu *et al.,* 1999; Brink, 2006). The roasted seeds of *T. fassoglense* are used as food and beverage, leaves for livestock fodder while pieces of root tuber are often traded as medicine in Kenya (Maundu *et al.,* 1999). Stems are used as cordage by some communities (Maundu *et al.,* 1999). The root extract has antimicrobial properties (Adongo *et al.,* 2012) as well as ability to halt the replication of HIV virus in human blood (Mecham *et al.,* 2007).

2.1.4 Nutritional composition

The nutritional composition of *T. fassoglense* seed is similar to those of *T. esculentum* with 45% protein and 24-40% oil (Dubois *et al.,* 1995; Okumu, 2011; Otieno *et al.,* 2015). Of concern is the relatively high content of phytate (3.5%) in seeds, an anti-nutritional compound which sequesters positive ions making them unavailable when the seed is consumed (Dubois *et al.,* 1995; Lott *et al.,* 2000). The dominant amino acid in *T. fassoglense* seed proteins is glutamic, aspartic acid as well as tyrosine while it is limiting in methionine and cysteine which contain the element sulphur (Dubois *et al.,* 1995).

2.1.5 *Tylosema fassoglense* seed

Mature legume seeds have three major components comprising seed coat, endosperm and embryo which include cotyledons constituting the major part (Figure 2:2). After fertilisation, the seed coat and endosperm are initially formed and subsequently the embryo. The seed coat is a transient storage organ for the growing embryo, accumulating starch and protein before storage activity shifts to the embryo. The embryo grows by cell division, expansion and accumulation of storage reserves during maturation. Generally, the endosperm disappears during maturation in leguminous species leaving cotyledons as the main food reserve. *Tylosema spp.,* peanut and soybean store energy in form of lipids compared to other legumes that mostly store starch. The stored lipids provide energy essential for growth upon germination (Bewley and Black, 1994).

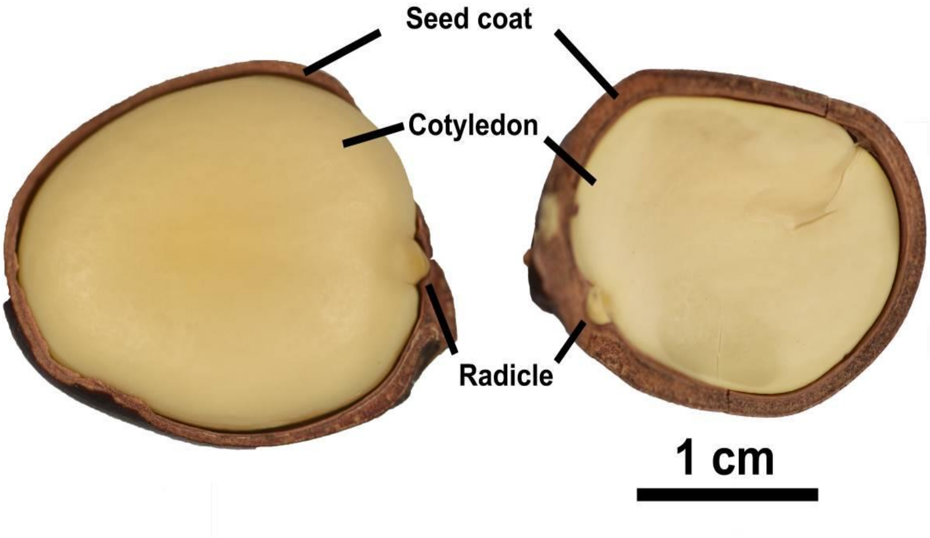

Figure 2:3: A sectioned seed of *Tylosema fassoglense* [Left] and *T. esculentum* [Right] showing the cotyledon, radicle and seed coat (Photo: Pablo-Barreiro Gomez)

2.2 Seed maturity, collecting and seed characteristics

Three phases have been recognized during seed development (Sacandé *et al.*,2004; Smith *et al.,* 2004): the first phase starts soon after pollination and fertilization and is characterized by rapid cell division and embryogenesis (lag phase). Further cell division as well as growth, rapid increase in fresh weight accumulation: proteins, lipids and carbohydrates take place during the second phase. The final phase depicts maximum cell size, weight and the moisture content (MC) starts to decrease which characterizes physiological maturity (Ellis and Pieta Filho, 1992). At physiological maturity, orthodox seeds become desiccation-tolerant and enters germination mode (Hay *et al.,* 2003; Smith *et al.,* 2004). The seeds also have increased longevity (Smith *et al.,* 2004) and an abscission layer forms between the embryo and seed covering structure (Yogeesha *et al.,* 2005). Unlike recalcitrant seeds, orthodox seeds attain increased desiccation tolerance as they undergo maturation drying to low moisture content while on the mother plant (Hong and Ellis, 1996). A number of physiological changes occur after maturity leading to the development of "hard seededness" in certain species. For example, the Fabaceae family produce soft and milky seeds when immature and hard when mature, so much so that they even fail to imbibe water and germinate (Muthoka *et al.,* 2003; Smith *et al.,* 2004). The maturity and harvesting stage can significantly affect germination (Demir *et al.*, 2010), longevity (Kochanek *et al.*, 2011) and storability (Whitehouse *et al.,* 2015) of a seed lot.

Seed maturity of most wild species is usually spread over a few weeks and sometimes months and often depends on floral morphology or position on the mother plant. Therefore, seed collecting of these species poses a serious challenge of collecting seeds at different maturity stages. It is therefore, necessary to monitor and collect fruits/seed at harvest maturity, for example at dispersal for dehiscent fruit (pod) or ripeness for drupe and berries and sorting out may be necessary before processing.

Generally, seed quality or characteristics at any point depicts the significance of factors that have acted upon it ranging from cultivar or genetic, environmental factors, to physical or processing. In crop production, a number of these determinants of seed quality are under human intervention, while in wild germplasm only physical factors can be controlled. Wild species seeds are often collected at unknown moisture content and are sometimes empty, insect-infested or diseased. This can subsequently impact on seed storability and germination results. Seed equilibrium relative humidity (eRH), moisture content and x-ray radiography have been widely used for quality screening of wild germplasm to guide post-harvest handling (FAO IPGRI, 1994; ISTA, 2017). The Fabaceae family are pod bearing plants that produce mainly orthodox or recalcitrant seed which are dispersed through an explosive mechanism (for orthodox seed) upon drying. Therefore, understanding of seed phenology and screening for seed characteristics of *T. fassoglense* is essential to guide seed collection and subsequently storage and propagation or germination testing.

2.3 Seed germination and dormancy

Seed germination is a complex process influenced by light, temperature, water and their interactions, which is sometimes limited by dormancy (Bewley *et al.*, 2013). The process starts with water imbibition, reactivation of enzymes and mobilization of food reserves, stored messenger ribonucleic acid (mRNAs) as well as deoxyribonucleic acid (DNA) repair (Rajjou *et al.*, 2012). Water imbibition of seed with a permeable seed coat occurs in three phases namely phase I, II and III (Preston *et al.*, 2009; Steinbrecher and Leubner-Metzger, 2016). Rapid initial imbibition facilitated by water potential difference between germination medium and seed occurs in phase I. The second or plateau phase is characterized by gradual increment of the seed mass. The embryo elongates along the axis and ruptures through the seed coat in third or final phase due to increased water absorption. This process culminates in seed germination that is radical protrusion.

Seed dormancy is the failure of a viable seed to germinate under suitable thermic and hydric conditions for the species (Finch-Savage and Leubner-Metzger, 2006) over a defined period (Baskin and Baskin, 2014). Seed dormancy is a characteristic that synchronizes germination to suitable environmental conditions (Finch-Savage and Leubner-Metzger, 2006) and prevents germination during seed development and maturation. However, under crop production the trait is an undesirable for rapid and uniform germination. Baskin and Baskin, (2014) proposed five classes of dormancy namely morphological, physical, physiological, morphophysiological and physiophysical. Seeds with underdeveloped embryos have morphological dormancy (MD) while physical dormancy (PY) is exhibited in seed with impermeable seed/fruit coat. Specific environmental stimuli such as warm and/or cold stratification, dry-after ripening are required by physiologically dormant seeds to germinate (PD). Morphophysiological results from a combination of MD + PD while physiophysical is a combination of PD + PY.

Physical dormancy is present in 18 angiosperm plant families, including Fabaceae (Baskin and Baskin, 2014). Seed dormancy is an adaptive strategy to environmental heterogeneity and variation within and between populations has been associated with maternal age plus nutrient availability (Matilla *et al*., 2005), position of inflorescence (Volis, 2014), seed mass and shape (Baloch *et al.,* 2001), duration since collection (Penfield and MacGregor, 2017) as well as environmental conditions during seed maturation (Donohue, 2009; Menegat *et al*., 2018; Hradilová *et al*., 2019). While plant breeding (selection) has eliminated dormancy in cultivated grain legumes (Bewley and Black, 1994), it still remains a challenge in propagating wild germplasm. Seed coat permeability has been widely explored in legume species, for example in soybean it is proposed that water enters through closed hilum and/or micropyle (Gama-Arachchige *et al.,* 2013), water gaps in the lens (Koizumi and Kano, 2014) or cracks in the cuticle of the testa (Ma *et al.,* 2004). In Robinia, Acacia and many pantropical legume species water enters the seed through the hilar slit and strophiole or water gap (Karaki *et al.,* 2012). Water enter seeds through cracks developed in the extrahilar region or hilum in Caesalpiniodeae and Mimosoideae subfamilies (Hu *et al.,* 2009). Physical dormancy-breaking pre-treatments in legume species such as scarification (i.e. use of sand paper, sulphuric acid, nail clipper), dry and/or wet heat, and passage through an animal gut have been explored to enhance germination (Travlos *et al.,* 2007; Kimura and Islam, 2012; Baskin and Baskin, 2014; de Morais *et al*., 2014). For example, Travlos *et al.,* (2007) reported moderate physical dormancy in *Tylosema esculentum* (Fabaceae sub-family Caesalpiniodeae) while scarification using sulphuric for 20 minutes or soaking for 20 hour in cold water improved germination. The information on how to germinate various species provides a better understanding of reproductive strategies, adaptation to local environment and physiological processes. Despite physical dormancy having been reported in the Family Fabaceae as well as the genus Tylosema in *T. estculentum*, study on the four remaining *Tylosema spp.* has not been conducted despite the potential of *T. fassoglense* as a future crop.

2.4 Environmental factors that influence seed germination

2.4.1 Role of light on seed germination

Several species have demonstrated insensitivity to light requirement for seed germination, while a few species require light to germinate (Baskin and Baskin, 2014). However, in natural habitats light requirements for germination may be complex and seasonal variations in light spectra or shading in habitat can trigger or inhibit germination (Fenner and Thompson, 2005). Light requirement is an abiotic factor influencing seed germination, storage soil bank longevity and dispersal (Fenner and Thompson, 2005; Baskin and Baskin, 2014). The role of light is both to trigger germination (Vleeshouwers and Bouwmeester, 2001) and release seed dormancy (Battla and Benech-Arnold, 2005). It follows that, the dormancy release process is the last step when seeds exposed to light experience changes that allows them to germinate in darkness (Bewley and Black, 1994; Leubner-Metzger, 2006). The role of light on seed germination has been linked with a protein called phytochrome that contains photoreceptors. The cycling of phytochrome between the active Pfr (phytochrome far red) and the inactive Pr (phytochrome red) form depends on light wavelength. The inactivation of the photoreceptor cells via phytochrome by exposure to far-red light can reverse the effect of red light and initiate the process of germination (Casal *et al.*, 2013). However, a combination of light and gibberellic acid (GA) has been reported to release physiological dormancy and enhance germination in some species (Kucera *et al.*, 2005; Leubner-Metzger, 2006).

Baskin and Baskin (2014) proposed three groups of plants: those that require light (positive photoblastic), those not sensitive to presence/absence of light (neutral photoblastic) and those that do not require light (negative photoblastic). The three categories offer a better concept in understanding seed germination response to light requirement in laboratory experiments. Milberg *et al.*, (2000) proposed relative light germination index (RLG) ranging between zero (negative photoblastic) and one (positive photoblastic) based on the proportion of seed germinating under light and dark. Light penetration into the soil is usually limited to a few millimetres (Cussans *et al*., 1996; Benvenuti, 2003) and therefore light requirements for seed germination can occur before or after the seed is covered with soil (Noronha *et al*., 1997; Baskin and Baskin, 2014;). Therefore, seeds use total light absence to detect burial depth and location near or on soil. However, large-seeded species have been reported to emerge from greater depths than light can penetrate (Del Arco *et al*., 1995; Milberg *et al*., 2000). The role of light in seed germination has been strongly linked to seed mass, which limits seed burial depth that will permit germination and emergence before the food reserves are depleted (Milberg *et al.*, 2000). Therefore, understanding light requirement for germination is important in domestication and conservation of wild species. However, there is insufficient information on germination ecology especially germination response to light for a number of indigenous grain legumes such as *T. esculentum*, *T. fassoglense* despite their huge potential for domestication.

2.4.2 Effect of temperature on germination

Temperature is a major environmental factor that influences seed germination rate, percentage and dormancy cycling (Baskin and Baskin, 2014). Naturally, dormancy cycling is primarily controlled by seasonal and diurnal temperature fluctuations. Seed germination rate after dormancy release is majorly determined by the incubation or germination temperature. Under field conditions, temperature has a strong implication on germination timing and seedling establishment (Walck *et al.*, 2011; Baskin and Baskin, 2014). Grain legumes have the ability to grow under diverse climates characterised by high temperatures, low water potential and poor soils (Cullis *et al.*, 2018). However, seed germination is quite sensitive to temperatures while the cardinal temperatures are species-specific with intra- and inter-species variations having been reported. The role of temperature on the seed germination processes can be quantified using thermal model through germination percentage and rate (Garcia-Huidobro *et al.*, 1982; Tribouillois *et al.*, 2016). Laboratory studies usually characterize seed germination response to three cardinal temperatures: base, optimum, and ceiling (T_b, T_o and T_c) and thermal time (Garcia-Huidobro *et al.*, 1982). The base (T_b) and ceiling (T_c) temperatures is that under and over, respectively, at which germination is zero, while germination is quite fast at optimum temperature (T_o). The other parameter is thermal time (°C h; degree Celsius hours or days); that is, the cumulative heat units that a given population (G) of seed needs to germinate.

The adaptation of species to local environments is controlled by the cardinal temperatures which synchronise germination timing to suitable conditions that will permit germination and seedling establishment (Walck *et al.*, 2011). Therefore, knowledge of variations in seed germination traits within and between populations is important for germplasm screening and predicting seed germination progress, environmental limits, planning seedbed preparation, measuring of physiological time and timing for sowing (Hardegree *et al.,* 2016). Studies conducted in a number of species have shown intra- and inter- species variation in cardinal temperatures. The differences in species cardinal temperatures is generally correlated to ecological and geographical factors which helps in understanding species adaptation to a particular environment (Hardegree *et al.,* 2013; Costa *et al.,* 2018). Characterisation of seed germination to cardinal temperatures have been conducted under constant or alternating temperatures for several species, but only a few studies have focussed on crop wild relatives and indigenous tropical legumes (Castillo-Lorenzo *et al*., 2019a,b; Dürr *et al*., 2015; Tribouillois *et al.,* 2016; Covell *et al*., 1986). Currently, there is no literature that considers seed germination of *Tylosema spp.* under varied temperatures despite the potential of *T. fassoglense* as a future crop. Therefore, a knowledge gap exists on cardinal temperatures and thermal time for this neglected and underutilized tropical grain legume.

2.4.3 Effect of water potential on germination

The other environmental factor that significantly impacts on seed germination is precipitation, which is influenced by the water potential difference between the germination media and the seed (Bradford, 2005). Similarly, seed germination response to water potential at optimal temperature can be quantified using the hydro model through germination percentage and rate (Bradford, 2005). The hydro model predicts the base water potential, below which germination is zero and hydro time (measured in Megapascal hours or days), that is cumulative water potential hours or days a seed population (G) requires to germinate (Gummerson, 1986; Bradford, 2005). The hydro model has been used to detect seed dormancy associated with change in germination temperature (Windauer *et al.,* 2007). Seed physiological response to environmental condition provides another definition of seed dormancy as an internal status that prevents germination under suitable hydric, thermic and oxic conditions (Benech-Arnold *et al.,* 2000).

The cultivated grain legumes have also the ability to grow under water stress, high temperature and poor soils associated with ASALs (Cullis *et al.,* 2018). However, seed germination is quite sensitive to water stress that hydro thresholds are known to be species-specific. Soil moisture in the ASALs is usually available for short periods following precipitation, therefore the ability of seed to germinate under such environments requires special adaptation mechanisms. The adaptation of germination requirements in wild species can occur in response to slight variations in the soil water potential. Therefore, information of inter- and intra-population variations in seed germination response to water potential is important for restoration planting and predicting seed germination progress, measuring of physiological time and environmental tolerance limits (Hardegree *et al.,* 2016). The effect of water potential on seed germination has been explored in many species, but a few have focussed on neglected indigenous grain legumes (Aguilar-Benitez *et al.,* 2014; Hu *et al.,* 2015; Castillo-Lorenzo *et al.,* 2019b). The variation in species seed base water potential and hydro time can occur across habitats and geographical locations (Evans and Etherington, 1990; Daws *et al.,* 2008; Hu *et al.,* 2015). According to Zeng *et al.,* (2010) the low tolerance to water stress at germination and seedling establishment stage by arid desert xerophytes has been regarded as an evolutionary strategy. The intra- and inter-species variation in water requirements and availability for germination partly reflects their adaptation to water stress conditions, which is correlated to the habitat (Baskin and Baskin, 2014). Seed germination responses to water stress has been reported to vary intra- and inter-species in grass (Hardegree *et al.,* 2013), in *Poa* spp (Springer and Goldman, 2016) and in *Rheum* spp. (Peng *et al.,* 2017) However, there is no literature that considers seed germination of *Tylosema spp.* under varied water potentials, despite the potential of *T. fassoglense* as future a crop and its ability to grow in water stressed environment.

2.5 Role of maternal environmental on seed functional traits

The core functions of seed are storage, germination and dispersal. The roles of the maternal environment are manifested in the “offspring” through seed physical and germination traits (Donohue *et al.,* 2009; He *et al.,* 2014). The abiotic factors that are known to impart maternal effects on seed traits are photoperiodicity, temperature, rainfall, nutritional status of the soil and competition among plants (Baskin and Baskin, 2014). Seed morphological traits such as size/mass and composition are controlled genetically (zygotically/maternally) (Li and Li, 2015), environmentally (Paul-Victor and Turnbull, 2009; He *et al.,* 2014; Richardson *et al.,* 2015; Li *et al.,* 2017) and may vary depending on inflorescence position and harvest season. These physical traits are often studied in relation to germination traits (Ambika *et al*., 2014) but are also considered market quality parameters (Finch-Savage and Bassel, 2015). *Tylosema spp.,* peanut and soybean store seed oil as a major energy source for the germinating seed and growing seedling, however its role in relation to seed germination traits has been less explored. The maternal environment influences the seed filling phase and particularly, mean seed mass and seed numbers per inflorescence have been positively correlated with annual rainfall or with mean annual temperature, MAT (Li *et al*., 2017). The prevailing temperature during the maturation phase has also been reported to influence seed dormancy (Fenner, 1991). For example, Richardson (2015) reported that seed mass is principally controlled by genetic factors with limited maternal environment effect on big sagebrush. Similarly, Paul-Victor and Turnbull, (2015) reported growth conditions affects seed size and a balance occurs between seed mass and seed numbers.

Previous studies have reported a lack of association between seed mass and germination rate or vigour and final percentage (Mondo *et al*., 2013; Rezapour *et al*., 2013; Kering and Zhang, 2015). However, a negative correlation was reported between seed mass and base water potential of neotropical forest species (Daws *et al*., 2008) and no correlation was found between seed mass and base temperature of *Aesculus hippocasternum* seeds from across Europe (Daws *et al*., 2004). Rosbakh and Poschlod (2015) also reported a strongly negative correlation between initial minimum temperature T_{min} of germination and habitat mean annual temperature MAT of 49 species from different habitats. The understanding of correlation between seed functional traits provides an opportunity in germplasm selection or improvement and site matching to suitable environmental condition to maximize germination. This information has not been documented for neglected and underutilized legume such as *T. fassoglense,* despite its potential to improve agro-biodiversity and legume production in less productive areas.

CHAPTER THREE

3.0 Dormancy and the role of light on germination of Sprawling bauhinia (*Tylosema fassoglense)*

3.1 Abstract

Seed dormancy and light requirements for germination is important for plant propagation, domestication as well as understanding germination ecology. This information has not been generated for *Tylosema fassoglense* [Family: Fabaceae], despite its potential as a future crop in Kenya. The objective of this study was to determine seed dormancy status and the role of light on the germination of three seed lots of *T. fassoglense.* Seed material was collected from Busia, Migori and Siaya counties in Kenya. Water imbibition and germination experiments were performed on scarified and non-scarified seeds whilst scarified seeds were germinated under 12/12 hour and 0/24 hour photoperiod at 25 °C. For each treatment, 10 seeds were sown onto moist germination paper in sandwich boxes and then sealed in polythene bags. The boxes were placed randomly in germination cabinet and regularly shuffled. Water uptake (g) and germination percentage (%) was recorded. Germination data were normalized by arc-sine transformation, subjected to analysis of variance and the means separated with Tukey's HSD ($p<0.05$) using SAS version 2002-2003. Scarification significantly ($p<0.05$) improved water uptake by 4 to 22 times as well as germination percentage from 30% to 90% (Migori seed lot) and from 70% to ≥90% (both Busia and Siaya seed lots). Seeds germinated to over 50% under both light and dark conditions while the relative light germination (RLG) index ranged from 0.46 to 0.59. Seeds of *T. fassoglense* possess physical dormancy that varies across populations and are probably neutral photoblastic. Scarification improves both imbibition and final germination percentage while burial sowing is possible under cultivation. These findings can help in propagation and domestication of *T. fassoglense* to benefit small-holder farmers of Kenya by supporting food security and sustainable agriculture.

Keywords: dormancy; germination; light requirement; potential crop; *Tylosema fassoglense*

3.2 Introduction

Seed dormancy is simply the failure of a viable seed to germinate under suitable conditions for the species within a defined period (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014). Plants have evolved a diverse range of seed dormancy as an adaption to environmental heterogeneity that synchronizes germination to suitable conditions as well as a bet-hedging strategy (Bewley *et al.,* 2013; Baskin and Baskin, 2014). The five classes of seed dormancy are; physical dormancy PY (i.e. presence of impermeable seed/fruit coat that prevents embryo imbibition); seed requiring special environmental condition to germinate (physiological dormancy PD); morphological dormancy MD (seed with underdeveloped embryos) and combinational dormancy (i.e. morphophysiological MP+PD and physiophysical PD + PY) (Baskin and Baskin, 2014). Physical dormancy which is caused by the presence of Malphigian cells that prevents water imbibition has been reported in 18 angiosperm plant families including Fabaceae (Baskin and Baskin, 2014; Smýkal *et al*., 2014). Seed dormancy is genetically controlled (Willis *et al.,* 2014), however variations within a species can occur intra- or inter-population and between seeds collected in different seasons from the same population (Lacerda *et al*., 2004; Smýkal *et al*., 2014). Selection of non-dormant seeds has eliminated dormancy in cultivated legumes (Bewley and Black, 1994), however dormancy remains a challenge in propagating wild species (Rodríguez-Arévalo *et al.,* 2017). Physical dormancy, variation and dormancy breaking pre-treatments is well documented in legume species (Travlos *et al*., 2007; Kimura and Islam, 2012; Gama-Arachchige *et al.,* 2013; de Morais *et al*., 2014; Richard *et al,* 2018; Hradilová *et al*., 2019). For example, Travlos *et al.,* (2007) reported moderate physical dormancy in *Tylosema esculentum* (Fabaceae), however literature on seed dormancy and germination ecology of *T. fassoglense* has not been documented despite its potential as a future crop in Kenya.

Light requirement is an environmental factor influencing seed germination, soil seed bank and dispersal in a number of species (Fenner and Thompson, 2005). According to Baskin and Baskin, (2014) three groups of plants exist based on light requirement: those that require light (positive photoblastic), those not sensitive to presence/absence of light (neutral photoblastic) and those that do not require light (negative photoblastic). Milberg *et al.,* (2000) also proposed relative light germination (RLG) index ranging from zero (negative photoblastic) to one (positive photoblastic) based on proportion of seed germinating under light and dark. The role of light in seed germination is also strongly linked to seed mass and shape, which limits seed burial depth that will permit seed germination and seedling emergence (Thompson and Hodgson, 1993; Milberg *et al.,* 2000). The concept of light requirements for germination has been explored on some wild species and vegetables (Sánchez-Bayo and King, 1994; Motsa *et al.,* 2015; Flores *et al.,* 2016; Costa *et al*., 2018). The knowledge of the role of light on germination is important in propagation and understanding germination ecology of wild germplasm (Baskin and Baskin, 2014). *Tylosema fassoglense* has the potential as future crop in Kenya; however, information on seed germination response to light to guide sowing under cultivation has not been generated.

Tylosema fassoglense [Family: Fabaceae] is a perennial tuberous vine endemic to sub-Saharan Africa (Castro *et al.,* 2005). In Kenya, *T. fassoglense* is widespread from the Coast, through Eastern, Rift Valley to the Lake Victoria Basin. It grows in scattered bushland, open grassland and along roadside on well drained sandy-loam soils (Maundu *et al*., 1999). The leaves are large, bilobed while flowers are creamy-yellow and dimorphic/heterostyllus (Hartley *et al.,* 2002). Flowering and seeding varies from one region to another. The pods bear 1 or 2 large black-brown seeds. *Tylosema fassoglense* has the potential as a future crop since the seeds which are consumed as a snack or beverage are high in oil (24-40%) and protein (30-40%) (Dubois *et al.,* 1994; Okumu, 2011; Otieno *et al.,* 2015). The tuber which acts as water reservoir is rich in starch and mainly exploited for medicinal purposes (Maundu *et al.,* 1999). Despite its huge potential as a future crop, information on seed dormancy and germination ecology of *T. fassoglense* to guide its propagation and domestication has not been generated.

Under crop production, good uniform germination and crop density is essential for management options applicable (Bewley and Black, 1994; Finch-Savage and Bassel, 2015). Therefore, the objective of this study was to determine seed dormancy status and role of light on germination of three seed lots of *T. fassoglense* collected from three counties in Kenya.

3.3 Material and methods

3.3.1 Seed source information

Seeds of *T. fassoglense* were collected from three populations (later referred to as seed lots) along the Lake Victoria basin in Busia, Migori and Siaya counties in Kenya where large populations occur (Otieno *et al.,* 2015) (Figure 3:1). The pods were sampled randomly from seeding plants between December 2017 and January 2018 in Busia and Migori sites and between April and June 2018 in Siaya site (Table 3:1) while the areas were geo-referenced using Global Positioning System GPS. Harvest maturity indicator of *T. fassoglense* was determined by pod physiological maturity when the pods colour turned from green to brown or dark brown and rattled when shaken. On hot sunny days, the mature pods snapped during harvesting to yield brown to dark brown or black hard seeds. The pods collected from these sources were put in paper bags and transported to Kenya Forestry Research Institute (KEFRI-Seed Centre) Muguga for processing. Seeds were extracted from the pods by drying at 25-30

°C in the glasshouse to allow them to dehisce naturally and those that failed to dehisce were opened manually by hand. Seeds were allowed to dry further in the glasshouse for a couple of weeks, sorted manually by hand and then stored at 10 °C. The seed lots were shipped to the Seed Conservation Department of the Royal Botanic Gardens, Kew, in the United Kingdom where the experiments were conducted.

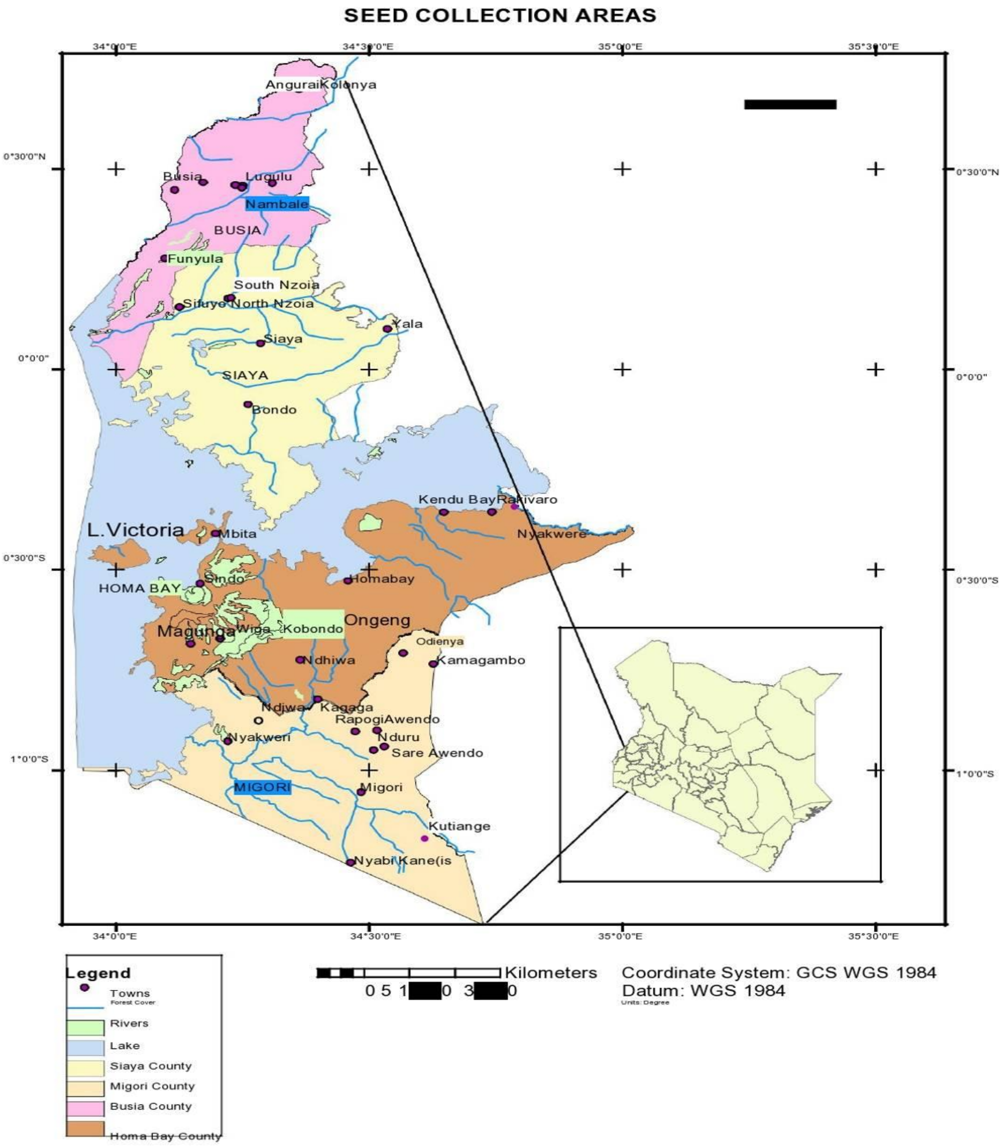

Figure 3:1: Map of counties where seeds of *Tylosema fassoglense* were collected in Kenya (Map: Abiud Sayah)

Table 3:1:Information of *Tylosema fassoglense* seed lots showing seed source, seed collection months (grey cells) and the average monthly precipitation (mm), maximum, mean and minimum temperatures for the collection sites (Source: Kenya Met. Dept. Satellite Data)

		Jan	Feb	Mar	April	May	June	July	August	Sep	Oct	Nov	Dec
Busia	**Max T℃**	28.86	28.82	28.84	28.32	27.80	27.32	27.04	27.12	27.49	28.06	28.08	28.06
	Min T℃	17.03	17.29	17.31	16.78	16.27	15.79	15.49	15.57	15.94	16.52	16.44	16.51
	Mean T℃	22.80	23.05	23.08	22.55	22.03	21.55	21.26	21.34	21.71	22.29	22.26	22.28
	Prec (mm)	52.71	79.33	136.64	231.43	212.71	104.11	79.53	111.92	115.86	135.44	147.95	88.44
Migori	**Max T℃**	27.67	27.90	27.91	27.40	26.89	26.42	25.94	26.13	26.71	27.28	27.15	27.07
	Min T℃	16.53	16.76	16.78	16.26	15.75	15.29	14.96	15.14	15.73	16.30	16.16	16.08
	MeanT℃	22.10	22.33	22.34	21.83	21.32	20.86	20.45	20.64	21.22	21.79	21.65	21.58
	Prec (mm)	61.69	81.86	139.13	208.84	162.69	71.48	48.36	61.61	82.50	108.04	150.61	99.04
Siaya	**Max T℃**	28.32	28.57	28.54	28.03	27.50	27.01	26.73	26.84	27.28	27.93	27.88	27.86
	Min T℃	17.39	17.64	17.60	17.09	15.57	16.08	15.85	15.96	16.40	17.05	17.00	16.98
	Mean T℃	22.85	23.10	23.07	22.56	22.03	21.55	21.29	21.40	21.84	22.49	22.44	22.42
	Prec (mm)	46.30	72.73	128.97	207.53	173.45	78.65	62.95	84.44	90.08	96.46	125.03	93.02

Climate Data for Busia and Migori are for the month of June 2017 to May 2018 while Siaya data covers Jan to Dec 2018

3.3.2 Seed lot characteristics

Immediately the seeds were received at Millennium Seed Bank-Laboratories-UK, seed lot assessment by x-ray radiography, equilibrium relative humidity eRH, moisture content and morphology were conducted. Seed x-ray radiography was performed on three replicates of each seed lot by placing random samples of seeds on a 240 x 180 x 2 mm transparent acrylic plate at a distance of 28.6 cm from radiation source (Faxitron MX-20DC12 cabinet X-ray system, Faxitron Bioptics, Tucson, AZ, USA). The seed radiographs were used to assess seed quality by examining the embryonic and endospermic tissues for: emptiness, mouldiness, insect infestation, damaged embryo or seed coat and diseased tissues (ISTA, 2017).

The seed eRH of *T. fassoglense* was measured on random samples of three replicates from the three seed lots in the drying room operating at *ca*. 15% Relative Humidity (RH) plus 15 °C (FAO IPGRI, 1994). The sample vial was three quarter filled and then inserted into the rotronic chamber (Rotronic AWVC-DIO Sensor Manufactured by Rotronic Limited, UK), with an accuracy of ±2% eRH for 30 minutes to allow the seeds to equilibrate before recording the eRH% (Karrfalt, 2010).

Moisture content, MC was determined on ten seeds weighed singly using a microbalance (0.0001 g; UMT2 Mettler, Toledo). The seeds were put in metal cups and then dried in an oven at 103 °C for 17 hours (ISTA, 2017). The oven dried seeds were allowed to cool over a closed drum containing silica gel *ca*. 0% RH for 30 minutes before reweighing. Seed moisture content (SMC) was calculated on fresh weight basis (fwb) according to ISTA, (2017) (Eq.3.1).

$$MC(fwb) = \left\{\frac{M1-M2}{M1}\right\} \times 100\% \qquad \text{(Eq.3.1)}$$

where, M1=wet seed weight and M2=dry seed weight

Seed morphological measurement was performed by using a Carl Zeiss camera to take images from different angles of 10 seeds randomly sampled from each seed lot and mounted on a cardboard. From the images, seed dimensions (i.e. length, width and thickness) were estimated using Axio vision computer software (Axio Vs40; Carl Zeiss Micro imaging 2010, Germany). Seed shape was expressed as the variance of length L, width W and thickness T according to Thompson and Hodgson, (1993) whereby a value of 0 = dimensionless or perfectly spherical and ≥0.3 = needle or disc-shaped. Length, width and thickness were first transformed by dividing each by length after which the variance was obtained as the average of the squared differences from the mean.

3.3.3 Imbibition of water

To determine seed coat permeability to water, imbibition rates of water were measured for scarified and non-scarified seeds. For scarification treatment, small part of the seed coat opposite to the micropyle was chipped off using a nail clipper. Ten individual seeds for each treatment (scarified and non-scarified) from the three seed lots were used in the experiment. Initial weight of an individual seed was measured using the microbalance (0.0001 g; UMT2 Mettler, Toledo). The seeds were sown onto moistened anchor steel blue seed germination blotter (Anchor Paper Company, Seed Solutions; Saint Paul, MN, USA) in sandwich boxes (17 x 11 x 5 cm) placed at 25 °C. Seeds were removed, blotted dry and weighed after 1, 3, 8, 23, 28, 32, 47 and 52 hours of keeping on moist media. Seeds were watered when needed by adding 20 ml and 10 ml of deionized distilled water on scarified and non-scarified seeds respectively. The amount of water uptake (g) was calculated following modified formula by Baskin and Baskin, (2004) (Eq. 3.2):

$$\triangle M_{\mathrm{t}} = (M_{\mathrm{t}} - M_i) \qquad \text{(Eq. 3.2)}$$

where ΔM_t is the mass increment at a given time “t”, M_t represents the mass at a time “t” and M_i is the initial mass at time “t” = 0 (dry at 15%RH).

3.3.4 Effect of manual scarification on germination

The experiment of dormancy-breaking comprised four replicates of 10 seeds each for scarified and non-scarified (one replicate was the same used during the imbibition test). The seeds were sown onto moist germination paper in sandwich boxes (as described in imbibition tests), wrapped in self-sealing polythene and placed randomly in germination cabinet. The boxes were incubated at 25 °C plus 12/12 hour photoperiod (radiometric flux density 50-100 W.m^{-2}). Seed germination was monitored daily for a period of 14 days. Cracked seeds in the non-scarified treatment were eliminated in the final germination count. The number of days to first count was recorded and final germination percentage was calculated as follows:

$$\frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown} \times 100\%. \qquad \text{(Eq. 3.3)}$$

3.3.5 Seed germination under light and darkness

The role of light on germination was determined on scarified seeds of each seed lot sown onto moist germination paper in sandwich boxes in four replicates (as described in imbibition tests).

For each seed lot, one set of four sandwich boxes was kept under 12/12 hour (light/dark) (radiometric flux density 50-100 W.m^{-2}) and a second set under 0/24 hour (total darkness). The dark treatment/photoperiod seeds were wrapped in aluminium foil while the other set were kept in light penetrating sandwich boxes and incubated at 25 °C. Germination for seed samples under dark photoperiod was scored in an isolated dark room under "safe" green light. The sandwich boxes were wrapped in self-sealing polythene bags to prevent moisture lose while the media was kept moist by adding 20 ml of deionised distilled water when needed. Germination percentages were calculated using (Eq. 3.3) and germination under light was compared against its absence using the relative light germination index (RLG) (Milberg *et al.*, 2000) (Eq. 3.4):

$$RLG = Gl \,/\, (Gl + Gd) \qquad \text{(Eq. 3.4)}$$

where Gl= proportion of germinated seeds in light conditions and Gd is its equivalent in dark conditions. The average seed germination percentage in presence of light and dark conditions was used for each seed lot. RLG can range from zero (negative photoblastism) and one (positive photoblastism)

3.4 Germination evaluation

Germination was recorded daily for the three seed lots until no more seeds germinated. Seed germination was defined as, radicle protrusion (>2 mm) and the germinated seed were allowed to develop into normal seedling in the sandwich boxes. According to ISTA (2017), a normal seedling is that which has grown to show essential structures (i.e. cotyledons, hypocotyls and roots) necessary for further development into a satisfactory plant and lacks visible abnormalities. Viability of non-germinated seeds were evaluated by cut test and seeds that were firm and full were reported as viable while rotten seeds were reported as dead.

3.5 Data analyses

All percentage data (seed eRH, MC, and germination) were arcsine transformed before analysis. Analysis of Variance (ANOVA) was conducted and the means separated with Tukey's HSD test where significance difference were observed ($p<0.05$). Data was analysed using SAS Version 2002-2003 (SAS Institute Inc. Cary, NC, USA)

3.6 Results

3.6.1 Seed lot characteristics

The examined x-ray radiographs indicated high seed quality in the three seed lots (Figure 3:2). The three seed lots had no signs of mouldiness or physical damage. There was no observable insect infestation and embryo damage across the three seed lots. The equilibrium relative humidity (eRH) of seeds sampled from Siaya seed lot was significantly ($p<0.05$) high compared to Busia and Migori seed lots. The mean eRH of *T. fassoglense* seed lots ranged from 56.03% (Migori seed lot) to 59.0% (Siaya seed lot) (Table 3:2). Seed moisture content (SMC) of *T. fassoglense* did not differ ($p>0.05$) significantly among the three seed lots. The mean moisture content of seeds sampled from the three seed lots ranged from 5.9 % (Migori seed lot) to 6.3% (Siaya seed lot). Seed variance or shape ranged from 0.32199 (Busia seed lot) to 0.35142 (Migori seed lot).

Table 3:2: Seed characteristics of the three seed lots of *Tylosema fassoglense:* moisture content, equilibrium relative humidity eRH, Variance and Viability(Cut test)

Seed lot	Moisture content %	eRH %	Variance	% Viable seed
Busia	6.09±0.67a	57.16±0.29a	0.32199±0.042a	90
Migori	5.90±0.90a	56.03±0.93b	0.35142±0.029a	90
Siaya	6.30±0.46a	59.0±0.1b	0.35050±0.026a	90

Figures in the same column with different letters are significantly different ($p<0.05$, n=10 for MC; variance and n=3 for eRH).

3.6.2 Imbibition of water

The average water uptake differed ($p<0.05$) significantly between scarified and non-scarified seeds after 1, 3, 8, 28, 32, 47 and 52 hours of keeping on moist media (Figure 3:3; Supporting data Appendix 1). Scarified seeds from Busia and Siaya seed lots imbibed three to four times more water compared to the non-scarified seeds from the same lot after 52 hours of incubation. Scarified seeds from Migori seed lot imbibed 22 times more compared to the non-scarified seeds from the same seed lot after 52 hours of incubation. For example, after 1 hour of incubation the average amount of water imbibed by scarified seeds ranged from 0.285 g (Busia) to 0.0638 g (Migori) while non-scarified seeds ranged from 0.0871 (Busia) to 0.0039 g (Migori). After 52 hours, the average amount of water imbibed by scarified seeds ranged from 3.4171 g (Busia) to 3.4393 g (Migori) while non-scarified seeds ranged from 1.0754 g (Busia) to 0.1515 g (Migori). Non-scarified seeds from Migori population imbibed the least amount of water.

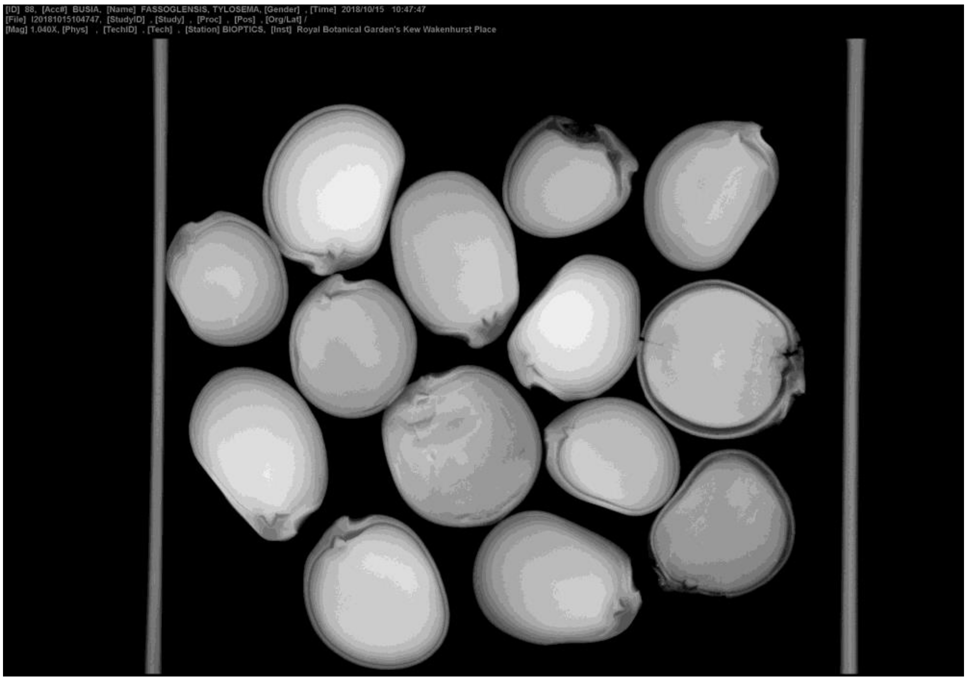

Figure 3:2: Seed x-ray radiograph of Tylosema fassoglense (Radiograph: V. Otieno)

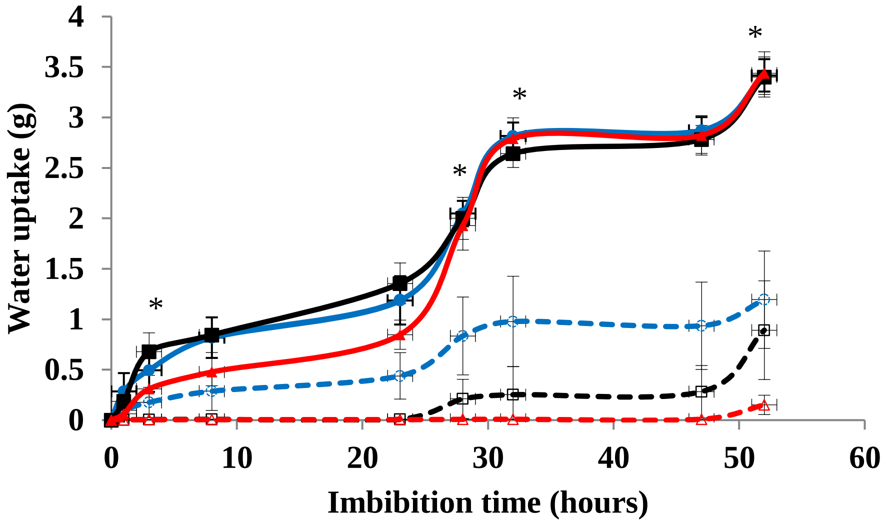

Figure 3:3: Water uptake by imbibition at 25°C plotted against time for scarified (continuous line) and non-scarified (dotted line) seeds of *Tylosema fassoglense* from three seed lots. Blue= Busia, Red=Migori and Black=Siaya seed lots. Points with asterisks (*) differ (*p<0.05*, n=10) statistically between scarified and non-scarfied seeds and vertical bars are standard error (±se)

3.6.3 Effect of manual scarification on germination

Despite the imbibition was much lower in the non-scarified seeds, this did not affect the germination capacity of the seeds since both, scarified and non-scarified began to germinate at the same time. However, seed scarification greatly affected the final germination with seeds attaining high germination percentages within five to six days. Non-scarified seed sampled from Migori seed lot had significantly (*$p<0.05$*) the lowest final germination (Figure 3:4). Scarified seeds from the three seed lots reached a high germination percentage (>90%) compared to non-scarified seeds (≤70%). Non-scarified seeds from Migori seed lot had the lowest germination percentage (30%) compared to both Busia and Siaya seed lots (70%). A cut test on non-germinated seed at the end of the experiment showed high viability 90% in all the seed lots tested (Table 3:2).

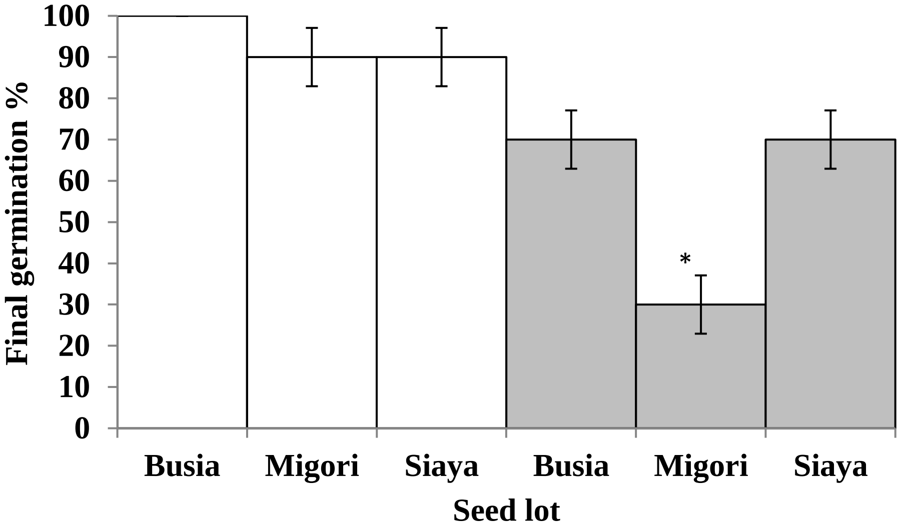

Figure 3:4: Germination percentages of scarified (clear bars) and non-scarified (grey bars) seeds of *Tylosema fassoglense* incubated under 12/12 hr photoperiod and 25 °C. Bar graph with asterisk (*) differ statistically (*$p<0.05$*, n=4) and vertical error bars are the standard error (±se)

3.6.4 Role of light on germination

Seed germination under the two photoperiods of light/dark and total darkness was not significantly (*$p>0.05$*) different among the seed lots. The final germination percentage of *T. fassoglense* seeds under both light/dark and under darkness was over 50% (Figure 3:5). Similarly, seed germination sensitivity to light determined by calculating relative light germination index, RLG ranged from 0.46 (Busia seed lot) to 0.57 (Migori seed lot)

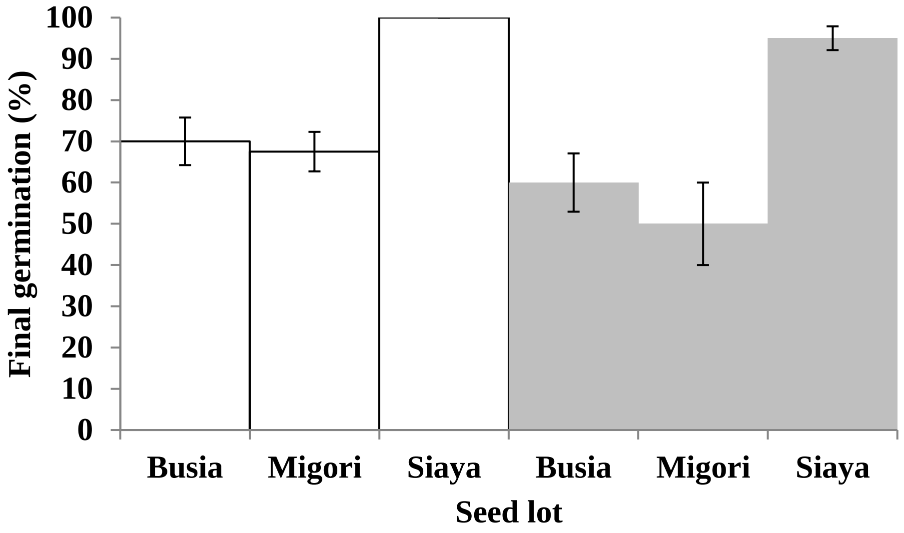

Figure 3:5: Germination percentages of scarified seeds of *Tylosema fassoglense* seed lots under 12/12 hour (clear bars) and 0/24 hour (grey bars) photoperiod. No significant (*p>0.05*, n=4) difference between the two photoperiods and vertical error bars are standard error (±se)

3.7. Discussion

3.7.1 Seed dormancy and dormancy-breaking

This study has demonstrated that seed scarification is effective in breaking dormancy in *T. fassoglense*. It confirms that *T. fassoglense* seeds have moderate physical dormancy (Baskin and Baskin, 2014). In addition, variation in physical dormancy was also observed in *T. fassoglense* seeds collected from different populations. Physical dormancy increases chances of seedling survival by promoting temporal distribution of seed germination due to variation in degree of coat permeability and different rates at which dormancy is overcome in a seed population (Baskin and Baskin 2014). Previous studies have been conducted to determine seed dormancy and effectiveness of dormancy breaking pre-treatment in legume species (Travlos *et al.*, 2007; Long *et al.,* 2012; Abubakar and Muhammad, 2013; Baskin and Baskin, 2014). For example, Abubakar and Muhammad, (2013) reported that scarification of *Tamarindus indica* (Fabaceae- Caesalpinoideae) using 50% sulphuric acid for 30 minutes resulted in higher germination percentage compared to hot water (100 °C) treatment for 30 minutes and the control. Travlos *et al*., (2007) reported that mechanical scarification using sandpaper, soaking seeds in cold water for 20 hours or in concentrated sulphuric acid for 20 minutes greatly increased speed and percentage germination of *T. esculentum* (Fabaceae- Caesalpinoideae). Similarly, Long *et al*., (2012) reported that scarification improved germination of *Astralagus arpilobus* (Fabaceae- Papilionoideae) from 4% to nearly 100%. The positive response to manual scarification by the three seed lots confirms the presence of some form of physical dormancy in *T. fassoglense.*

On the other hand, physical dormancy variations have been studied on a few legumes (Lacerda et al., 2004; Richard *et al*., 2018; Hradilová *et al*., 2019). According to Richard *et al*., (2018) variation in “hard seededness” and seed coat thickness in three seed lots of *Desmanthus virgatus* (Fabaceae- Mimosoideae) was linked to the seed source and maternal environment. Hradilová *et al*., (2019) reported variation in germination (ranging between 0-100%) and seed coat thickness (80-140 mm) across the different environment envelopes in 96 accessions of wild pea (*Pisum sativum* sbsp. elatius). Similarly, Lacerda *et al.,* (2004) reported dormancy variation in *Senna multijuga* (Fabaceae- Caesalpinioideae) and *Plathymenia reticulata* (Fabaceae- Mimosoideae) at population level with seed germination of 9-35% and 40-62% respectively for non-scarified seeds and germination improved >84% for scarified seeds. Among the seed lots of *T. fassoglense*, there was variation in seed water imbibition and final germination percentage between Migori seed lot and those of Busia as well as Siaya seed lots. Since non-scarified seeds from both Busia and Siaya seed lots had high and similar germination percentage, we concluded that Migori seed lot may possess more dormancy. The prevailing temperatures during seed filling phase strongly affects seed coat pigmentation and dormancy (Toorop *et al.,* 2012; MacGregor *et al.,* 2015; Springthorpe and Penfield, 2015). For example, Toorop *et al.,* (2012) reported a transition in seed coat colour during seed set that alters dormancy and correlates with flowering in *Carpsella bursa-pastoris.* Similarly, MacGregor *et al.,* (2015) reported seed coat pigmentation is affected by temperature during seed development in *Arabidopsis sp.* Additionally, Springthorpe and Penfield, (2015) reported temperature during seed set strongly affects seed dormancy in *Arabidopsis thaliana*, concluding that lower temperatures almost always results in lower germination. Besides temperature, seed collection year and storage duration are known to influence seed dormancy even when working with same population (Lacerda *et al*., 2004; Penfield and MacGregor, 2017). The observed difference in dormancy level between Migori seed lot and Busia as well as Siaya seed lots may be attributed to the prevailing temperatures during seed filling phase since seeds from Busia and Migori were collected and stored for similar duration. The monthly maximum temperatures preceding seed maturity in Migori were 1 °C lower than both Busia and Siaya. However altitude, salinity, nutrient levels and genotype can also affect dormancy level but were not considered in this experiment (Fenner, 1991; Wang *et al.,* 2012). Future studies on flowering and fruiting phenology as well as genetic diversity among several populations may shade more light on this observation. Hard seed coat is an undesirable trait in agricultural production and in food processing, therefore in selecting suitable populations for domestication Busia and Siaya seed lots would be the preferred.

3.7.2 Sensitivity to light presence

Regarding light, this study has demonstrated that *T. fassoglense* seeds are probably neutral photoblastic (Baskin and Baskin, 2014). Seeds of *T. fassoglense* incubated under both light/dark and dark conditions had good germination percentages. Several species have been shown to be indifferent to light, however a small proportion exist in a seed population of neutral photoblastic that require light for germination (Fenner and Thompson, 2005). For example, Sánchez-Bayo and King, (1994) reported seeds of *Acacia horrida* (Fabaceae- Mimosoideae) responded with indifference to light. Motsa *et al.*, (2015) reported light positively affected germination of *Brassica rapa* L. subsp. Chinensis, *Citrullus lanatus* Thunb., *Solanum retroflexum* Dun. seed and optimal germination would occur if seeds were sown at or near soil surface. The results in this study indicate that seeds of *T. fassoglense* are probably neutral photoblastic and therefore can germinate under light/dark or dark conditions while burial sowing under agricultural ecosystem is practicable. However, further studies involving more light regimes may provide better understanding on this observation.

In addition, seed mass and shape has also been shown to be correlated to germination light requirement and soil seed bank formation for other taxa (Milberg *et al.*, 2000; Thompson *et al*., 1993). The results of *T. fassoglense* seed weight, relative light germination (RLG) index range and seed variance were characteristic of neutral photoblastic species. For example, Milberg *et al*., (2000) reported that germination became less dependent on light with increasing seed mass in 54 species. Gómez-Barreiro *et al.,* (2019) reported light had an impact at constant temperature on germination of *Flacourtia indica* (Salicaceae) based on relative light germination index. Thompson and Hodgson, (1993) reported that seed weighing less than 15 mg and had a variance of less than 0.18 are likely to form soil seed bank in 97 species. Similarly, Flores *et al., (*2016) reported that seed with higher seed mass were less dependent on light for germination in 13 species (10-Asparagaceae sp; 3-Cactaceae). From the results of this study, burial sowing can be practised while cultivating/domesticating *T. fassoglense* and achieve good germination percentage. However, future study should focus on seed germination in relation to sowing depth, which can affect crop emergence and stand establishment. This information will be useful in domestication of *T. fassoglense* since burial seed sowing is the common practise under crop production.

3.8 Conclusion

The results suggest that *T. fassoglense* possess physical dormancy that can potentially affect germination and stand establishment under crop production. Seed dormancy variation was also observed among the three seed lots, a trait that can be exploited in selection during domestication. Scarification positively improved water imbibition and the final germination of *T. fassoglense* seeds which will help improve its propagation. Seeds of *T. fassoglense* are probably neutral photoblastic. This trait is common in cultivated crops and may positively facilitate its domestication since burial sowing is common practise under cropping ecosystem. The information generated in this study can help the propagation and domestication of *T. fassoglense* to benefit small-holder farmers of Kenya by supporting food security and sustainable agriculture.

CHAPTER FOUR

4.0 Germination response to temperature and water potential of Sprawling bauhinia (*Tylosema fassoglense)*

4.1 Abstract

The knowledge of cardinal parameters is critical for understanding environmental tolerance limits for germinating plant species. However, this information is not readily available for *Tylosema fassoglense* [Family: Fabaceae]; despite its potential as a future crop. The findings of the study was primarily intended to benefit small-holder farmers of Kenya, by improving nutrition and enhancing sustainable agriculture while contributing to good health and well-being. The objective of this study was to characterize seed germination responses to temperature and water potential of three seed lots of *T. fassoglense.* Seeds were collected from Busia, Migori and Siaya counties in Kenya. Scarified seeds were germinated at a wide range of constant temperatures from 10 to 45 °C with intervals of 5 °C and water potentials from 0, -0.25, -0.5, -1.0, -1.5 Megapascal (MPa) at 25 °C. Final germination percentage (normalized) and germination rates were analyzed by ANOVA and means separated with Tukey's HSD or Duncan's multiple range test ($p<0.05$). Seeds of *T. fassoglense* had a wide range of germination temperature of 20 °C (from 15 to 35 °C). Low (10 °C) and high (≥ 40 °C) temperatures significantly reduced germination in the three seed lots. Busia seed lot had the lowest final germination of 5% at 10 °C while germination was zero at 45 °C in all the seed lots tested. The calculated base, optimal and ceiling temperatures (T_b, T_o and T_c) ranges were 4.05 - 8.0 °C; 33.61 - 35.75 °C; and 46.54 - 47.24 °C, respectively. Suboptimal and supraoptimal thermal time to 50% germination, ($\theta_{T(50)}$) ranged between 76.19 – 89.02 °C d (degree Celsius days) and 30.3-41.89 °C d respectively. Water potential of -0.5 MPa significantly reduced final germination to less than 50% in all seed lots while germination was zero at both -1.0 and -1.5 MPa. The base water potential (Ψ_b) and cumulative hydro time to 20% germination ($\theta_{H(20)}$) was between -0.92 to -0.97 MPa and 3.95-4.11 Megapascal days (MPa d) respectively. The optimum temperature range for germination was 30-35 °C and seeds were tolerant to low water potential up to -0.5 MPa. However, further studies will need to be carried out on germination in the supraoptimal temperature range and water potential between 0 and -1.0 MPa. The findings in this study will be useful in future research contributing to the cultivation and domestication of *T. fassoglense.*

Keywords: cardinal parameters; potential crop; *Tylosema fassoglense*

4.2 Introduction

Tylosema fassoglense (Fabaceae) is a perennial tuberous vine endemic to sub-Saharan Africa (SSA) (Castro *et al*., 2005) with immense potential for domestication. *Tylosema fassoglense* seeds are a good source of protein and oil, while the tuber is rich in starch. The large cotyledons are rich in protein (20-40%), lipids (20-30%), and minerals (Dubois *et al*., 1995; Brink, 2006; Okumu, 2011; Otieno *et al.,* 2015). The seed has been consumed for many millennia in Kenya (Maundu *et al.,* 1999) and may be a source of protein in many subsistence diets and potential uses in modern nutraceutical. The seed oil has nutritional value and might have industrial and pharmaceutical use. Young tubers have been exploited as source of food and water during drought. The large tubers also act as water reservoir for the plant during drought when the above ground biomass is dead. *Tylosema fassoglense* is well adapted to semi-arid and arid regions of sub-Sahara Africa (Castro *et al*., 2005; Sinou *et al.,* 2009). Research conducted on *T. fassoglense* has focussed on its economic importance and potential uses with little emphasis on its propagation. However, information on seed germination traits and seedling establishment which are important in plant production cycle under different pedoclimates remains unavailable for this species. The understanding of the ability of this legume species to complete this important stage and environmental tolerance limits is crucial in its domestication.

The two primary environmental factors that control seed germination rate and final percentage are temperature and water potential (Baskin and Baskin, 2014). Temperature influence seed dormancy cycling (initiation and termination) and both the rate and final germination percentage (Baskin and Baskin, 2014). Under crop production extreme temperature can decrease emergence and consequently yield and quality. Seed germination response to temperature can be examined through the final germination percentage and germination rate (Dürr *et al*., 2015). The range of temperatures favourable for seed germination of a particular species can be described by cardinal temperatures: base temperature (T_b) and ceiling temperature (T_c) below and above which germination-physiological process do not proceed to completion and therefore germination is null and optimal temperature (T_o) at which germination is fastest as well as thermal time ($\theta_{T(G)}$) that is the heat units in degree days a seed population (G) accumulates to germinate (Garcia-Huidobro *et al*., 1982). The cardinal temperatures are known to be species-specific and are valuable tools in predicting plant-climate relationships, analysing stand establishment, species modelling and weed establishment (Trudgill *et al.,* 2005; Hardegree, 2013; Dürr *et al*., 2015). However, intra- and inter-species variations reflect the different climate and ecogeographic region of the seed (Daws *et al*., 2008). Several studies have characterized seed germination in relation with temperature response in many crops, trees, shrubs and herbs (Covell *et al.*, 1986; Ellis *et al.,* 1986; Raveneua *et al*., 2011; Hu *et al.,* 2015; Zhang *et al.,* 2015; Tribouillois *et al*., 2016; Castillo-Lorenzo *et al*., 2019a, b), however such information has not been generated for a number of species including *T. fassoglense.*

Similarly, water availability is another environmental factor that controls both seed germination and rate at optimal temperature and is dependent on the difference between soil water potential and the seed (Baskin and Baskin, 2014). Low soil water potential can potentially decrease emergence and eventually crop yield and quality. Grain legumes have demonstrated ability to be tolerant to production under moisture stressed environment (Cullis *et al.*, 2018), however seed germination is quite sensitive to water availability. Seed germination response to water potential can be quantified using hydro model through germination percentage and rate (Gummerson, 1986; Bradford, 2005). The range of water potential suitable for seed germination of a particular species can be characterised through theoretical threshold (Ψ_b) below which germination-physiological process do not proceed to completion and therefore germination is zero and hydro time ($\theta_{H(G)}$) in Megapascal days needed for a given seed population (G) to germinate (Bradford, 2005). The base water potential (Ψ_b) is known to be species-specific and is useful in predicting germination progress and planting time while intra- and inter-species variation is often associated with seed ecogeographic region (Hu *et al.,* 2015). The hydro time model predicts germination under varied water potential for cultivated crop, crop wild relatives and a few tree species (Zuk-Golaszewska *et al.,* 2007; Raveneau *et al*., 2011; Aguilar-Benitez *et al.,* 2014; Hu *et al*., 2015; Durr *et al*., 2015; Castillo-Lorenzo *et al*., 2019a, b). However, no data has been generated for neglected and underutilized legumes such as *T. fassoglense* despite its' potential to benefit smallholder farmers by improving nutrition and enhancing sustainable agriculture in Kenya.

The objective of this study was to quantify the response of germination to temperature and water potential and to calculate germination parameters; T_b, T_o, T_c and $\theta_{T(G)}$; Ψ_b and $\theta_{H(G)}$ for three seed lots of *T. fassoglense*.

4.3. Materials and Methods

4.3.1 Seed lot information

The seeds lots of *T. fassoglense* used in this experiment to determine germination response to temperature and water potential were the same as those used in chapter three (Refer to: 3.3.1 Seed lot information)

4.3.2 Experiments on seed germination

To avoid working with physically dormant seeds, thermal and hydro experiments were conducted using scarified seeds of *T. fassoglense*. Germination experiments were conducted in germination cabinets (Series 1A: Cool Incubators, LMS, Kent UK) with 10 scarified seeds placed on steel blue germination blotter (Anchor Paper Company, Seed Solutions; Saint Paul, MN, USA) in sandwich boxes (17 x 11 x 5 cm) replicated four times for each seed lot at each of the tested temperature and water potential. When testing for temperatures, the steel blue germination blotter was moistened with 50 ml of deionised distilled water and the sandwich boxes were then wrapped in self-sealing polythene bags to prevent moisture loss. The boxes were then placed randomly in eight germination cabinets operating at constant temperature ranging from 10 °C to 45 °C ±2 °C at intervals of 5 °C and 12/12 hour photoperiod (radiometric flux density 50-100 $W.m^2$). The germination media was kept moistened by adding 20 ml of deionized distilled water when needed and the boxes kept in self-sealing plastic bags.

Whilst testing for water potential, the seeds were kept at constant temperature (25 °C) to limit temperature effect on germination time between seed lots. Four concentrations of polyethylene glycol, PEG-8000 (M.W. 7000-9000; specific gravity 1.2±0.1; pH, (5% water) 6-7; Fischer Scientific, Belgium) (not verified with osmometer) were created according to Michel and Kaufmann (1973) following the equation (Eq. 4.1) by Hardegree and Emmerich (1990)

$$\psi = 0.130[\mathrm{PEG}]^2\mathrm{T} - 13.7[\mathrm{PEG}]^2 \qquad \text{(Eq. 4.1)}$$

Ψ = water potential of a solution of PEG 8000 (molecular weight of 8000 g/mol) in water (MPa), [PEG] = concentration of PEG in water (g PEG 8000/g $H_2O_{(l)}$) and T = temperature in degrees Celsius (°C) at which the solution will be used. For different water potentials PEG-8000 was weighed and dissolved in pre-measured volume of water (density of water = 1 g/ml) by heating on hot plate constantly stirred by magnetic stirrer as follows: -0.25 MPa (123.68 g PEG/800 g H_2O); -0.50 MPa (174.99 g PEG/800 g H_2O), -0.10 MPa (247.48 g PEG/800 g

H_2O) and -1.50 MPa (303.09 g PEG/800 g H_2O). The PEG-8000 solutions were allowed to cool overnight.

The germination media was moistened by adding 50 ml of each [PEG] and water to control before sowing. To maintain relatively constant water potential, 20 ml of [PEG] solution of each concentration was added when needed to the steel blue germination paper and water to the control.

4.3.3 Germination evaluation

Germination was recorded daily for the three seed lots until no more seeds germinated. Seeds were considered germinated, if the radicle protruded more than 2 mm and the germinated seeds were allowed to develop into normal seedling in the sandwich boxes. According to ISTA (2017), a normal seedling is that which has grown to show essential structures (i.e. cotyledons, hypocotyls and roots) vital for further development into a satisfactory plant and lacks visible abnormalities. Viability of non-germinated seeds were evaluated by cut test and seeds that were firm and full were reported as viable while rotten seed were reported as dead.

4.3.4 Calculations

Seed germination was characterised as germination percentages, GPs and germination rates, GRs. For temperature treatments, cumulative germination progress over time for each seed lot and each replicate (50th percentile) or each temperature were fitted to the Boltzmann distribution function (Eq. 2) using Origin 9.1 software (Origin, 2013) from which the GRs were estimated

$$T_{50} = dx \cdot \ln\left(\frac{A_1 - A_2}{Y_{50} - A_2} - 1\right) + X_{50} \qquad \text{(Eq. 4.2)}$$

where *d*x= slope of the curve, A_1= germination at t=0 (and therefore is zero), A_2= estimated final germination, Y_{50}= 50^{th} percentile of the maximum germination obtained and X_{50}= time to 50% germination

Thermal model quantifies seed germination responses to temperature (T) according to equations 4.3 and 4.4 (Garcia-Huidobro *et al.*, 1982). For temperature, GR was calculated for each replicate at (GR: $1/t_{50}$), the reciprocal of the time the population (g) required to attain a percentage germination of viable seed. When the GRs were regressed against temperature, a positive (suboptimal temperatures) or negative (supraoptimal temperatures) regression lines were obtained. The intersect of the two regression lines defined the optimal temperature (T_o)

where GR is highest and hence germination is fastest (Eq. 4.5). The T_b and T_c were estimated from the intercept of both regression lines where GR is zero and hence below and above these temperatures germination-physiological processes do not proceed to completion (Garcia-Huidobo *et al.*, 1982; Covell *et al.*, 1986).

For thermal analysis, data was separated into a suboptimal and a supraoptimal temperature range using the temperature with the highest GR. Thermal time ($\theta_{T(50)}$) was calculated as the inverse of the regression slopes described by $(1 / \mathrm{t_g} = ax + b)$ (Gummerson *et al.*, 1986).

$$\theta_{\mathrm{T(g)}} = (\mathrm{T} - \mathrm{T_b})\mathrm{t_g} \qquad \text{(Eq. 4.3)}$$

$$\mathrm{GR_g} = 1 / \mathrm{t_g} = (\mathrm{T} - \mathrm{T_b}) / \theta_{\mathrm{T(g)}} \qquad \text{(Eq. 4.4)}$$

$$T_{opt} = (\alpha.T_b + \beta.T_c)/(\alpha + \beta) \qquad \text{(Eq. 4.5)}$$

Where $\theta_{\mathrm{T(g)}}$ is the cumulative heat unit (degree Celsius days, °C d) a seed population requires for a given fraction (g) to complete germination in time (t); T, T_b, T_{opt} and T_c is the incubation, base, optimum and ceiling temperatures respectively and α and β are slope parameters.

Similarly, the hydro time model quantifies seed germination responses to water potential (Ψ) according to equations 4.6 and 4.7 (Gummerson, 1986; Bradford, 2005). Cumulative germination progress over time for each seed lot and for each replicate (20th percentile) was fitted to the Boltzmann distribution function as for temperature threshold above.

When the GRs (20th percentile since most of the germination were <50%) were regressed against the water potential values, a positive regression line was obtained and the intercept estimated the base water potential, $\Psi_{b(20)}$, at which germination-physiological progress does not proceed to completion and hence germination is zero and the inverse of the slope estimated the hydro time ($\theta_{H(20)}$).

$$\theta_{\mathrm{H(g)}} = \left(\psi - \psi_{\mathrm{b(g)}}\right)\mathrm{t_g}$$

(Eq. 4.6)

$$\mathrm{GR} = 1 / \mathrm{t_g} = \left(\psi - \psi_{\mathrm{b(g)}}\right) / \theta_{\mathrm{H}} \qquad \text{(Eq. 4.7)}$$

Where $\theta_{H(g)}$ is the hydro time (Megapascal days, MPa d) a seed population requires for a given fraction (g) to complete germination, Ψ is the actual water potential of the germination medium (MPa), $\Psi_{b(g)}$ is the base water potential below which germination-physiological processes of seed fraction g do not proceed to completion and hence germination is zero, and t_g is the germination time (days) of the corresponding fraction g.

4.4 Data analysis

Germination data were arcsine transformed and analysis of variance (ANOVA) performed for each seed lot and temperature (T) or water potential (Ψ). The means were compared using Tukey's HSD test for final germination and cardinal parameters and Duncan's multiple range test for GR ($1/t_{50}$ or$1/t_{20}$) ($p<0.05$). Data was analysed using SAS Version 2002-2003 (SAS Institute Inc. Cary, NC, USA).

4.5. Results

4.5.1 Seed germination response to temperature

Cumulative seed germination dynamics was greatly influenced by germination temperature in all the three seed lots (Figure 4:1, 2, 3). The three seed lots of *T. fassoglense* started germinating earlier (2 to 6 days after sowing) at temperatures varying from 15 °C to 40 °C attaining a high final germination percentage faster (4 to 12 days after sowing) than at other temperatures. Seed lots from Busia and Migori attained the highest germination at 15 °C compared to other temperatures. The three seed lots of *T. fassoglense,* kept at 10 °C began to germinate later (24 days after sowing) and attained a final germination after 54 days. The number of days to germination decreased with increase in temperature up to optima and then increased with further increase in temperature.

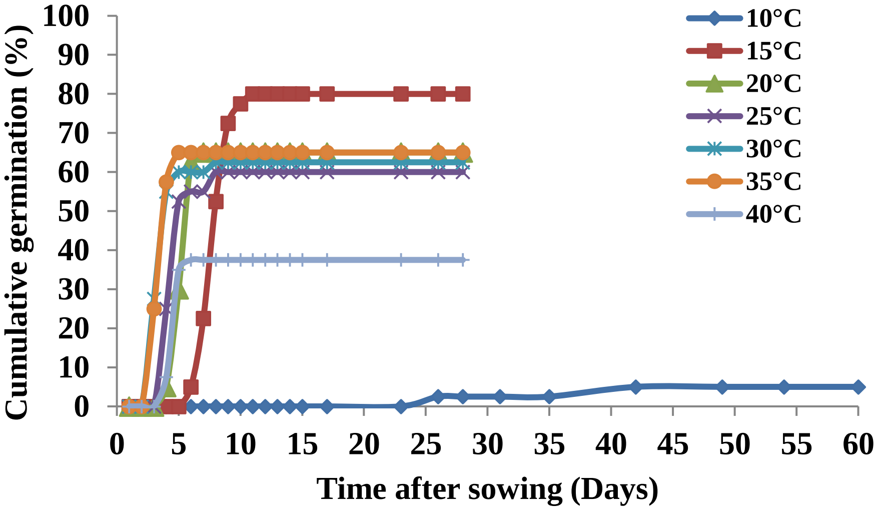

Figure 4:1: Cumulative germination time course of *Tylosema fassoglense* seeds germinated at different temperatures under 12/12 hour photoperiod for Busia seed lot.

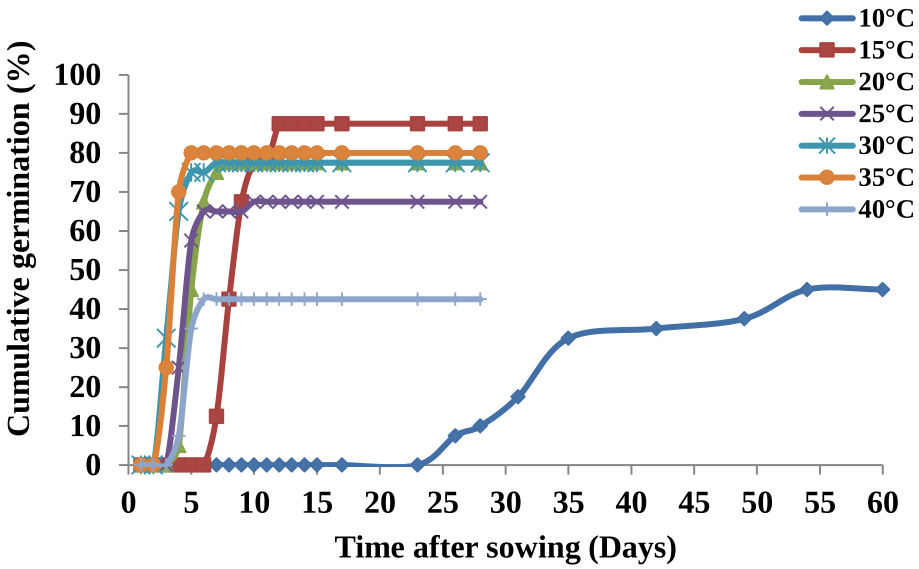

Figure 4:2: Cumulative germination time course of *Tylosema fassoglense* seeds germinated at different temperatures under 12/12 hour photoperiod for Migori seed lot.

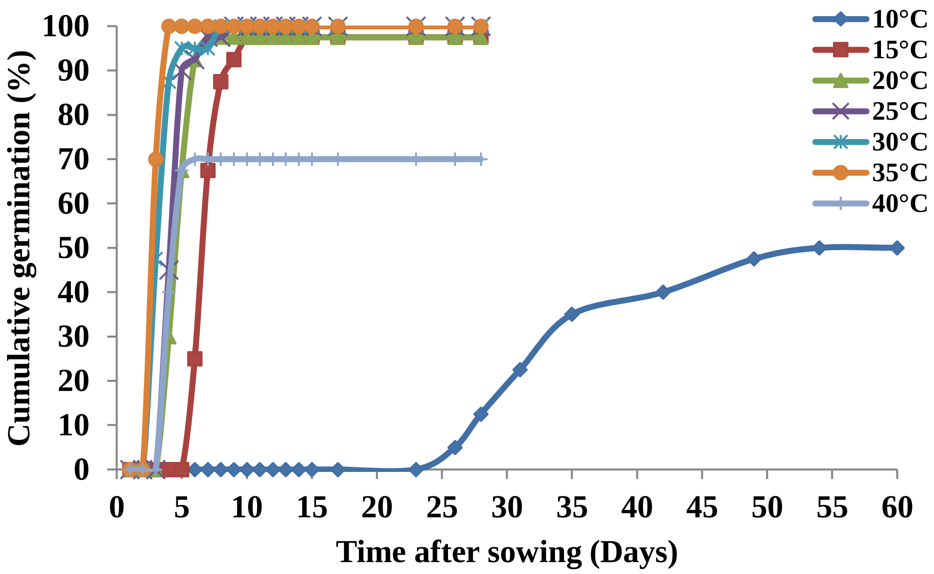

Figure 4:3: Cumulative germination time course of *Tylosema fassoglense* seeds germinated at different temperatures under 12/12 hour photoperiod for Siaya seed lot.

The final germination percentages were statistically similar at temperature ranges from 15 °C to 35 °C at the seed lot level, which sharply declined at 10 °C or 40 °C (Figure 4: 4, 5, 6). The final germination was significantly reduced ($p<0.05$) at 10 °C and 40 °C in both Busia and Siaya seed lots. However, in Migori seed lot the final germination percentage was statistically similar at 10 °C and 40 °C. The mean final germination was above 65% for both Busia and

Migori and 98% for Siaya seed lot at temperatures ranging from 15 °C to 35 °C. In addition, all the seed lots did not germinate at 45 °C. Busia seed lot was the most sensitive to low temperature with 5% germination at 10 °C. It was also observed that seeds germinated at 15 °C and 20 °C had lower fungal infection in all the three seed lots than at higher temperatures.

Generally, the seed germination rate of the three seed lots increased linearly from 10 to 35 °C and then decreased as temperature increased (Figure 4: 4, 5, 6). The highest germination rates GRs, (50th percentile) for the three seed lots was observed between 30 °C and 35 °C (that is 0.311/day, 0.295/day and 0.337/day for Busia, Migori and Siaya seed lots respectively). Below or above these temperatures the GR decreased in all the three seed lots. The germination rate of Siaya seed lot was the fastest, while Migori slowest at optimal temperature.

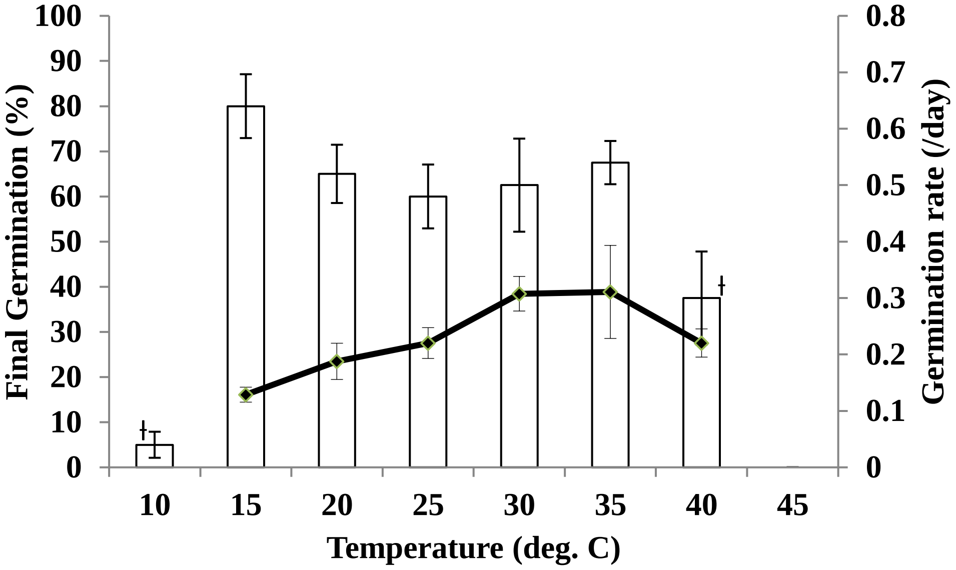

Figure 4:4: Final germination percentage (▭) and germination rate (— 1/t50) of *Tylosema fassoglense* seed germinated at varied temperature for Busia seed lot. Points with * and ƚ are significantly (*p<0.05*) different and vertical bars are standard error (±se)

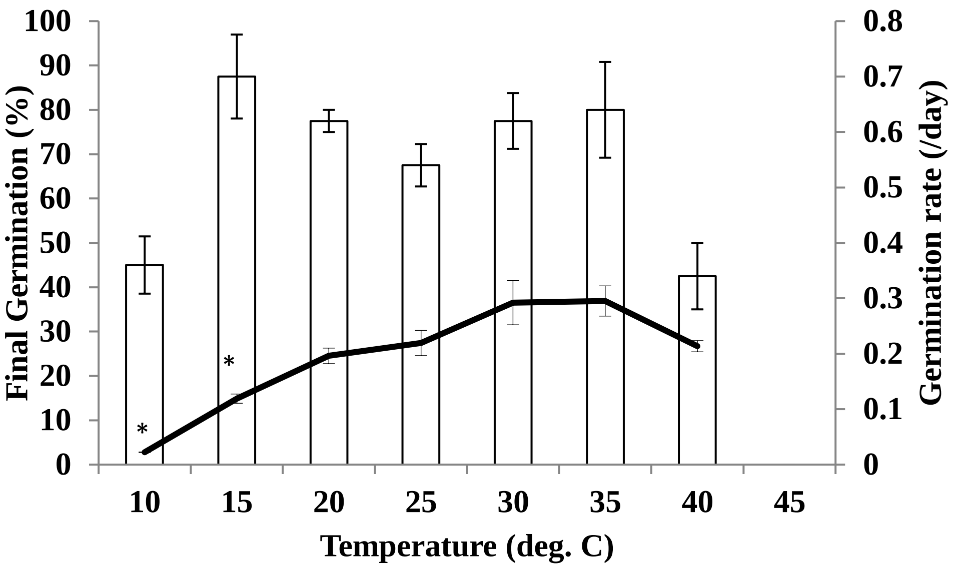

Figure 4:5: Final germination percentage (▭) and germination rate (— 1/t50) of *Tylosema fassoglense* seed germinated at varied temperature for Migori seed lot. Points with asterisks (*) are significantly (*p<0.05*) different and vertical bars are standard error (±se).

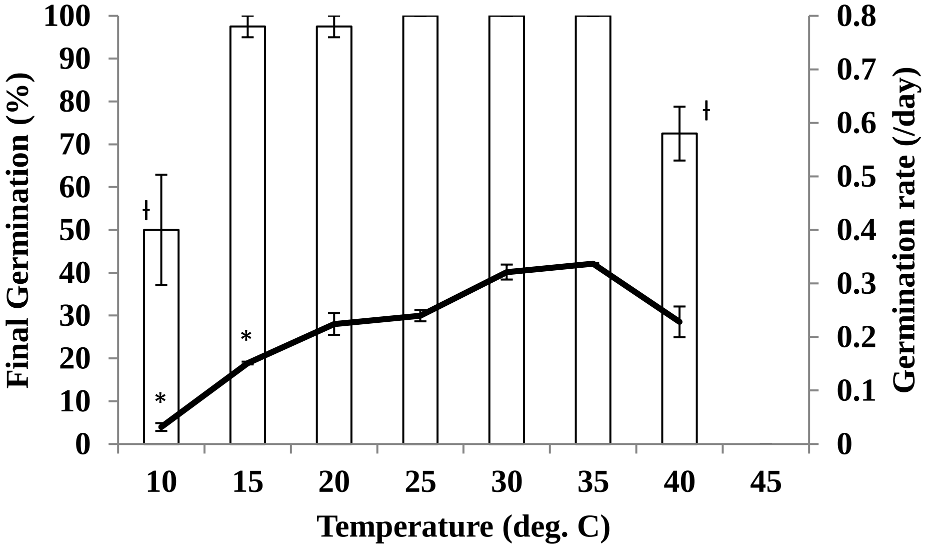

Figure 4:6: Final germination percentage (▭) and germination rate (— 1/t50) of *Tylosema fassoglense* seed germinated at varied temperature for Siaya seed lot. Points with * and ƚ are significantly (p<0.05) different and vertical bars are standard error (±se).

The fits of GR 50th percentile for each replicate (Supporting data Appendix 2) allowed for precise determination of cardinal temperatures: T_b, T_o, T_c; and $\theta_{T(50)}$ (Table 4:1). Inter population seed germination traits of the three seed lots of *T. fassoglense* showed variation in cardinal temperatures. The mean base temperature (T_b) for germination differed ($p<0.05$)

statistically between Siaya and Busia seed lots. The T_b for Migori seed lot was statistically similar to both Busia and Siaya seed lots. Base temperature varied by 3.95 °C ranging from 4.05° (Siaya seed lot) to 8.0 °C (Busia seed lot). The mean optimal temperature (T_o) did not differ ($p>0.05$) significantly among the three seed lots tested. The optimal temperature, T_o ranged from 33.61 °C (Busia seed lot) to 35.75 °C (Siaya seed lot). The mean ceiling temperature (T_c) was not statistically ($p>0.05$) different among the three seed lots and ranged between 46.54 °C (Siaya seed lot) to 47.24 °C (Busia seed lot). The mean thermal time ($\theta_{T(50)}$) was not significantly ($p>0.05$) different among the seed lots tested for both the suboptimal ($\theta_{Tsuboptimal}$) as well as supraoptimal ($\theta_{Tsupraoptimal}$) temperature range (Table 4:2). Generally, Siaya seeds were slower to germinate (longer $\theta_{T(50)}$) whilst Busia seeds were faster to germinate (shorter $\theta_{T(50)}$) in terms of θ_T in the suboptimal temperature range.

Table 4:1: Seed germination thresholds for three seed lots of *Tylosema fassoglense*; Cardinal temperatures (base T_b, optimal T_o and ceiling T_c) and thermal time (θ_T) in degree Celsius days (°Cd) at suboptimal and supraoptimal range for four replicates at 50th percentile ($1/t_{50}$)

Seed Lot	**Cardinal Temperatures (°C)**			**Thermal time (°Cd)**	
	T_b	**T_o**	**T_c**	**$\theta_{Tsuboptimal}$**	**$\theta_{Tsupraoptimal}$**
Busia	8.00±1.59a	33.61±2.27a	47.24±1.90a	76.19±8.47a	41.89±17.61a
Migori	6.40±1.59ab	34.21±1.29a	47.07±2.06a	87.07±16.31a	39.34±8.8a
Siaya	4.05±0.66b	35.75±0.27a	46.54±0.2a	89.02±3.79a	30.3±0a

Figures in the same column with different letters are statistically different ($p<0.05$, n=4) and standard error (±se)

4.5.2 Seed germination response to water potential

Similarly, water potential affected germination of the scarified seed of *T. fassoglense* from the three seed lots (Figure 4: 7, 8, 9). The number of days to germination increased with decrease in water potential. For example, seed germination commenced three days after sowing at 0 MPa but days to germination increased to between six and ten days at -0.25 MPa and -0.5 MPa across the three seed lots. Seed germination for the three seed lots was zero at water potentials -1.0 MPa and -1.5 MPa for the entire period of incubation.

The final germination percentage of all three seed lots declined with decrease in water potential. Final germination percentage differed ($p<0.05$) statistically between water potentials of 0 MPa and -0.5 MPa in the three seed lots tested (Figure 4:10). However, the final germination was not significantly ($p>0.05$) different for Busia and Migori seed lots at water potentials 0 and -0.25 MPa. For example, at 0 MPa final germination was 60, 68 and 100% but declined to 33, 15 and 45% at -0.5 MPa for Busia, Migori and Siaya seed lots respectively.

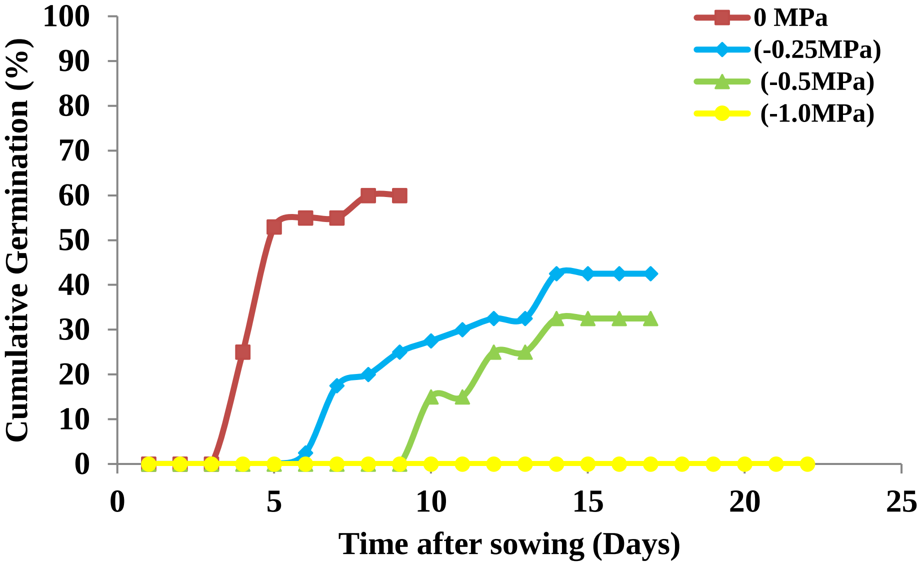

Figure 4:7: Cumulative germination time course of *Tylosema fassoglense* seeds germinated at different water potentials, 25 °C and 12/12 hour photoperiod for Busia seed lot.

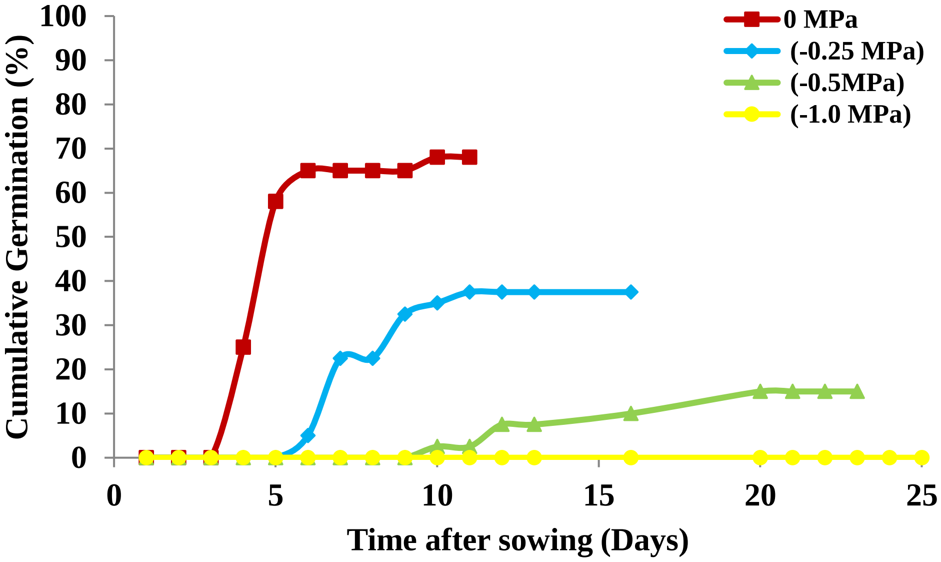

Figure 4:8: Cumulative germination time course of *Tylosema fassoglense* seeds germinated at different water potentials, 25 °C and 12/12hour photoperiod for Migori seed lot

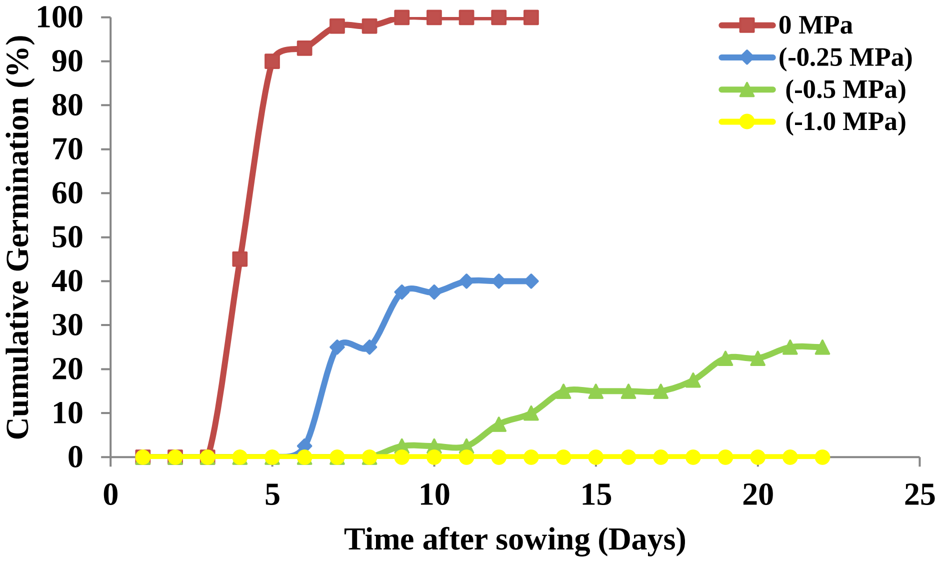

Figure 4:9: Cumulative germination time course of *Tylosema fassoglense* seeds germinated at different water potentials, 25 °C and 12/12hour photoperiod for Siaya seed lot

Seed germination rate GR (20th percentile) decreased with water potential in all the three seed lots of *T. fassoglene,* which enabled determine the base water potential (Supporting Data Appendix 3). The germination rate was significantly ($p<0.05$) different between the different water potential for each seed lot (Table 4:2). For example, the mean germination rate for Busia, Migori and Siaya populations decreased from 0.255/day, 0.256/day and 0.267/day at water potential 0 MPa to 0.059/day, 0.087/day and 0.08/day at -0.5 MPa respectively. Generally, GRs were highest at 0 MPa but decreased at more negative water potentials.

Table 4:2: Mean seed germination rate at 20th percentile for four replicates of *Tylosema fassoglense* seed from three seed lots germinated at different water potentials

Seed lot	**Water potential (MPa)**				
	0	**-0.25**	**-0.5**	**-1.0**	**-1.5**
Busia	0.255a	0.112b	0.059c	0	0
Migori	0.256a	0.131b	0.087c	0	0
Siaya	0.267a	0.142b	0.08c	0	0

Figures in the same row with different letters are significantly ($p<0.05$, n=4) different. GRs at -1.0 MPa and -1.5 MPa were assumed to be zero since no seed germinated.

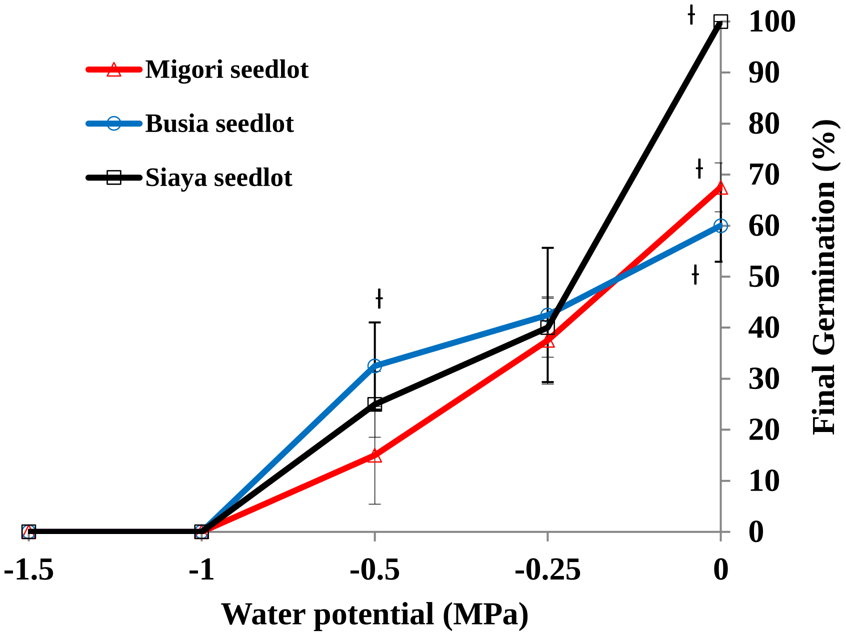

Figure 4:10: Effect of varied water potential on final germination percentage of *Tylosema fassoglense* from three seed lots. Points with differ (p<0.05) statistically at seed lot level, error bars are standard error (±se)

The estimated base water potential $\Psi_{b(20)}$ (20^{th} percentile) were staitistically similar ranging from -0.92 (Busia seed lot) to -0.97 MPa (Migori seed lot) (Table 4:3). The hydro time to 20% germination ($\theta_{H(20)}$) was also statistically similar ranging from 3.95±0.04a (Siaya seed lot) to 4.11±0.03 (Migori seed lot). In this case the three seed lots would respond in a similar way under low water potential and germinate within the same duration (hydro time).

Table 4:3: Hydro time model parameters (base water potential and hydro time) for the three seed lots of *Tylosema fassoglense* calculated at the $1/t_{20}$ germination percentile

Seed lot	Hydro thresholds	
	Ψ_b(MPa)	**θ_H(MPad)**
Busia	-0.92±0.00a	3.96±0.08a
Migori	-0.97±0.03a	4.11±0.03a
Siaya	-0.95±0.03a	3.95±0.04a

Figures in the same column with similar letters are not significantly different (*p>0.05,* n=4) and standard error (±se)

4.6. Discussion

4.6.1 Thermal threshold

This study has demonstrated that the cardinal temperature range for time to 50% germination of *T. fassoglense* for the three seed lots examined were affected by temperature and germination declined or ceased outside the optimal range of 30 to 35 °C. Both high (40 to 45 °C) and low (10 °C) temperatures negatively impacted on final germination percentage. In addition, inter population variation in seed germination base temperature, T_b was also observed among the three seed lots tested.

The cardinal temperatures and $\theta_{T(50)}$ were determined, and comparison made for three seed lots of *T. fassoglense.* These important data were not available in literature for this species, despite its potential for domestication. The trends observed in *T. fassoglense* seed germination in response to several temperatures and cardinal temperatures were generally consistent with those of other legume species (Covell *et al*., 1986; Ellis *et al.*, 1986; Raveneau *et al*., 2011; Dürr *et al*., 2014; Motsa *et al*., 2015; Tribouillois *et al*., 2016; Castillo-Lorenzo *et al*., 2019). For example, Covell *et al*., (1986) estimated T_b for four grain legumes ranged between 0.0 °C (chick pea) and 8.5 °C (cowpea) and Tribouillois *et al*., (2016) while studying several species in the family Fabaceae, reported T_b range of 0.0 °C (*Onobrychis viciifolia*) and 7.3 °C (*Pisum sativum PFX*). Moreover, Raveneau *et al*., (2011) reported a range of -2.0 °C (Pea cultivar) and 9.6 °C (Bean cultivar). Similarly, Hu *et al*., (2015) comparing germination of Fabaceae species from two habitats, reported T_b range of -0.6 °C (*Vicia sativa*) and 8.0 °C (*Sophora alopecuroides*). The calculated T_b was lower than the proposed ≥10 °C for tropical species (Trudgill *et al.* 2005) and the intra-species variation was close to 3.5 °C observed by Moot, (2000) in pasture species. The T_b observed in *T. fassoglense* depicts adaptation to tropical climate and variation may be associated with population/genotype, age of seed and generally seed quality of the seed lots. However, further investigation needs to be conducted on this species since literature has shown marked differences in threshold temperatures for germination and seedling growth (Brunel *et al*., 2011; Raveneau *et al*., 2011). In addition, the estimated T_b for Busia seed lot might not be the best since germination at 10 °C was quite low leading to assumption of GR to be zero. Therefore, investigation at more temperatures between 10° and 15 °C needs to be conducted for this seed lot.

The calculated T_o range and T_c were similarly high and comparable to tropical grain legumes. The relatively high T_o and T_c for crops have been associated with human selection for adaptation to the wide range of environmental conditions inhabited by man (Dürr *et al*., 2014). For example, Covell *et al*., (1986) estimated T_o in four legume species ranged between 31.8-33.0°C (Chickpea) and 34.0-34.5 (Soybean). Tribouillois *et al*., (2016) reported a range between 20.0±1.1 (*Vicia villosa*) and 31.8±2.2 (*Lens nigricans*) while studying several legumes. Similarly, Hu *et al.*, (2015) reported a range between 19.0±0.2°C (*V. unijuga*) and 31.5±0.5°C (*Glycyrhiza uralensis*) while comparing species in Fabaceae family from two contrasting habitats. Additionally, Covell *et al.*, (1986) estimated T_c of four legume crop in the range of 31.8-34.4°C (Lentil) and 48.0-60.0°C (Chickpea) and Hu *et al*, (2015) reported T_c in the range of 31.1±0.4°C (*V. angustifolia*) and 36 °C (*Amopiptanthus mongolicus).* The *T. fassoglense* seeds germinated at 40 °C failed to convert into normal seedlings due inhibition of hypocotyls elongation. Moreover, the seeds did not germinate at 45 °C despite a higher estimated T_c and this may be associated with thermal inhibition at this temperature. However, there is need for further investigation at temperatures between 35 °C and 45 °C to generate more data points for a more precise estimation of T_c and θ_T in the supraoptimal temperature range.

The calculated cumulative thermal time to 50% germination ($\theta_{T(50)}$) in the suboptimal temperature varied among the three seed lots by 12.83 °C d (ranging from 76.19 to 89.02 °C d) and as a measure of seed vigour can be used in selecting germplasm for domestication. According to ISTA, (2017) seed vigour is the ability of a seed lot to germinate and establish under varied environmental conditions. Besides adaptation to varied environments, the performance of a seed lot can be determined by germination rate plus thermal times (Finch-Savage and Bassel, 2015). Domestication (selection) of crops has resulted in shorter thermal time and fastest germination as opposed to wild species that use adaptation mechanisms to spread germination (Dürr *et al.*, 2015; Tribouillois *et al.*, 2016; Castillo-Lorenzo *et al.*, 2019b). For example, Castillo-Lorenzo *et al.*, (2019b) while comparing germination of crop wild relatives and cultivars reported that thermal time for wild sunflower genotypes was greater than 56.25 °C d and less than 34.58 °C d in cultivars. Similarly, Dürr *et al.*, (2015) reported a group of wild species with thermal time greater than 75 °C d and attributed it to underlying dormancy. Generally seed sample from Busia seed lot were faster to germinate (shorter θ_T) compared with Migori and Siaya seed lots, however the three seed lots will reach the thermal time required in same length of time. In the supraoptimal temperature range, the calculated thermal time ($\theta_{T(50)}$) varied but did not differ significantly among the seed lot. The estimates of $\theta_{T(\text{supraoptimal})}$ were calculated with only a few temperatures tested that resulted in fewer data points. Therefore, there is a need to further conduct germination in the supraoptimal temperature range. Generally, the observed variations in cardinal temperatures may be attributed to genotype and underlying environmental factors of the seed production site (see Daws *et al.*, 2008; Hu *et al.,* 2015; Castillo-Lorenzo *et al.,* 2019b). The rate of seed germination under different temperatures, when moisture is not limiting, determines a species ability to establish and gives competitive advantage for limited resources such as nutrients, light and soil moisture. Therefore, in selecting for performance the seed lot from Busia site would be preferred over the other two lots.

This is the first attempt to generate data on seed germination characteristics on thermal thresholds for *T. fassoglense* and comparison made across seed lots. Based on the current findings, in the presence of adequate soil moisture *T. fassoglense* has wide germination temperatures comparable to those of cultivated crops despite being in the wild. This also shows that the species can adapt to cultivation under changing climate scenarios (Walck *et al.*, 2011), since a shift in median temperature (25 °C) will not significantly impact on final germination but only affect days to germination.

4.6.2 Hydro threshold

This study has also demonstrated that the base water potential for time to 20% germination of *T. fassoglense* seeds was affected by water potential and germination declined or ceased at water potential below -0.5 MPa. Additionally, inter-population variations in base water potential and hydro time was also observed.

The calculated Ψ_b and hydro time for *T. fassoglense* seeds were similarly comparable to those of cultivated tropical legumes. These data were not readily available in literature, despite this species huge potential for domestication. Previous studies have reported a wide range of base water potential for other species and intra- or inter-species variations (Daws *et al.*, 2008; Brunel *et al.*, 2011; Hu *et al.*, 2015; Tribouillois *et al.*, 2016; Castillo-Lorenzo *et al.*, 2019a, b). For example, Tribouillois *et al.*, (2016) reported a Ψ_b range of -0.1 MPa (*Vicia faba* var. LAURA) and -2.6 MPa (*Secale cereale*) while Castillo-Lorenzo *et al.*, (2019a) reported a Ψ_b range of -1.5 MPa in *Brassica spp*. from different ecogeographic zones. Hu *et al.*, (2015) reported Ψ_b range of -0.64 MPa (*Vicia amoena*) and -1.36 MPa (*V. sativa*) among Fabaceae species, which differed significantly between species but not between habitats. Similarly, Daws *et al.*, (2008) reported values for 15 neotropical species range of -1.07 MPa (*Clidemia quinquenervia*) to -2.06 MPa (*Apeiba tibourbou*) concluding that species with more negative Ψ_b posses the ability to germinate under drier environment as opposed to those with higher Ψ_b that will germinate in wet conditions.

The non-significant difference in Ψ_b and $\theta_{H(20)}$ of *T. fassoglense* seed lots may be attributed to the fact that seeds have originated from similar ecogeographic region (Daws *et al.*, 2008; Hu *et al.*, 2015; Castillo-Lorenzo *et al*., 2019a). However, sensitivity to low water potential by *T. fassoglense* may be attributed to germination timing to coincide with availability of sufficient moisture coupled with rapid germination (Daws *et al*., 2008; Dürr *et al*., 2015) and formation of mucilage to store extra moisture for germination and seedling establishment. Further investigations need to be conducted with seeds from different geographic locations considering water potentials between 0 and -1.0 MPa to confirm the estimation of hydro parameters and a better understanding of diversity among seed lots.

The hydro and thermal parameters generated for *T. fassoglense* will be critical for domestication especially in understanding the species germination physiology, environmental tolerance limits, timing for sowing and selection of suitable populations with desirable traits. Seed sowing of *T. fassoglense* should therefore coincide with rainfall for good germination and stand establishment.

4.7 Conclusions

The findings of this study indicate that cardinal temperatures and water potential had significant effect on *T. fassoglense* germination rate and final percentage. Seed germination rate was fastest at optimum temperature and high water potential. Seed of *T. fassoglense* had wide range of germination temperature and both high T_b and Ψ_b characterize adaptation to tropical climate that were similar to those of cultivated tropical grain legumes. These parameters are useful tools in species modeling and understanding seedling establishment in crop production. These data will be critical in the cultivation and domestication of *T. fassoglense* especially in predicting days to germination, optimal temperatures and soil moisture for sowing.

CHAPTER FIVE

5.0 Interrelationships among seed functional traits of sprawling bauhinia (*Tylosema fassoglense)*.

5.1 Abstract

Understanding the interrelationships amongst seed physical and germination traits is important in domestication of wild species. *Tylosema fassoglense* [Family: Fabaceae] has the potential as a future crop; however correlations amongst its seed functional traits has not been studied. The objective of this study was to determine the seed physical traits and to test correlations among the seed functional traits. Seed material was collected from Busia, Migori and Siaya counties in Kenya. Seed weight was determined on 100 individual seed while dimensions and oil content were measured on 10 individual seeds. Seed physical traits were compared among the seed lots and correlation was determined among seed functional traits. Percentage data were normalized by arcsine transformation, analyzed by ANOVA and means separated with Tukey's HSD ($p<0.05$). Pearson Correlation moment ($p<0.05$) was tested between physical and germination traits and interrelationships amongst germination traits. Seed sample from Siaya had significantly ($p<0.05$) more oil than both samples from Busia and Migori seed lots while seed mass coefficient of variation was highest in Busia seed lot. Seed sample from Busia were significantly ($p<0.05$) thicker than both samples from Migori and Siaya seed lots. Negative correlations were observed between seed oil content and thermal time to 50% germination ($\theta_{T(50)}$); between seed variance and hydro time to 20% ($\theta_{H(20)}$); and between mass and base temperature (T_b) (however not significant). Negatively significant ($p<0.05$) correlations were observed between T_b and θ_T; base water potential (Ψ_b) and θ_T and non-significant positive correlation between T_b and Ψ_b. Seed source had significant effects on seed mass and dimensions but not on the oil content. Larger seed required more cumulative hydro or thermal time. Seed with high thresholds (T_b and Ψ_b) need high temperature and water potential to germinate. Siaya seed lot had good physical traits while Busia seed lot had good germination traits. The results of correlation among seed functional traits will assist in selection of germplasm with superior traits for domestication and guide future breeding programmes.

Keywords: interrelationships; potential crop; seed traits; *Tylosema fassoglense*

5.2 Introduction

Tylosema fassoglense (Fabaceae) is a perennial vine widely distributed from Ethiopia to Central Africa and South Africa (Castro *et al*., 2005; Brink, 2006). The species produces edible seeds and tubers and is considered a neglected and underutilized species, NUS. The large seeds up to 2.0 g contain protein (20-40%), lipids (20-30%) while the tuber is rich in carbohydrates (Dubois *et al.,* 1995; Okumu, 2011; Otieno *et al.,* 2015). The seeds have been collected from the wild; roasted and consumed as a snack or beverage and roots for medicinal purposes (Maundu *et al.,* 1999). The seed oil and protein has nutritional value and might have industrial and pharmaceutical use (Okumu, 2011; Otieno *et al.,* 2015). The root tubers have been exploited as a source of carbohydrate and water during drought (Brink, 2006). *Tylosema fassoglense* is widely distributed locally from coastal low lands to the drylands and around Lake Victoria basin (Maundu *et al.*, 1999; Otieno *et al*., 2015). Studies previously conducted on *T. fassoglense* have revealed its immense potential for domestication. However, information on diversity on seed functional trait across populations has not been generated. The knowledge of inter-population variation in seed functional traits and interrelationships among seed traits of this potential grain legume is not only important in population selection for germplasm source for domestication but also in future breeding for desirable agronomic traits.

The plant life cycle at some stage is dependent on the major functions of seed; storage, dispersal, germination as well as seedling establishment. The variation in timing for seed germination by different species results in plants colonising different habitats and adapting to varied abiotic conditions during growth and reproduction cycle. The roles of the maternal environment are manifested on the "offspring" through seed morphological and germination traits (Donohue, 2009; He *et al.,* 2014). The abiotic factors that are known to impart maternal effects on seed traits are photoperiodicity, temperature, rainfall, nutritional status of the soil and competition among plants (Baskin and Baskin, 2014).

Seed physical/morphological traits such as size, mass, shape and composition are influenced by maternal environment (Donohue, 2009). Seed mass and shape are functional traits most often studied in relation to seed germination (Fenner, 1991; Ambika *et al*., 2014) but also considered a market quality parameter (Finch-Savage and Bassel, 2015). The most often studied seed germination traits are base temperature T_b, base water potential Ψ_b, cumulative hydro time (θ_H) in Megapascal days (MP d) as well as cumulative thermal time θ_T in degree Celsius days (°C d) (Garcia-Huidobro *et al.,*1982; Bradford, 2005). The T_b and Ψ_b are temperature and water potential respectively below which germination is null and they are known to be species specific. While $\theta_{T(g)}$ and $\theta_{H(g)}$ is the cumulative heat units and water potential units respectively a seed population/percentile (g) requires to germinate. These germination parameters are important in understanding plant-climate relations, predicting seed germination physiology and planning for sowing or conservation programmes (Walck *et al.,* 2011; Hardegree *et al.* 2013). According to International Seed Testing Association, ISTA, (2017) seed vigour is the ability of a seed lot to germinate and establish under diverse physical conditions. However, Finch-Savage and Bassel, (2015) brought the concept of performance which can be measured from germination rate, hydro and thermal times.

The oil stored in seed is a major carbon source for energy production during seedling growth (Bewley and Black, 1994), however its role in relation to seed germination traits has been less analysed. The maternal environment influences seed filling phase and particularly, mean seed mass and seed numbers per inflorescence have been positively correlated with annual rainfall or with mean annual temperature, MAT (Li *et al*., 2017). The prevailing temperature during maturation phase has also been reported to influence seed dormancy (Fenner, 1991; Penfield and MacGregor, 2017). Daws *et al*., (2008) reported a negative correlation between seed mass and both base water potential and cumulative thermal time to 50% germination of neotropical forest species. The correlation between seed physical and germination traits and interrelationships among seed germination traits have been conducted on wild species and crops (Daws *et al.,* 2008; Dürr *et al*., 2015; Gardarin *et al.*, 2016; Castillo-Lorenzo *et al*., 2019a, b). *Tylosema fassoglense* is widely distributed across different agroecological zones (AEZ) in Kenya and therefore variation in seed physical and germination traits were highly suspected. However, literature on the diversity of *T. fassoglense* seed traits (seed mass, shape and oil content) and correlation among seed functional traits remains scanty.

The objective of this study was to determine seed physical traits (mass, oil content, variance) and examine correlation among seed physical traits and germination traits (extracted from Chapter 4) and interrelationship amongst germination traits.

5.3 Materials and method

5.3.1 Seed material information

Seed lots of *Tylosema fassoglense* used in this experiment to assess interrelationships amongst seed traits were the same as those used in Chapter 3 (Refer to: 3.3.1 Seed lot information)

5.3.2 Seed physical traits

5.3.2.1 Seed (Length, width, thickness) and shape

A Carl Zeiss camera was used to take images of 10 dry seeds mounted on a cardboard from different angles for morphological measurements. The measurements recorded for each seed included length, thickness and width. From the images, dimensions were estimated using Axio vision software (Axio Vs40; Carl Zeiss Micro imaging 2010, Germany). Seed shape was expressed as the variance of length, thickness and width according Thompson and Hodgson, (1993) whereby a value of 0 = dimensionless or perfectly spherical and ≥0.3 = needle or disc-shaped. Seed length, thickness and width were first transformed by dividing each by length. Seed shape or variance was obtained as the average of the squared differences from the mean.

5.3.2.2 Seed oil content

The oil content was measured on 10 individual seeds sampled from each seed lot, previously kept in a drying room operating at 15% RH and 15 °C (FAO IPGRI, 1994). The oil content was determined on individual seed cut into small portions (including testa) using secateurs using time-domain nuclear magnetic resonance spectroscopy, TD-NMR (Borisjuk *et al.*, 2011). Seed portions which weighed between 1.4368 g to 2.9352 g were put in an 18 mm probe tube (H20-18-25-A1) and the oil quantified by acquisition of 16 scans, time delay of 2 seconds at 40 °C using Bruker mq20 minispec with a 0.47 Tesla magnet of frequency 20 MHz (Bruker, Coventry, UK). The machine was calibrated with sunflower oil before measurements were done on the samples. The data obtained were expressed as percent oil content on weight basis (w/w)

5.3.2.3 Seed mass

One hundred seeds were randomly sampled from each seed lot and the weight of single seed was recorded using microbalance (0.0001 g; UMT2 Mettler, Toledo). The mean weight was determined for each seed lot and comparison made across the different seed lots. In addition, to obtain matching data set for correlation with germination traits, average weights of 25 seeds were calculated for each seed lot.

5.3.3 Correlation among seed traits

The interrelationships between physical and germination traits: seed mass and base water potential; and seed mass and base temperature; seed oil and thermal time (Refer to germination trait data in Chapter 4) was determined. The correlations among seed germination traits: base temperature and base water potential; base temperature and thermal time; base water potential and hydro time; and base temperature and thermal time were also tested.

5.4 Results

5.4.1 Seed physical traits

Seed physical traits varied among the three seed lots of *T. fassoglense* (Table 5:1). Seed sampled from Busia seed lot were significantly ($p<0.05$) thicker than both Migori and Siaya seed lots. The mean thickness of seeds ranged from 0.87233±0.18 cm to 0.73444±0.08 cm. However, non-significant difference in seed length and width was observed among the three seed lots. The mean seed length and width ranged from 2.28478±0.14 to 2.14984±0.17 cm and from 1.90512±0.17 to 1.86913±0.14 cm respectively. Seed variance (a measure of shape) varied from 0.32 to 0.35 among the seed lots tested. Seed sampled from Siaya seed lot was significantly ($p<0.05$) heavier than both Busia and Migori seed lots. The mean seed mass varied by 0.31395 g ranging from 2.39603 g (Siaya seed lot) and 2.08208 g (Migori seed lot). The coefficient of variation, CV in seed mass was highest in Busia seed lot and lowest in Siaya seed lot. Seed oil content was not statistically ($p>0.05$) different among the three seed lots examined. The mean oil content of *T. fassoglense* seed varied by 2.86%, ranging from 18.3169% (Busia seed lot) to 21.1773% (Siaya seed lot) (Table 5:1).

Table 5:1: Comparison of seed physical traits for *Tylosema fassoglense* seeds collected from three counties in Kenya

Seed lot	**Seed Dimensions (cm)**			**Seed Variance**	**Seed mass (g)**	**CV**	**Oil content (%)**
	Length	**Width**	**Height**				
Busia	2.24772± 0.19a	1.89936± 0.06a	0.87233± 0.18a	0.32199± 0.425a	2.12295± 0.67b	31.75	18.3169± 3.54a
Migori	2.14984± 0.17a	1.86913± 0.14a	0.73444± 0.08b	0.35142± 0.029a	2.08208± 0.45b	21.55	20.8517± 2.94a
Siaya	2.28478± 0.14a	1.90512± 0.17a	0.75214± 0.09ab	0.35050± 0.026a	2.39603± 0.52a	20.96	21.1773± 3.37a

Figures in the same column with different letters are statistically ($p<0.05$) different (mass, n=100; oil content, n=10 and dimensions, n=10) and standard deviation (±sd).

5.4.3 Interrelationships between seed traits

Thermal time, $\theta_{T(50)}$ was significantly negatively correlated to both T_b and Ψ_b ($p<0.05$, r=-0.73, r=-0.73 respectively) (Table 5:3). Negative correlations were observed between $\theta_{T(50)}$ and oil content; seed mass and T_b; seed variance and Ψ_b while positive correlations was observed between $\theta_{T(50)}$ and seed mass; $\theta_{T(20)}$ and mass; Ψ_b and variance; Ψ_b and both mass and T_b, however these were not significant.

Table 5:2: Correlations matrix between *Tylosema fassoglense* seed functional traits

Seed traits	$\theta_{T(50)}$	T_b	$\theta_{H(20)}$	Ψ_b
Oil	-0.46^{ns}			
Mass	0.21^{ns}	-0.05^{ns}	0.35^{ns}	0.19^{ns}
Variance			0.30^{ns}	-0.34^{ns}
T_b	-0.73*		-0.10^{ns}	0.50^{ns}
Ψ_b	-0.74*		-0.38^{ns}	

Physical traits: oil content, mass and variance; germination traits: $\theta_{T(50)}$, T_b, $\theta_{H(20)}$ and Ψ_b. Figures with asterisk (*) are statistically ($p<0.05$, n=12) different and ^{ns}not significant ($p>0.05$, n=12)

5.5 Discussion

5.5.1 Seed physical traits

This study has demonstrated that seed physical traits of *T. fassoglense* varied among the seed lots. It confirms that inter-population variation in seed morphological traits is present in *T. fassoglense*. The results for seed mass of *T. fassoglense* were within the range reported by Okumu, (2011) of 2.64±0.35g for seeds collected from Siaya county in Kenya. However, in this case seed sampled from Siaya seed lot were significantly heavier than samples from both Busia and Migori seed lots. On the other hand, seed sampled from Busia and Migori seed lots differed significantly in thickness. The results of seed length and width in this study were within the range as those reported by Okumu, (2011) of 2.32±0.18 and 1.94±0.21cm for length and width respectively on seed collected from Siaya county, Kenya. Seed size and mass are controlled genetically (Li and Li (2015) and variation occur due to genetic/environment interactions (Paul-Victor and Turnbull, 2009; He *et al.,* 2014; Richardson *et al.,* 2015;). For example, Richardson *et al.,* (2015) reported that seed mass is genetically controlled with limited maternal environment effect on big sagebrush. The variations observed in seed mass and thickness of *T. fassoglense* may be attributed to differences in maternal habitat, soil nutrient and prevailing climate during seed filling phase. Seed size and mass are important agronomic traits that reflect genetic, physiological and ecological components and affects yields, quality and market price (Ambika *et al.,*2014; Finch-Savage and Bassel, 2015). Therefore, in selecting population with good agronomic traits for domestication Siaya seed lot would be the preferred over both Busia and Migori seed lots.

Seed oil content was not significantly different among the three seed lots of *T. fassoglense* tested. The results of oil content values of *T. fassoglense* were lower compared to earlier reports. Seed oil biosynthesis has been reported to be genetically controlled and usually occur in the embryonic axis, with variations linked to genotype, season, maternal environment and extraction as well as measurement method. For example Dubois *et al*., (1995) reported 24-30% oil on *T. fassoglense* (seed source not documented), Okumu, (2011) reported 22±0.3% on a sample obtained from Siaya county in Kenya. Otieno *et al*., (2015) reported 36.4% on single seeds samples collected from Homabay, Mombasa, Siaya and Taita Taveta counties in Kenya. Brink, (2006) also reported 24-30% oil content in *T. fassoglense* seed collected in Burundi and DR Congo. In extraction method, Okumu, (2011) used pre-weighed fat cups containing petroleum to extract oil at 60 °C and recorded weight gained by the fat cups after drying at 103 °C while Otieno *et al*., (2015) used sohxlet extraction. In this study time-domain nuclear magnetic resonance spectroscopy (TD-NMR; Borisjuk *et al*., 2011) which uses whole seed was used in quantifying seed oil content.The observed variations in oil may be attributed to extraction method, measurement method and genotype while low variation in seed oil content among the three seed lots tested may be due to similarity in genotype and climate in the three counties.

5.5.2 Interrelationships between seed traits

This study has demonstrated that seed physical and germination traits are correlated and interrelationship occurred among seed germination traits in *T. fassoglense* seeds. It confirms that *T. fassoglense* seed source significantly influenced both seed physical and germination traits. The study of the interrelationships among seed functional traits has been explored in crops and wild species (Kos and Poschlod, 2010; Dürr *et al.*, 2015; Gardarin *et al.*, 2016; Castillo-Lorenzo *et al.*, 2019a, b). It was hypothesized that seed water imbibition and germination on top of paper (TP) was likely to be influenced by seed shape which is also related to surface area in contact with the media. In testing the correlation between seed shape and hydro time, we found a positive correlation. The three seed lots tested here had a large variance and short hydro time and thus are likely to germinate very fast and less likely to form soil seed bank. This observation was in agreement with report that seed shape increases time to germination thus slow-germinating species are likely to form soil seed bank (Kos and Poschlod, 2010).

On the other hand, seed mass was positively correlated to $\theta_{T(50)}$, $\theta_{H(20)}$, Ψ_b and negatively correlated to T_b. This result suggests that the heavier the seeds (i.e. *T. fassoglense* seeds) the more cumulative thermal time or hydro time needed for germination and the lower T_b and Ψ_b (not significant). This observed trend was similar to that reported by Castillo-Lorenzo *et al.*, (2019a) on 10 *Brassica spp.* In this case Siaya seed lot were the heaviest, had the lowest T_b, highest thermal time to 50% germination and therefore would require longer cumulative thermal time to germinate compared to the other two seed lots. However, further research should incorporate more seed lots from different geographical locations to understand better the correlations between seed traits at species level.

Seed oil (Triacylglycerols, TAGs) provide carbon skeleton during seedling establishment and it is believed small proportion is metabolized through p-oxidation to provide energy to the growing embryo (Bewley and Black, 1994). In relation to seed oil content, a negative correlation was recorded between oil content and thermal time ($\theta_{T(50)}$). Previous studies have reported either negative or no correlation between oil content and thermal time in other species. For example, Castllo-Lorenzo *et al.,* (2019b) reported a negative correlation between oil content and thermal time in 10 *Helianthus spp.* (5 native species and 5 crop cultivars). Gardarin *et al.*, (2016) reported a weak, non-significant relation between time to mid-germination and lipid content. Dürr *et al.*, (2015) reported that oily seeds are not usually faster to germinate and non-oily seed of grass species and oily seed of aromatic and medicinal plants had similar thermal time. Furthermore, seed oil catabolism has been predicted to occur during later stages of seed germination especially during seedling establishment (Bewley and Black, 1994). The results in this study suggested that seed with high oil content require shorter cumulative thermal to 50% germination which were similar to those reported in other species (Gardarin *et al.,* 2016; Castillo-Lorenzo *et al.* 2019b). Interestingly, Busia seed lot had a lower oil content and shorter thermal time. This result may be supported by Dürr *et al.,* (2015) observation that oily seeds are not always faster to germinate. Gardarin *et al.,* (2016) also reported that germinaton arises from the interplay of several factors; seed size, storage reserve location and seed longevity. Therefore, it was not possible to make conclusion on the results of this test where three seed lots of one species was used. The inclusion of more seed lots from varied habitats in future studies may provide more information on this relationship.

Additionally, interrelationships were observed among the seed germination traits of *T. fassoglense*. In this study, $\theta_{T(50)}$ was significantly negatively correlated to both T_b and Ψ_b while $\theta_{H(20)}$ was also negatively correlated to both T_b and Ψ_b; and T_b was positively correlated with Ψ_b. This result depicted that the lower the base thresholds (T_b and Ψ_b), the longer the germination time. In addition, seed with lower T_b has the capacity to germinate at warmer temperatures compared to the ability of those with high T_b to germinate at lower temperatures. The positive correlation between T_b and Ψ_b is probably an adaptation strategy to tropical climate where species require both high temperature and water potential. These observations were similar with those reported on other taxa (Dürr *et al.*, 2015; Castillo-Lorenzo *et al.*, 2019a). For example, Castillo-Lorenzo *et al.,* (2019a) reported negative correlation between T_b and θ_T; and between θ_H and Ψ_b in 10 *Brassica spp.* Similarly, in a study of several species from wide ecogeographic location Dürr *et al.,* (2015) reported a negative correlation between T_b and θ_T and as an adaptation strategy, species with low T_b (from temperate climate) are likely to germinate at high temperatures. The positive correlation can be attributed to adaptation to tropical climate, meaning *T. fassoglense* require both warm and wet conditions to germinate. However, further research should incorporate more seed lots of *T. fassoglense* from different habitats and ecogeographic locations to better understand intra-species interrelationships among seed functional traits.

5.6 Conclusion

The seed maternal environmental effect has resulted in seed functional trait diversity among the three seed lots of *T. fassoglense* allowing for selection of populations with desirable traits for domestication. Seed collected from Siaya county had better physical traits (mass and oil content) and germination (low T_b-adapted to wider environment) desirable for cultivation over those from Busia and Migori counties. Seeds sampled from Busia seed lot had low oil content, large variation in seed mass (Co-variance) and shorter thermal time (fast germination) compared to other seed lots. The information generated here will be useful in germplasm sourcing, domestication and future breeding programmes of *T. fassoglense*.

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 GENERAL DISCUSSION AND CONCLUSIONS

Manual scarification of *T. fassoglense* seeds using a nail clipper significantly improved water imbibition and final germination percentage. This positive response to scarification showed that *T. fassoglense* seed possess physical dormancy (Baskin and Baskin, 2014). Scarification (mechanical and acid) has proven to be effective in physical dormancy-break in Fabaceae species (Travlos *et al.,* 2007; Kimura and Islam, 2012; de Morais *et al.,* 2016). Inter-population variation in "hard seededness" was observed among the three seed lots of *T. fassoglense* tested. The non-scarified seeds sampled from Migori seed lot imbibed the least and had the lowest final germination percentage. Inter-population variations in physical dormancy have been reported in Fabaceae species (Lacerda et al., 2004; Richard *et al*., 2018; Hradilová *et al*., 2019). The inter-population variation in physical dormancy observed in *T. fassoglense* can be attributed to several factors among them genetic/environmental, storage duration and collection time (Gama-Arachchige *et al.,* 2013; Smýkal *et al.,* 2014). This inter-population difference in dormancy level can help explain the interplay of genetic and environmental factors therefore can be exploited in selecting populations for domestication. Plant domestication has been shown to rely on a few seed traits such as indehiscent pods, dormancy, size and quality (Bewley and Black, 1994). The result on dormancy was supported by Travlos *et al*., (2007) who reported moderate physical dormancy in *T. esculentum* (Fabaceae sub-family Caesalpiniodeae), however inter-population variation is reported here for the first time on *T. fassoglense*

The germination of *T. fassoglense* seeds was not affected by the two photoperiods (light/dark or dark) tested and therefore is probably neutral photoblastic (Baskin and Baskin, 2014). Several species have been shown to be indifferent to light requirement, however germination of seed after dispersal in the wild occurs when the chances of seedling survival is high (Fenner and Thompsom, 2005). Seed germination response to light in relation to seed size and shape has been explored in other taxa (Sánchez-Bayo and King 1994; Thompson *et al.,* 1993; Milberg *et al,* 2000; Flores *et al.,* 2016). The result obtained on *T. fassoglense* was in agreement with earlier report on other species that large seeds are less dependent on light requirement while disc shape (large variance) prevents penetration of the seed in soil crevices to form soil seed bank. Although these results showed that *T. fassoglense* are probably neutral photoblastic, there is need for further research using more light regimes. However, *T. fassoglense* seeds can be buried under cultivation or tested under 12/12 hour or 0/24 hour conditions and attain good germination results.

The incubation temperature influenced seed germination of *T. fassoglense* and the cardinal temperature range determined for time to 50% germination for the three seed lots was sensitive to temperatures. The observed optimal temperature for germination was 30 - 35 °C in the three seed lots tested. Seeds of *T. fassoglense* had a wide temperature for germination ranging from 15 to 35 °C at which the final percentage was not significantly different. Seed germination response to temperature by *T. fassoglense* was similar to those of other crops, trees and shrubs (Covell *et al.,* 1986; Dürr *et al.,* 2015; Tribouillois *et al.,* 2016; Castillo-Lorenzo *et al.,* 2019a, b). The calculated, T_b, T_o and T_c were comparable to those of tropical legume crops and wild legume species (Covell *et al.,* 1986; Dürr *et al.,* 2015; Tribouillois *et al.,* 2016). The calculated base water potential (Ψ_b) for *T. fassoglense* was comparable to those of cultivated or tropical legume species while seed germination response to varied water potential was similar to those of other crops and other wild taxa (Daws *et al*., 2008; Brunel *et al*., 2011; Hu *et al*., 2015; Castillo-Lorenzo *et al*., 2019b). The Ψ_b and hydro time ($\theta_{H(20)}$) of *T. fassoglense* were not significantly different among the three seed lots tested. This observation can be attributed to seed of the same species and ecogeographic region. According to Hu *et al*., (2015) and Daws *et al*., (2008) variations occur inter-species and in seeds of the same species from different ecogeographic locations. The high T_b and Ψ_b are adaptive traits to tropical climates for rapid germination when the soil water potential rises above the threshold (Dürr *et al.,* 2015). This study has generated important data on cardinal temperatures and hydro parameters of *T. fassoglense*, which has not been done before. These results will be useful in understanding the environmental limits, species modelling especially predicting germination and timing for sowing under varied pedoclimates.

Seeds of *T. fassoglense* collected from the three counties varied in mass, thickness, shape as well as oil content. The physical traits of *T. fassoglense* seeds that are directly influenced by the production environment correlated with germination traits. The seed mass of the three seed lots were negatively correlated, first with base temperature and second positively correlated with cumulative hydro times ($\theta_{H(20)}$), base water potential and cumulative thermal times ($\theta_{T(50)}$). In addition, seed shape was negatively correlated to base water potential and positively correlated with hydro time. The associations observed in *T. fassoglense* physical and germination traits are similar to those observed in other taxa (Norden *et al.,* 2009; Dürr *et al.,* 2015; Gardarin *et al.,* 2016; Castillo-Lorenzo *et al*., 2019a, b). The result suggested that heavier seed need to accumulate more thermal and hydro times to germinate and had a lower base temperature as well as high base water potential (Castillo-Lorenzo *et al*., 2019a, b). This observation can be explained from adaptation point of view, that large-seeded tropical species germinate and establish very fast under sporadic rainfalls and high temperatures (Daws *et al.,* 2008). It was hypothesized that seed with high variance need to accumulate less hydro time and had lower base water potential. However, in testing this hypothesis, variance was positively correlated with hydro time and negatively correlated with base water potential. In this case seed sampled from Busia seed lot had a smaller variance and a smaller base water potential compared to both Migori and Siaya seed lots. This observation may be attributed to the non-significant difference in hydro parameters and low dataset and therefore, future study using more seed lots from varied ecogeographic locations may shed more light. Seed oil content has also been correlated with thermal time (Dürr *et al.,* 2015; Castillo-Lorenzo *et al.,* 2019a). The result here suggested that seeds high in oil are fast to germinate (short thermal time). Seed oil is known to be broken down through p-oxidation for provision of carbon skeleton (Bewley and Black, 1994). Interestingly, *T. fassoglense* obtained from Busia seed lot had low oil content and shorter thermal time (fast to germinate) and this was in agreement with Dürr *et al.,* (2015) observation that oily seeds were not always fast in germination. Additionally, seed germination may be influenced by several factors among them seed shape, size and storage reserve location (Gardarin *et al.,* 2016). The weak relationships observed between seed physical and germination traits may be attributed to the similarity in geographic location of the three sites and low dataset. Therefore, further investigations on correlation between seed functional traits of *T. fassoglense* should consider populations from diverse ecogeographic locations. These data will be useful in germplasm selection of *T. fassoglense* for domestication and future breeding for desired seed traits.

Seed quality is not only an important physical trait in food production and seed marketing but equally critical physiological trait in crop production (Ambika *et al.,* 2014; Finch-Savage and Bassel, 2015). The most essential seed quality parameters often considered are; purity, thousand seed weight, genetic purity, germination (measured by vigour and viability) (ISTA, 2017). According to ISTA (2017), seed vigour is the capacity of seed lot to germinate and establish under diverse environmental conditions and is quite important in selecting germplasm for domestication. Besides adaptation, seed vigour as a measure of performance can be inferred from germination rate as well as cumulative hydro and thermal times (Finch-Savage and

Bassel, 2015). The base temperature values of *T. fassoglense* were negatively correlated with cumulative thermal time ($\theta_{T(50)}$) and hydro time ($\theta_{H(20)}$). The base water potential was also negatively correlated with cumulative thermal time ($\theta_{T(50)}$) and hydro time ($\theta_{H(20)}$). On the other hand, base temperature was positively correlated with base water potential. The results of this study were similar with those reported for other taxa (Dürr *et al.,* 2015; Castillo-Lorenzo *et al.,* 2019b). This result showed the higher the threshold (base temperature and base water potential), the shorter the time required for germination (thermal and hydro time). The positive correlation between T_b and Ψ_b suggested that tropical species seeds would require both warm and wet conditions to germinate (Castillo-Lorenzo *et al.,* 2019b). This is an adaptive trait to tropical climate, whereby seed germinates and establishes very fast under sporadic rainfall and high temperatures (Daws *et al.,* 2008). The weak correlation in seed germination traits observed in *T. fassoglense* collected across the three sites may be attributed to low dataset and seeds originating from similar geographical location characterised by slight difference in climate. Therefore, further research targeting seed lots from varied agroecological zones AEZ may shed more light to these findings.

This information on seed biology, germination ecology and interrelationships among seed traits will be useful in propagating and domesticating *T. fassoglense. Tylosema fassoglense* is adapted to harsh environment and poor soils (Brink, 2006), therefore its cultivation in marginal production areas will increase its seed and tuber production thus contributing to food security, agrobiodiversity as well as improved livelihood and sustainable agriculture. Promoting domestication and consumption of *T. fassoglense* has the potential to alleviate malnutrition among the local communities since the seeds and tubers are high in macro- and micronutrients (Otieno *et al.,* 2015).

6.2 RECOMMENDATIONS

1. Seeds of *T. fassoglense* should be scarified for better germination results either for field establishment or laboratory germination test.
2. While selecting population for germplasm source with low dormancy levels, seeds from both Busia and Siaya sites should be collected.
3. Burial seed sowing of *T. fassoglense* can be practiced under field condition while seed testing under laboratory can be conducted under light/dark or dark conditions.
4. Seeds of *T. fassoglense* should be sown when soil temperatures are between 15 °C and 35 °C for high germination percentage.

5. Seed sowing of *T. fassoglense* should be done when the soil water potential is zero up to -0.5 MPa for good germination.
6. Seed lot from Siaya county had better physical and germination traits than both Busia and Migori seed lots.
7. Future study on light requirement for germination of *T. fassoglense* should consider more light regimes
8. Further studies for field sowing should explore effect of seed burial depth on germination of *T. fassoglense*.
9. Further thermal model experiment should be conducted in the supra-optimal temperature range to ensure better estimation of thermal time (supra-optimal) and ceiling temperature for germinating *T. fassoglense*
10. Further thermal model experiment should be conducted in the suboptimal temperature range for Busia seed lot to ensure better estimation of base temperature, T_b
11. Future studies on water potential should target values within the range of zero to -1 with possible division of 0.2 units for better estimation of the base water potential and hydro time of *T. fassoglense*
12. Correlations between seed physical and physiological traits should target more seed lots of *T. fassoglense* collected from varied ecogeographic areas to capture diversity and generate sufficient dataset.

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LIST OFAPPENDICES

Appendix: 1: Mean seed mass increment (g) by water imbibition of scarified and non-scarified seed of *Tylosema fassoglense* from three seed lots measured at different times (hr) and kept on moistened steel blue germination paper at 25 ℃

	Time (hours)/ mass increase								
Pop_Treatment	**0**	**1**	**3**	**8**	**23**	**28**	**32**	**47**	**52**
Busia Scarified	0	0.2850a	0.4958a	0.8178a	1.1873a	2.0475a	2.816a	2.8721a	3.4171a
Migori Scarified	0	0.0638a	0.3093a	0.4772ab	0.8469ab	1.9291a	2.7914a	2.8228a	3.4393a
Siaya Scarified	0	0.1852a	0.6673a	0.8441a	1.3555a	2.0002a	2.6411a	2.7824a	3.4014a
Busia Control	0	0.0871b	0.1584b	0.2585ab	0.3952bc	0.7517b	0.8807b	0.8425b	1.0754b
Migori Control	0	0.0039b	0.0044b	0.005b	0.005c	0.0079b	0.0086b	0.0093b	0.1515b
Siaya Control	0	0.0040b	0.0060b	0.0082b	0.0091c	0.2108b	0.2522b	0.2823b	0.8917b

Figures in the same column with different letters are significantly different (*p*<0.05, n=10)

Appendix 2: Estimated regression line parameters: y = ax + b for germination rates ($1/T_{50}$) plotted against temperature for four replicates of the different seed lots of *Tylosema fassoglense*

Seed lot	**Replicate**	**Suboptimal**			**Supra-optimal**		
		a	**b**	**R^2**	**a**	**b**	**R^2**
Busia	**1**	0.012	-0.075	0.923	-.032	1.479	0.922
	2	0.014	-0.128	0.909	-0.02	0.967	0.89
	3	0.015	-0.143	0.968	-0.042	1.895	0.996
	4	0.012	-0.085	0.889	-0.016	0.788	0.678
Migori	**1**	0.012	-0.069	0.953	-0.033	1.501	0.968
	2	0.013	-0.099	0.918	-0.021	0.995	0.9
	3	0.013	-0.101	0.992	-0.018	0.899	0.799
	4	0.009	-0.04	0.918	-0.03	1.385	.947
Siaya	**1**	0.011	-0.043	0.93	-0.033	1.543	0.924
	2	0.011	-0.049	0.926	-0.033	1.527	0.987
	3	0.011	-0.035	0.892	-0.033	1.536	0.918
	4	0.012	-0.056	0.946	-0.033	1.537	0.985

Appendix 3: Estimated regression parameters: y = ax + b for germination rates ($1/T_{20}$) plotted against water potential for four replicates of the different seed lots of *Tylosema fassoglense*

Seed lot	**Replicate**	**a**	**b**	**R^2**
Busia	1	0.24	0.224	0.94
	2	0.265	0.243	0.905
	3	0.251	0.231	0.923
	4	0.256	0.236	0.915
Migori	1	0.242	0.227	0.958
	2	0.242	0.23	0.939
	3	0.247	0.229	0.92
	4	0.239	0.225	0.95
Siaya	1	0.25	0.229	0.899
	2	0.253	0.236	0.914
	3	0.26	0.239	0.921
	4	0.249	0.228	0.926