SEED GERMINATION OF MORAMA BEAN (*Tylosema esculentum* (Burch) A. Schreib) FROM DIFFERENT COLLECTION SITES IN BOTSWANA

A dissertation submitted in partial fulfillment of the requirements for the award of the Master of Science Degree in Crop Science (Agronomy)

BY

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CERTIFICATION

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DECLARATION

I hereby declare that this thesis is my own work except where highlighted. To the best of my knowledge this work has not been awarded or submitted for a degree or master of degree at any other universities.

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Author`s Signature Date

APPROVAL

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Dean of faculty`s name	Date	Signature
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DEDICATION

I dedicate this dissertation to my parents, sister, younger brother, my two nephews and a niece who have been there for me every step of the way and believing in me. My best friends Mrs Epe Obakeng-Kemolatlhe and Mrs Tshepo Buzwani deserves a special dedication as they encouraged and motivated me every time I was low. I also devote this beautiful work to my mentor Mr Kolobetso Balopi who encouraged and supported me during the difficult times I encountered as a graduate student. Together with their support I wouldn't be where I am. I will also like to dedicate it to everyone who believes they can achieve anything they put their heart to.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
Cd	Heat units accumulated in a specific time
Cm	Centimetres
G	Grams
GP	Total Germination Percentage
GPS	Global Positioning System
GR	Germination Rate
GT_{50}	Number of Days to 50% to Germination
K.Farm	Kerebotswe Farm
LSD	Least Significant Difference
m	Meters
MD	Morphological Dormancy
ml	Millilitres
mm	Millimetres
MPD	Morphophysiological dormancy
^{o}C	Degrees Celsius
PD	Physiological Dormancy
PY	Physical Dormancy
SD	Standard Deviation
T_b	Base Temperature
T_c	Ceiling Temperature
TG	Total Germination

T_o	Optimum Temperature
UPP	Useful Plants Project
Θ_T	Sub-optimal thermal time
Θ_{Tsupra}	Supra-optimal thermal time

ABSTRACT

Understanding the seed biology and germination ecology of plants is critical for bringing them into cultivation, yet this information is insufficient for *Tylosema esculentum* despite its potential as a future crop. *T. esculentum* (Morama bean) is a multi-use, drought-tolerant legume highly valued by local communities. It is adapted to arid regions in Botswana, Namibia and South Africa, where it is traditionally wild harvested for its natural and economic value. *Tylosema esculentum* belongs to the family Fabaceae and is found growing in many parts of Botswana, Namibia and South Africa. Seeds for this study were collected from Charleshill, Malwelwe, Malatswai and Kerebotse Farm in Botswana. The experiment was conducted in the laboratory at Royal Botanic Gardens, Kew in the Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, United Kingdom.

The main objective of the study is to evaluate seed germination of *Tylosema esculentum* from different collection sites. The study determined the temperature thresholds of the different collection sites for seed germination, rate of imbibition between scarified seeds and non-scarified seeds. Further to this, the study focused on the effect of light at 25^{o}C for all the collection sites and comparing the seed moisture content, weight and morphology of seeds of *T. esculentum* between the collection sites.

The seed germination results show that the highest germination rate is between 25^{o}C and 35^{o}C for all the collection sites. There was a significant difference in the optimum temperature (T_o) in the cardinal temperatures while there was no difference in minimum (or base, T_b) and maximum (or ceiling T_c) temperatures for the collection sites. For the thermal time there was no significant difference in both the sub-optimal and supra-optimal range of temperatures between the collection sites. The imbibition rate for all the collection sites showed that there was higher water intake for scarified seeds as compared to non-scarified seeds. There was no

difference in germination rate and germination percentage between light and dark treatments as the results showed that they were high for all the collection sites. The morphology of seeds showed that there was no significant difference between the collection sites, whereas weight and moisture content showed a significant difference among the collection sites.

Keywords: Collection sites, germination, scarification, temperature, *Tylosema esculentum*

CHAPTER ONE

INTRODUCTION

1.1. General Background

Most of the wild species have been overlooked when it comes to research and development which is shame as they can be improved through research and breeding to be used as important sources of food or raw materials for biofuels. One of the important wild arid land plants that grows naturally in the wild and plays a major role in the lives of the rural inhabitants in Southern Africa is morama bean. Morama bean *(Tylosema esculentum)* is a tuberous hardy perennial leguminous oilseed crop that is widespread in Southern Africa, including Botswana, Namibia and South Africa (Mpotokwane *et al.,* 2013). The botanical name of the species, *esculentum* which means "edible" was given because it produces tubers and seeds that are consumed not only by indigenous people but also browsing stock and game animals that feed on the green seeds and tuberous stems. It has a lot of synonyms depending on the tribes or location in which it is found. Botanically it is referred to as *Tylosema esculentum* while it has varying common names like Marama bean and gemsbok bean (English); maramaboontjie, elandsboontjie and braaiboontjite (Afrikaans); marama and morama (Tswana); maramama (Thonga), Tsi and tsin! (Kung San); gami (Khoi); ozombanui (Herero) (Van Wyk and Gericke, 2000).

1.2. Taxonomy of *Tylosema esculentum*

T. esculentum belongs to the family Fabaceae (Leguminosae), subfamily Ceasalpinoidae, and genus *Tylosema* (Hartley *et al.,* 2002). The Fabaceae family contributes significantly to human nutrition as the family is made up of major palatable legumes and oilseeds such as soybean *(Glycine max*), peanut *(Arachis hypogaea),* mung bean *(Vigna radiata),* chickpea (*Cicer arietinum*), and lentil *(Lens culinaris)* as well as vegetable crops such as common bean

(Phaseolus vulgaris) and pea *(Pisum sativum)* and forages such as alfalfa *(Medicago sativa)* (Paterson *et al.,* 2000). There are five recognised species of Morama bean found in the genus *Tylosema* being *Tylosema esculentum* (Burchell) A. Schreiber, *Tylosema fassoglense* (Kotschy) Torre and Hillic, *Tylosema humifusa* (Pichi-Serm and Roti-Michael) Brena and *Tylosema argentea* (Chiov) Brena occurring in eastern, central tropical and Southern Africa (Coetzer and Ross, 1977). The fifth species reviewed by Castro *et al*. (2005) is referred to as *Tylosema angolense* Silveira and Castro.

Compared to other legumes like cowpeas which are nodulating and able to fix nitrogen, *T. esculentum* is the only legume which is unable to nodulate or fix nitrogen thus it does not obtain its nitrogen from symbiotic fixation with soil rhizobia, rather it scavenges nitrogen efficiently from low concentrations in the soil and rapidly builds reserves in the large tubers to serve as a buffer for formation of protein-rich organs (Dakora *et al.,* 1999). *T. esculentum* has been observed to be the only species in the genus *Tylosema* specific to arid regions of Southern Africa that is non nodulating (Takundwa *et al*. 2010) but able to produce seeds and tubers that are consumed after roasting and cooking.

1.2.1. *Tylosema esculentum* plant

The *T. esculentum* plant is not yet domesticated but seeds and tubers are harvested from the plants that grow naturally in the wild. Just like most undomesticated species, the plant is low yielding and it has a creeping growth pattern with stems that can grow from 3m to 6m long from its large tuber with forked tendrils opposite the leaves, which facilitate climbing (Castro *et al.,* 2005). As a perennial, the plant`s upper green foliage dies off during the off-season and regenerates during the rainy season every year from a large underground tuber. The tubers normally have a reddish-brown bark and are usually tapered to a thinner neckline structure near the soil surface from where the annual branches sprout during the rainy season

(Chimwamurombe, 2011). The storage root or tuber is a source of water and food and can attain a weight between 10kg to 12kg in a few years (Bousquet, 1982). In older plants, tubers weighing between 80kg to 250kg have been reported (Biesele and Murry, 1983). The leaves of the plant are deeply two lobed, hairless and firm in texture while the flowers are bright yellow and born along the stems each with erect petals and stamens (Chimwamurombe, 2011). The *T. esculentum* produces pods that are green and soft during the vegetative stages and later dries and becomes brown and hard with two to six large dark brown seeds per pod and the seeds weigh 2g to 3g (Wehmeyer *et al.,* 1969). The seeds have a hard coat about 2mm that acts as a protective shell to resist mechanical damage and it encloses the delicious and nutritious white nut (Van Wyk and Gericke, 2000).

1.3. Distribution of *Tylosema esculentum*

According to the National Research Council (2006) and Castro *et al*. (2005) a large population of *Tylosema esculentum* is found in Botswana (Central Kgalagadi) and Namibia, while a small population is found in Zimbabwe and South African provinces of Limpopo, North West and Gauteng. *T. esculentum* has been collected for consumption by indigenous people. The distribution of the genus may therefore be linked to the migration of these peoples from one place to another (Schapera, 1937). Nepolo *et al*. (2009) stated that it is possible that the widespread, but patchy distribution of *T. esculentum* in Namibia may be associated with the historical movements of traditional users of the plant. According to Bousquet (1982) in Kalahari region where *T. esculentum* is mostly found, it does not grow everywhere but it confines itself to isolated patches of sandy soil. Nepolo *et al.* (2009) stated that *T. esculentum* in Namibia is found growing in open savannah veld in competition with tall grasses, shrubs and small trees.

T. esculentum has the advantage of surviving and producing in very harsh conditions that characterise the desert which has highly unpredictable and low rainfall. *T. esculentum* is

confined to these areas because of differences in environmental conditions of other habitats such as the amount of rainfall, evaporation of water from the soil surface, temperature and soil types which are not ideal for it. These differences in the environmental conditions make it difficult for the species to maintain a population. *T. esculentum* grows best at altitudes between 1 000m and 1 500m with 300mm to 700mm of rainfall and minimum temperature above 15°C and maximum of 33°C (Müseler and Schönfeldt, 2006). Keegan and Van Staden (1981) found that since *T. esculentum* has the great ability to survive under unfavourable conditions, it can be considered suitable for cultivation especially under preventive conditions for other crops. It mostly grows in sandy soils which have limited water holding capacity and in areas that are frequently exposed to very high light intensity, extreme temperatures and prolonged drought (Mitchell *et al.,* 2005).

1.4. Uses of *Tylosema esculentum*

The plant is one of the neglected and underutilised traditional food resources which forms part of the diet for the indigenous population in Southern Africa (Holse *et al*., 2010). It contributes 75% of the total vegetable content of the diet for tribes in the Kalahari (Chimwamurombe, 2011). According to the National Research Council (2006) *T. esculentum* and other components of the plant have been involved in the human diet as long as the people have been in contact with these plants and that includes people living in Southern Africa such as Herero, Tswana, Khoi San and other Bantu speaking peoples.

As a legume, the seeds of *T. esculentum* are rich in protein and oil content making it a good alternative consumable protein source and compares well with other protein foods like soybean (Mmonatau, 2005). The beans are roasted and consumed or pounded and boiled with water to make porridge (Vietmeyer, 1986). A batch of sun-dried beans can be roasted in the shell for a

few minutes in hot ashes and sand of the cooking fire and a slight burst of steam from the roasting beans indicate that they are ready for eating (Bousquet, 1982). The beans are then removed from the ashes and are opened with a tap of a stick or rock. Oil extracts from the seeds have pleasant odour and can be used in food and cosmetics industries (Amarteifio and Moholo, 1998). The seeds of *T. esculentum* are normally not eaten raw due to the slimy texture and the disagreeable taste (Chimwamurombe, 2011). Other products can be made from the *T. esculentum* include morama flour, morama butter, morama cookies, morama yoghurt, morama milk and snack roasted nuts (Jackson *et al*., 2010). The farmers may use the morama bean as a supplement for fattening pigs (Elfant *et al*., 1985).

The root tuber is a rich source of water and starch (Chimwamurombe, 2011). Chingwaru *et al*. (2007) noted that the *T. esculentum* bean and tuber extracts have been used in traditional African medicine to treat diarrhoea and for general upkeep of human health. In addition, in some villages, elders use this species as treatment or preventing some illness such as headaches and women`s reproductive system problems. It is also reported to be a potential source of phytonutrients, which have shown to contribute positively to human health (Chingwaru *et al*., 2007; Jackson *et al*., 2010). Including *T. esculentum* in the daily diet has many beneficial physiological effects in controlling and preventing several metabolic diseases such as diabetes mellitus, coronary heart diseases and colon cancer (Muzur *et al*., 1998).

1.5. Reproductive life cycle of *Tylosema esculentum*

Since *T. esculentum* is a perennial legume, it takes a longer period for it to reach maturity. Under its natural state, it requires eight to twenty-one days to germinate on conducive wet soils and eighteen to twenty-four months to fully reach reproductive maturity (Chimwamurombe, 2011). After germination, the plant grows vegetative for the next five to six months and during this period, a tuber will be growing underground that helps sustain the plant with nutrients

during the winter period when the runners die back and shrivel off (Chimwamurombe, 2011). The tuber remains underground in winter and when next rains start, the new runners begin to grow from the tubers and produce flowers. The yellow flowers are pollinated by a solitary carpenter bee leading to new set of pods developing to full maturity and thereafter, the runners die back again in winter and regrow the following rainy season (Chimwamurombe, 2011).

1.6. Chemical composition of *Tylosema esculentum*

T. esculentum is a good source of oil and good quality protein. The bean oil is stated to be rich in mono and di-unsaturated fatty acids and contains no cholesterol (Keegan and Van Staden, 1981). The protein content of *T. esculentum* is considered to range from 32% to 45% while the oil content ranges from 30% to 42% (Keegan and Van Staden, 1981). The bean also contains significant amounts of vitamins (A, B3, B6, folic acid, B12 and E) and minerals (iodine, calcium, iron, zinc, phosphate and magnesium) (Müseler and Schönfeldt, 2006; Mazimba *et al*., 2011). The moisture content of *T. esculentum* is very low as the dry matter ranges from 94.4% to 98.7% (Bower *et al*., 1988; Holse *et al*., 2010). Variation in moisture content may be due to external factors such as soil composition, climate, harvesting time and maturation state of beans (Jackson *et al*., 2010).

1.7. Justification of Study

T. esculentum is considered one of the important food resources for people living in areas of the Kalahari Desert and areas that are along the poor semi-arid soils in Botswana. Even though it is important for these inhabitants, research and domestication of the crop has been lacking or neglected this far. According to Jackson *et al*. (2010) little research has been conducted locally on *T. esculentum* since it is found in the wild and it is only consumed by a small percentage of the population in Botswana.

There are several studies on *T. esculentum*, however, information on germination responses of seeds is still insufficient in Botswana. In the project, the seeds are germinated under a wide range of temperatures to determine the thermal time and cardinal temperatures from different collection sites. Further to this, the effect of germination of *T. esculentum* under varying light intensities is not well known. Since *T. esculentum* is found in different locations of Botswana that have different environmental conditions, comparing the cardinal temperatures, thermal time, seed morphology, seed weight and seed moisture from different collection sites will come in handy for the plant breeder and crop producers.

The knowledge obtained will improve or supplement the efficacy of seed germination of *T. esculentum*. Documenting the results of *T. esculentum* will be the starting point in doing extensive research on the germination requirement of physically dormant seeds. This will be important in the improvement of the crop so that it is not limited to the natural habitats, but also cultivated to diversify the nation's economic and food supply sources. This will include, but not limited to, commercialization of the species for protein and oil production.

1.8. Objectives

1.8.1. Main Objective

The main objective of the study is to evaluate seed germination of *Tylosema esculentum* from different collection sites.

1.8.2. Specific Objectives

1.8.2.1. To compare imbibition curves of scarified and non-scarified *T. esculentum* seeds from different collection sites.

1.8.2.2. To determine seed germination of *T. esculentum* from different collection sites as influenced by light.

1.8.2.3. To compare seed moisture content and weight of *T. esculentum* between the collection sites.

1.8.2.4. To compare morphology of seeds between the collection sites.

1.8.2.5. To determine seed germination thermal time and cardinal temperatures for each seed.

1.8.3. Hypotheses

1.8.3.1. **Null hypotheses:** There is no difference in imbibition curves for scarified and non-scarified *T. esculentum* seeds from different collection sites.

Alternative hypotheses: There is a difference in imbibition curves for scarified and non-scarified *T. esculentum* seeds from different collection sites.

1.8.3.2. **Null hypotheses:** Light do not affect germination of scarified *T. esculentum* seeds from different collection sites.

Alternative hypotheses: Light affect germination of scarified *T. esculentum* seeds from different collection sites.

1.8.3.3. **Null hypotheses:** Seed moisture and weight of *T. esculentum* are not the same from different collection sites.

Alternative hypotheses: Seed moisture and weight of *T. esculentum* are the same from different collection sites.

1.8.3.4. **Null hypotheses:** There is no difference in morphology of seeds from different collection sites.

Alternative hypotheses: There is a difference in morphology of seeds from different collection sites.

1.8.3.5. **Null hypotheses:** Temperature does not differently affect germination performance of *T. esculentum* seeds from different collection sites.

Alternative hypotheses: Temperature does differently affect the germination performance of *T. esculentum* seeds from different collection sites.

CHAPTER TWO

LITERATURE REVIEW

2.1. Seed Germination

Every plant species has its own ecological requirements for seed germination (Baskin and Baskin, 2014). Germination begins with the uptake of water by the dry seed and is completed when the embryonic axis, usually the radicle, extends to penetrate the structures that surround it (Bewley and Black, 1994); Nonogaki *et al*., 2010). According to Bradford (1990) imbibition is a triphasic process, with rapid initial water uptake (Phase 1), followed by a plateau phase with little change in water content (Phase 2) and a subsequent increase in water content coincident with radicle growth (Phase 3). Phase 2 can be completed when the embryo has initiated the radicle elongation and its length is extended by dormancy, low or high temperatures, water deficit or abscisic acid. Other factors that may promote germination will reduce the length of Phase 2 (Bradford, 1990).

Water is one of the most important components for seed germination because mature seeds are often extremely dry and need to take in significant amounts of water relative to the seed dry weight, before cellular metabolism and growth can start. Khasa (1993), found out that hard seed coats can completely prevent the imbibition of the germination process. A dormant seed contains 10% to 15% of water and is generally dehydrated. So, the dormant seed must absorb water to become active and germinate (Bewley, 1997). Water makes the seed coat soft, causes it to rupture after swelling and start germination (Koger *et al.,* 2004). Water is also needed to bring in the dissolved oxygen for use by the growing embryo. According to Chachalis and Reddy (2000) environmental factors such as temperatures, light, pH and soil moisture are best known to affect seed germination. According to Fenner and Thompson (2005) the knowledge

of seed germination response to environmental factors is required not only for understanding and predicting the ecological adaptation of the species but for also formulating effective strategies for restoration. Seed germination and seedling emergence may also be affected by the planting depth of the seed.

Bradford (1995) defines the germination rate as the inverse of the time to radicle emergence of a specific fraction or percentage of the population. As seeds in the given population do not germinate at the same time, germination rate must be defined with reference to a germination percentage of the seed population (Bradford, 1990). Germination rate can be expressed in various ways but no one way is universally superior in its linear relationship to temperature (Scott *et al.,* 1984). Other approaches rely on the sigmoidal shape that cumulative germination takes over time for most species (Scott *et al.,* 1984).

2.2 Seed Dormancy

Seed dormancy is the failure of an intact viable seed to complete germination under favourable conditions and it is controlled by several environmental factors such as light, temperature and the duration of seed storage (Bewley, 1997). Baskin and Baskin (1998) describes physiological dormancy (PD) as seeds that require physiological changes such as hormonal or chill or warm stratification (Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). Nikolaeva (1977) states that seeds with PD contain physiological inhibiting mechanism in the embryo that stops emergence of the radicle. Morphological dormancy (MD) happens when the embryo is immature or underdeveloped while physical dormancy (PY) is when the seeds have impermeable seed coats (Baskin and Baskin, 1998). Morphophysiological dormancy (MPD) happens in seeds with differentiated, immature embryos that are linear, rudimentary or spatulate

in shape. Nikolaeva (1969) describes mechanical dormancy to be caused by the existence of a hard, woody (stony) fruit wall and chemical dormant seeds to be caused by inhibitors contained in the pericarp.

The morama bean structure is made up of a very hard protective seed coat, embryo and two cotyledons that are like other legumes such as soybeans. The hard seed coat of many leguminous species offers important ecological advantages that favours the accumulation of persistent seed banks in the soil, spreads germination over time and increases the chance that some seeds will germinate, establish and complete the life cycle successfully (Gutterman, 1993).The failure to germinate has evolved differently across species through adaptation to the prevailing environment so that the germination occurs when conditions for establishing a new plant generation are likely to be suitable (Li and Foley, 1997). According to Demel (1998) the hard seed coat of many leguminous species has evolved to resist unfavourable conditions such as heat caused by fire, strong teeth of dispersing animals, severe drought and mechanical damage. The germination of *T. esculentum* crop is usually delayed or takes a very long time and several researches has been conducted to establish and improve the germination of the seeds.

2.3 Breaking Seed Dormancy

In nature, seed dormancy can be broken by melting and freezing of soil, soil microbial activity, forest fires, animal digestive systems and other factors (Mousavi *et al*., 2011). However, these activities may take several years to break the seed coat and stimulate germination (Mousavi *et al*., 2011). The mechanisms of breaking seed dormancy vary from species to species (Mng'omba *et al*., 2007). Germination of many leguminous species of arid and semi-arid

regions is limited by hard seed coats which require breaking it down for seeds to imbibe water. According to Demel (1998) the aim of performing different treatments on hard seed coat is to allow the seed coat to be permeable to water by acting on specific weak spots.

Legume seeds contain a mechanism of physical dormancy with water impermeable seed or fruit coats (Baskin, 2003). Seed coat impermeability usually is associated with the presence of one or more layers of palisade cells (Baskin and Baskin, 1998). Mechanical and chemical scarification can be used to break physical dormancy (Finch-Savage and Leubner-Metzger, 2006). This is supported by Rouhi *et al*. (2010) who reported that some pre-treatments, such as mechanical and chemical scarification have been proved to promote the germination of the hard-seeded species.

Mng'omba *et al*. (2007) reported that hot water, sulphuric acid, filing and abrasion pre-treatments have been used to scarify the hard seed coats. Seeds of some species especially those indigenous to semi-arid and tropical regions, containing hard and thick coat germinated at a higher percentage when soaked in sulphuric acid and hot water (Nejadsahebi *et al.*, 2007). Fang *et al*. (2006) reported a high seed germination percentage (98%) for 'sweet tea tree' (*Cyclocarya paliurus* (Batal) Iljinskaja) after the seeds were scarified in concentrated sulphuric acid for ten hours. Owonubi *et al*. (2005) reported that soaking of *Azadirachta indica* seeds for 1 and 12 hours in sulphuric acid resulted in increasing rate of seed germination. Muhammad and Amusa (2003) observed that germination percentage was highest when *Tamarindus indica* L. seeds containing hard seed coat were soaked in 98% sulphuric acid concentration for 30 minutes. Aliero (2004) studied the effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa*. He observed

that prior treatment of seeds with sulphuric acid, wet heat and mechanical scarification induce germination of the dormant seeds. He found that prolonged emersion of seeds in sulphuric acid maybe injurious to the seed coat as the acid may rupture the vital parts of the embryo.

Travlos *et al.* (2007) investigated germination behavior of untreated *T. esculentum* seeds compared to seeds undergoing various dormancy-breaking treatments. The results indicated that seed germination was greatest when scratching and cracking of the seed coat with sandpaper. The highly positive responses of marama seeds to the mechanical treatment clearly indicated that there is a moderate coat-imposed dormancy in this species. They also found that immersion of the seeds in water for 20 hours and in concentrated sulphuric acid for 20 min were also most effective treatments for morama seeds.

Lebutswe *et al*. (2003) observed that the germination rate and percentage of *T. esculentum* can be greatly increased by subjecting the seeds to mechanical scarification, water immersion or acid treatment. In Greece, Travlos *et al.* (2007) observed that the speed and percentage of *T. esculentum* seed germination was greatly increased by an immersion in hot water for 2 to 4 min or dry heating for 5 min at 100 to 150°C, while higher temperatures proved harmful to some seeds. They concluded that the highly positive responses of marama seeds to treatments indicated that there is a moderate coat-imposed dormancy in this species

Travlos and Economou (2006) found that the partial removal of the hard seed covering structures by means of the massive abrasion can be considered as the most effective treatment in stimulating germination and emergence in *Medicago arborea*. Seed scarification of morama seeds with a sandpaper before planting could also be used in the field to improve germination. Gul *et al*. (2018) also found that seed dormancy in bean can be broken by mechanical method

like rubbing the seed in the laboratory as well as treating with tetrazolium, while Zare *et al.* (2011) found that the most effective methods for breaking seed dormancy in *Prosopis koelziana* and *Prosopis juliflora* was scarification with sandy paper and sulphuric acid.

Nourmohammadi *et al*. (2019) studies the effects of 20 physical dormancy breaking treatments on germination of *Gleditsia caspica* (Caspian locust) seeds and seedling growth. They found that immersion in concentrated sulfuric acid (98%) for 60 minutes was optimal for all germination traits and for all seedling traits except root length. After seed coat removal or treatment with sulfuric acid (for 45 to 120 minutes), 99–100% of the seeds germinated, but seedling growth traits after 5 months were significantly lower in the former than in the latter treatment. Sikhondze *et al.* (2020) investigated the effects of different pre-germination methods on seed germination of guava. They found that pre-germination treatments using sulphuric acid 20% for three minutes was the effective method for breaking seed dormancy and promoting growth and development of guava seeds. The highest germination percentage was obtained from seeds treated with sulphuric acid.

Kheloufi *et al*. (2018) investigated germination of *Astragalus armatus* Willd. subsp. *armatus* which is an endemic shrub of the Northern Africa. Pre-sowing treatments included immersion in concentrated sulphuric acid for 30, 60 and 90 minutes, and immersion in hot water. They found that untreated seeds of both ecotypes of *A. armatus* failed to germinate, for both populations, the most effective treatment was immersion in sulphuric acid for 60 minutes.

The beneficial effect of hot water bath is common among perennial legumes widespread in arid and semi-arid zones (Muhammad and Amusa, 2003). Sabongari (2001) found that the immersion of dry seeds in hot water may cause rupture of the coat wall permitting water to penetrate the seed tissues, thus causing physiological changes and subsequent germination of the embryo.

Emongor *et al*. (2004) evaluated the effects of sulphuric acid, nitric acid, hot water, gibberellic acid and ethephon on the germination of *Corchorus tridens* seeds. They found that seeds treated with sulphuric acid for 10, 20, and 30 min significantly broke the seed dormancy and promoted the germination as well as hot water. They concluded that that *Corchurus tridens* seeds from Botswana have hard seed coat or impervious seed coat dormancy but not physiological dormancy as an adaptation to arid and desert conditions.

2.4 Temperature on Seed Germination

Temperature is one of the most important factors regulating germination of non-dormant seeds in irrigated, annual agroecosystems at the beginning of the growth season where light, nutrients and moisture are more typically not growth limiting (Garcia-Huidobro *et al.,* 1982). For many species, constant or alternating temperature regimes can affect seed germination differently (Probert, 2000). Ritchie and Nesmith (1991) states that the combination of temperature and time is the more appropriate unit of measure for predicting plant development than the time alone. Temperature has a primary influence on seed dormancy and germination affecting both the capacity for germination by regulating dormancy and the rate or speed of germination in non-dormant seeds (Alvarado and Bradford, 2002).

Dubal *et al.* (2016) evaluated the physiological quality of seeds and isozyme expression in seedlings of three bean's genotypes (Cario-ca, BRS Expedito and IPR Tuiuiú) under the influence of germination temperature of 15, 20, 25, 30 and 35 °C. They found that as the temperature rose, seeds of IPR Tuiuiú and BRS Expedito had reduced germination. The increase on temperature significantly affected the germination speed index of the three genotypes, leading to a greater increase in the germination. They also found that the seeds exhibit better performance when exposed to temperature of 30 °C compared to lower temperatures. The similar results were found by Machado Neto *et al.* (2006) studying the effect of temperature on germination of bean seeds, obtaining a decrease in germination at a temperature of 35 °C, becoming null at the temperature of 39 °C.

The range of temperatures over which the seeds of a species can germinate is described by the cardinal temperatures (Bewley and Black, 1994). The cardinal temperatures for germination generally are related to the range of environmental conditions which a given species is adapted and they match germination timing to favourable conditions for subsequent seedling growth and development (Hu *et al.*, 2015). Black *et al.* (2006) found that cardinal temperatures measure the range of temperatures that contribute to the development of a plant. The three cardinal temperatures that characterize germination response to temperature of most species are; the minimum, optimum and maximum temperatures. According to Bradford (1995) the sub-optimal temperature range thermal time model (heat units accumulated in time) can be used to characterize the times to germination at different suboptimal temperatures. The minimum (or base, T_b) and maximum (or ceiling, T_c) temperatures are those temperatures at which germination will not occur below and above respectively while the optimum temperature (T_o) is the point at which germination is the most rapid (Bradford, 2002). Covell *et al. (*1986) describes sub-optimal temperature range as the range between T_b and T_c. There are large

variations in cardinal temperatures among and within species (Hardegree, 2006) and the variations are often related to ecological and geographical factors and thus provide ecological significance for understanding adaptation to local environments (Qui *et al.,* 2010). The temperature range between the T_b and T_c is very sensitive to the dormancy status of seeds often being narrow in dormant seeds and widening as dormancy is lost (Vegis, 1964). Ellis *et al.* (1987) stated that there is little variation in T_b among individual non-dormant seeds.

2.5 Other Factors Affecting Seed Germination

Other factors influencing seed germination are water, light and gases (Probert, 2000; Chauhan *et al.,* 2006). Water is important because mature seeds are often extremely dry and need to take in significant amount of water relative to the seed dry weight, before cellular metabolism and growth can start in the seed. So, the dormant seed must absorb water to become active and exhibit germination (Bewley, 1997). Water is also needed to bring in the dissolved oxygen for use by the growing embryo.

In the dormant condition the seeds respiratory rate is very low and so oxygen is required in very small quantities. But for germination, oxygen is needed in large quantities and the seeds obtains the oxygen from the air. Seeds sown deeply in soil, fail to germinate because of lack of oxygen, and ploughing aerates the soil and helps in good germination.

Light has varied effects on germinating seeds of different plants. Some seeds need light for germination, while in some seed's germination is hindered by light (Koger, 2004). According to Baskin and Baskin (1988) most of the plant species germinate under light and darkness.

Silveira and Fernandes (2006) found out that there was no difference in comparison in light and dark conditions in *M. foliolosa*. Depending on the species, some seeds germinate at higher percentages in darkness as compared to light (Baskin and Baskin, 2014).

Due to the high nutrient value of the seeds and tubers rich in protein, oil and starch, morama bean has the potential to become one productive and economical crop in arid areas where few conventional crops can survive (Ketshajwang *et al.*, 1998). Growing morama in heavy clay loam soils has to be avoided as it is unsuitable for the growth and emergence. The growth of morama bean is supported by soils that are low in organic matter and nutrients. The morama plant can develop a tuber that can weigh up to 10kg (Mmonatau, 2005). This implies that a single plant can require more space for cultivation as compared to other legumes.

Most of the natural growing conditions of the crops are characterised as relatively poor soils, with low mineral nutrient soils (Ramolemana *et al.* 2007) thus the morama have shown no response to phosphorus and nitrogen fertilization (Ramolemana *et al.*, 2003). The plant has the ability to extract necessary growth nutrients from poor soils to meet its requirement for shoot and growth development (Ramolemana, 2013). Morama bean grows best on a sandy loamy soil where waterlogging is not a problem (Jackson *et al.* 2010) also prefers sandy soils which have poor water holding capacity that is frequently exposed to very high light intensity, extreme temperatures and prolonged drought (Mitchell *et al.,* 2005). Researches are underway to domesticate morama and further come up with desirable cultivars that are high yielding and early maturing as it is still not yet cultivated with its potential contribution to food security (Chimwamurombe, 2008; Nepolo *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Description of Experimental Site

The experiments were conducted in a laboratory at the Royal Botanic Gardens, Kew in the Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, United Kingdom.

3.2. *Tylosema esculentum* (Morama Bean) Collection

Wild mature *T. esculentum* seeds were collected from four collection sites in Botswana (Figure 3.1). The collected seeds were labelled accordingly as per the collection manual; GPS location and coordinates, date of collection, habitat type, soil description and vegetation description. A handheld Global Positioning System (GPS) was used to record the coordinates of collections site of all the samples for further reference and future collections. The recorded points were transformed into a database program (Map Source) and marked on the map to show the collection sites. Additional information was also sourced from other institutions working on *T. esculentum* like the Natural Plant Genetic Resources in Botswana and the Useful Plants Project (UPP) database and the data was drawn on the map to show the overall distribution of *T. esculentum* in Botswana.

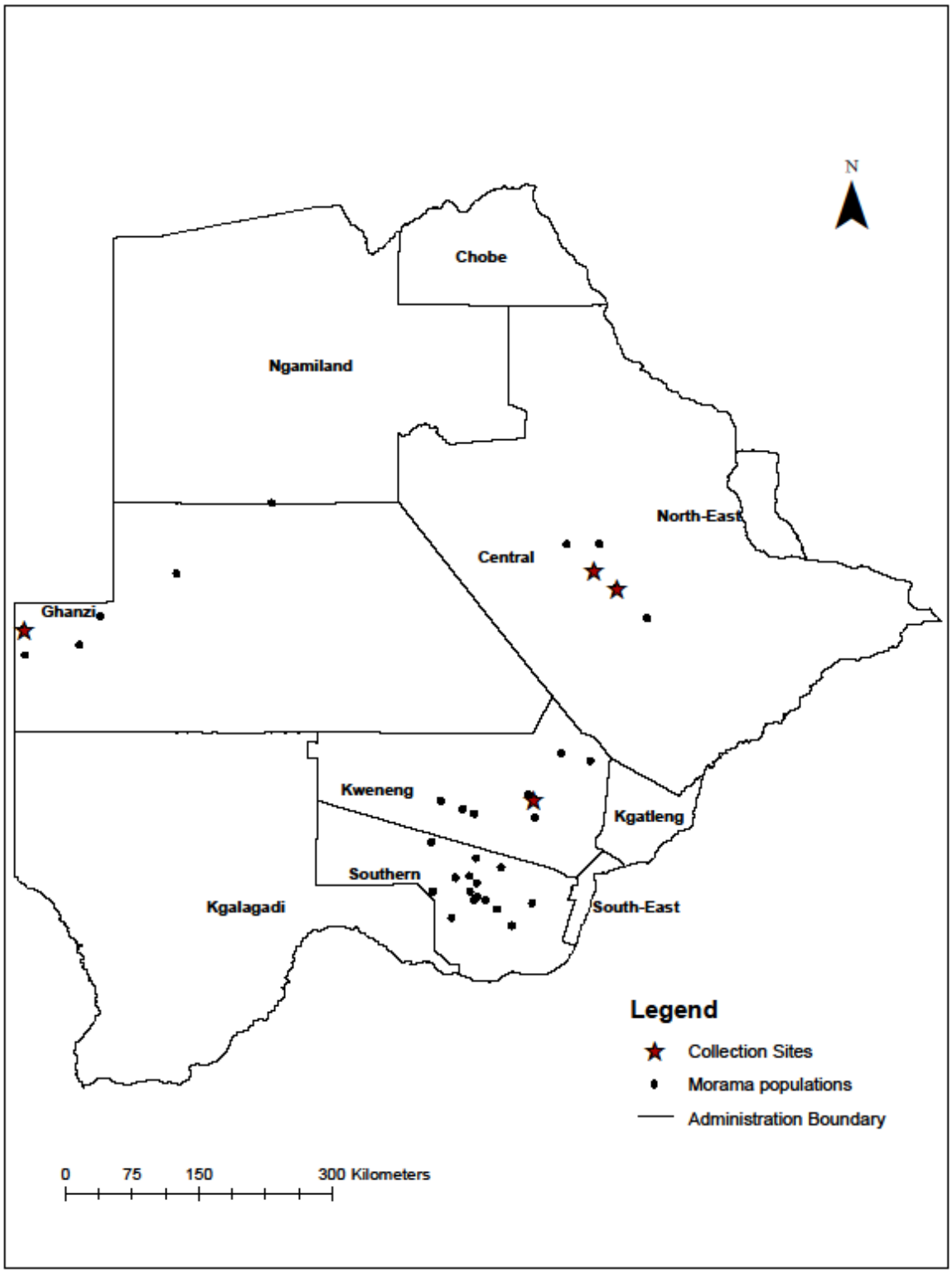

Figure 3. 1: Map showing the distribution of *Tylosema esculentum* in Botswana

Table 3. 1: Geographical coordinates of the distribution of *Tylosema esculentum* in Botswana. Highlighted locations are studied in the project.

Location	**South**	**East**
Boatlaname	-23.60306	25.82222
Charleshill	**-22.27639**	**20.09278**
Diphuduhudu	-23.52111	25.52833
Ditawana	-21.41667	25.91222
Gantsi	-21.71238	21.63934
Gasita	-25.09987	24.88199
Jwaneng	-24.5858	24.66736
Kerebotswe Farm	**-21.4044.9**	**25.5140.4**
Kole gate	-21.0019	22.59619
Konowe	-25.18603	24.41986
Lefhoko	-24.83527	24.67254
Letlhakane	-21.4167	25.5833
Maboane	-24.08705	24.5309
Madige	-25.01136	24.76626
Magagarape	-24.13233	24.64739
Mahotshwane	-24.42006	24.21805
Makhi	-22.16193	26.40019
Malatswai	**-21.85806**	**26.08667**
Malwelwe	**-23.98722**	**25.24833**
Mamuno Borderline	-22.40375	19.91494
Mantshwabisi	-24.17236	25.26227
Maokane	-24.92341	24.60998
Maratswane	-23.94282	25.19997
Mochaanyana	-24.97578	24.68194
Mokhomba	-24.76543	24.60042
Mosadimogolo	-25.00568	24.64797
Naledi	-24.6821	24.92122
Samane	-24.78208	24.45664
Takatokwane	-24.0044	24.30691
Thankane	-24.91612	24.22781
Tsatsu	-25.26418	25.02745
Tshootsha	-22.1438	20.8685
Tsonyane	-25.04053	25.23634
Xanagas	-22.43139	20.655

3.3. Climate Data

The data for precipitation and temperatures (minimum, mean and maximum) was obtained from the WorldClim by the use of GPS coordinates of the collection sites for months of September to April which is the growing season for *T. esculentum*. Mean monthly precipitation and mean temperatures for the four collection sites were calculated by dividing the sum of all nine means of months by nine which is the growing season of *T. esculentum.* The lowest and highest values were also noted to identify the range of temperatures of the four collection sites.

3.4. Imbibition Test

Scarification of the seeds was performed using a drill (RS 183-5813) with a mini circular saw tool to make an aperture in the seed coat and forceps were used to hold the seed in place. Ten scarified seeds and ten non-scarified seeds (control) from four different collection sites were weighed on the balance (GR-202 from A&D Company, Limited) to determine their individual mass. The seeds were then placed on the steel blue germination blotters (23 x 15 cm), folded into a zig-zag shape to support the seeds in plastic sandwich boxes (17 x 11.5 x 5.5 cm) and moistened with 50 ml of distilled water. The sandwich boxes were placed in incubators at a constant temperature of 25±2°C, with 12/12 hours photoperiod. The seeds were weighed after 1 hour, 3 hours, 5 hours, 7 hours, 23 hours, 28 hours, 32 hours and 47 hours to determine the amount of water the seeds have imbibed. An additional 20 ml of distilled water was applied whenever there was a need to keep the germination paper moist. The data recorded was analysed to compare the water uptake for scarified seeds and control (non-scarified) seeds for each collection site. There was a total of eight experimental units where each of the four collection sites had two scarification treatments (scarified and non-scarified). Each experimental unit contained ten seeds, thus a total of eighty seeds in the experiment.

3.5. Effect of Light on *Tylosema esculentum* Seed Germination

Two light treatments (light and darkness) were set at a constant temperature of 25±2^{o}C to determine the effect of light on germination of *T. esculentum* seeds from four collection sites. Scarification of the seeds was used, and they were scarified using the same method as that in Section 3.4. Four replicates of ten seeds were used for each treatment. The sandwich boxes for the dark treatment were covered with aluminium foil to avoid exposing the seeds to light and those for light treatment were placed in incubators of 12 hours photoperiod (light and darkness). Therefore, there was a total of 32 experimental units (four collection sites by two treatments by four replicates).

An additional 20 ml of distilled water was applied whenever there was a need to keep the germination paper moist. The seeds were given the same management across the treatments and monitored for germination on a daily basis with recording the number of germinated seeds in each treatment. For the dark treatment, scoring for germination was done in a dark room with a green light so as not to affect the treatment process. The seeds of *T. esculentum* were considered to have germinated when the healthy, white radicle has emerged through the tough outer protective layer and has grown to about 1mm. The data recorded was analysed to compare the cumulative germination under light and dark treatments for each collection site.

3.6. Effect of Temperature on *Tylosema esculentum* Seed Germination

The germination experiments were conducted at different temperature levels starting at 10±2°C increasing by 5°C up to 45±2°C for four collection sites. Scarification of the seeds was used, and the scarification was performed following the same method used in Section 3.4. Four replicates of ten seeds were used for each treatment. The sandwich boxes were placed in incubators of 12/12 hours photoperiod. An additional 20 ml of distilled water was applied whenever there was a need to keep the germination paper moist. There was a total of 128

experimental units **(**four collection sites by eight treatments i.e.; 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45 °C by four replicates).

The seeds were given the same management across the treatments and monitored for germination on a daily basis with recording the number of germinated seeds in each treatment. The data recorded from each experiment was analysed to compare the total germination percentage, t50 (Time to 50% germination), germination rate, cardinal temperatures and thermal time from the four collection sites.

3.6.1. Total Germination percentage

Total Germination percentage (GP) is an estimate of the viability of a population of seeds. It is calculated using the following equation:

GP = (seeds germinated / total seeds sown) x 100

3.6.2. t50

This is the time the population needs to reach 50% of germination. It is calculated from cumulative germination curve (sigmoidal line) using the following equation (Boltzman equation):

$$t_{50} = dx * \ln\left(\frac{A1-A2}{y_{50}-A2} - 1\right) + X_0$$

Where;

A1 = initial germination at time 0

A2 = Total germination of the population

X_0 = is the time when 50% of the populations germinates for each sigmoidal curve (not considering the total germination of the population)

dx = slope (returned by the equation)

ln = natural logarithm

y = the percentile we want to calculate (50)

3.6.3. Germination Rate (GR)

This is the inverse of the time the population needs to reach specified percentile. It is calculated using the following equation:

$GR_g = 1/t_g$

Where;

GR_g = Germination Rate

t_g = the time to completion of germination percentile *g*

3.6.4. Cardinal Temperatures

Germination rate was plotted against temperatures to obtain linear regression lines. Two equations for the linear regression lines (sub-optimal range and supra-optimal range) were used to determine the cardinal temperatures T_b, T_c and T_o. Supra-optimal range is the range between T_o and T_c.

Linear regression lines;

y = -ax + b (supra-optimal range)

y = ax-b (sub-optimal range)

Where;

a = slope

b = intercept

$T_b = -b/a$

$T_c = b/-a$

T_o = x when ax-b = ax+b

3.6.5. Thermal Time

Thermal time is the ability of seeds to accumulate heat units to germinate in each time. It was determined by the inverse of the absolute values of the slope from the linear regression lines (sub and supra-optimal respectively).

3.7. Average Weight of 100 Seeds from Different Collection Sites

A total of 100 *T. esculentum* seeds each from four collection sites were individually weighed to determine the mass of the seeds. The data was analysed to compare the average seed weight of the collection sites.

3.8. Moisture Content of Seeds from Different Collection Sites

Ten individual *T. esculentum* seeds from four different collection sites were initially weighed to determine their fresh weight. The seeds were oven dried at 103°C for 17 hours and put in dry silica gel to allow them to cool for 1 hour. They were then weighed to determine their dry weight. Seed moisture content was calculated using the following equation:

MC = [(fresh weight-dry weight)/fresh weight]*100

3.9. Morphology of Seeds from Different Collection Sites

Ten seeds of *T. esculentum* from four collection sites were photographed using a camera (Nikon). The images were analysed using Axiovision software (AxioVs40, Carl Zeiss Micro Imaging 2010, Germany) to measure the seed length, seed width and seed thickness. The data were analysed to compare the means of seed length, seed width and seed thickness of seeds from different collection sites.

3.10. Data Analysis

Analysis of variance (ANOVA) was evaluated using the SAS 9.2 statistical software (SAS Institute, 2004) using the PROC GLM procedures. Treatment means were compared using Least Significant Difference (LSD) at risk level of 5% ($p \leq 0.05$). The $p \leq 0.05$ was used to derive the significant difference unless stated otherwise. The cumulative seed germination curves were calculated using Origin 8.6 software following Boltzmann equation to calculate t50 and Germination rate (GR).

CHAPTER FOUR

RESULTS

4.1 Climate Data of Seed Collection Sites

The climate data was obtained using GPS coordinates of the collection sites. The values of the monthly mean precipitation and temperatures are means of nine months from September to April, which is the growing season of *T. esculentum* (Table 4.1). The values for temperature ranges are the lowest temperatures and highest temperatures recorded from the collection sites in the nine months of data collection.

4.1.1 Monthly Precipitation

The mean monthly precipitation (PPT) recorded varied between the collection sites (Table 4.1). Among the four collection sites, Malwelwe had the highest mean monthly precipitation of 50.6 mm followed by Malatswai with mean monthly precipitation of 49 mm. The mean monthly precipitation of K.Farm was slightly lower at 47.5 mm while Charleshill had the lowest mean monthly precipitation of 41.4 mm.

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4.1.2 Monthly Temperatures

The mean monthly temperatures recorded showed minimal difference in minimum temperatures recorded from the collection sites as the values ranged from 15.1°C to 15.7°C (Table 4.1). The mean temperatures recorded among the collection sites were of the same range of 22.4°C to 23.1°C. There was minimal difference in maximum mean temperature recorded among the collection sites as the temperatures ranged from 29.6°C to 31.1°C. There was minimal difference in temperature ranges recorded from the collection sites. The lowest temperatures recorded from the collection sites ranged from 9.6°C to 11.5°C whereas the highest temperatures recorded ranged from 31.2°C to 33°C.

Table 4. 1: Mean monthly precipitation (PPT) and Temperatures (T) obtained from the collection sites of *T. esculentum.*

	Monthly mean	**Monthly Mean T (°C)**			**T Ranges (°C)**	
Collection site	**PPT (mm)**	**Minimum**	**Mean**	**Maximum**	**Lowest**	**Highest**
Charleshill	41.4	15.1	23.1	31.1	9.6	33.0
Malwelwe	50.6	15.1	22.4	29.8	10.1	31.2
Malatswai	49.0	15.5	22.6	29.6	11.2	31.5
K. Farm	47.5	15.7	22.4	29.9	11.5	31.8

4.2 Moisture Content and Weight of Seeds from Different Collection sites

The moisture content among the seed from different collection sites differed significantly ($p<0.05$, Table 4.2). Malatswai and K.Farm had a higher seed moisture content of 2.4% followed by Malwelwe (2.2%) and Charleshill had the lowest seed moisture of 2.1%. The results show that there is a significant difference ($p<0.0001$) amongst the seeds from different collection sites for seed weight (Table 4.2). Charleshill had the highest seed weight of 3g followed by Malwelwe with 2.8g, while Malatswai and K.Farm had the lowest seed weight of 2.4g each.

Table 4. 2: Weight and moisture content of *T. esculentum* seeds from different collection sites

Collection Site	**Moisture Content (%)**	**Seed Weight (g)**
Charleshill	2.1b	3.0a
Malwelwe	2.2ab	2.8b
Malatswai	2.4a	2.4c
K.Farm	2.4a	2.4c
P-value	*	***

The alphabets following the figures in columns are for mean comparison and the same alphabet shows that there is no significant difference at ($p\geq0.05$). ***, **, * Indicate significantly different at $p<0.0001$, $p<0.01$ and $p<0.05$ respectively; and **ns** – not significantly different at $p>0.05$.
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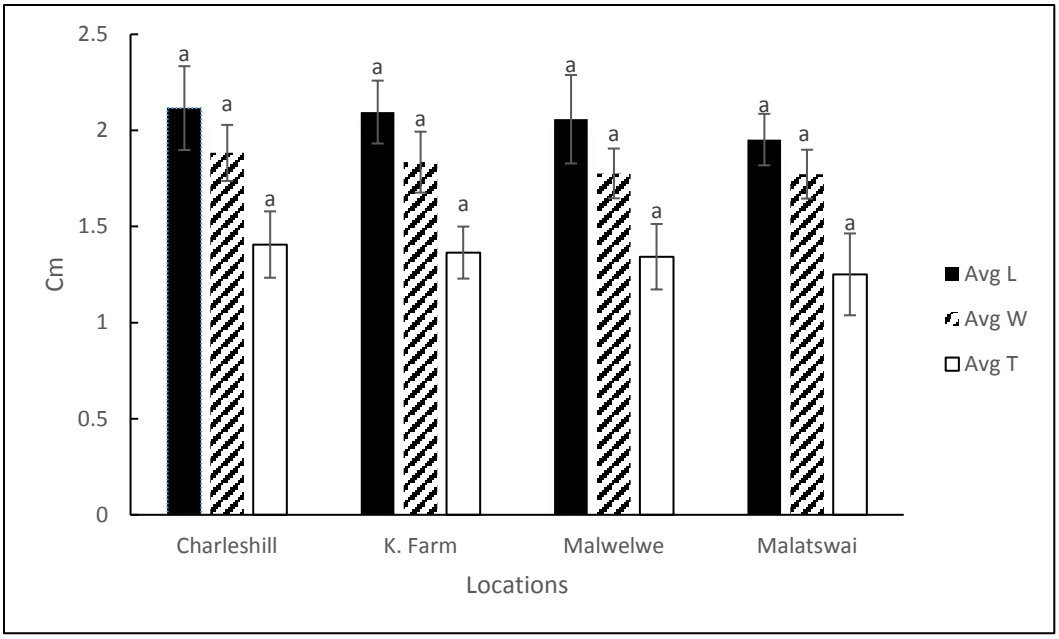

Figure 4. 1: Morphology of seeds (length=L, width= W and thickness = T) of *T. esculentum* from different locations**. "**a" on top of the bars represents mean comparison between seed measurements. Error bars indicating ±SD.

4.3 Morphology of Seeds from Different Collection Sites

There was no significant difference ($p \geq 0.05$) in the seed length, nor in seed width or seed thickness among the collection sites (Figure 4.1). The means for the morphology of seed length ranged from 1.95 cm to 2.11 cm, seed width (1.77 cm to 1.88 cm) and seed thickness (1.25 cm to 1.41 cm) for the collection sites.

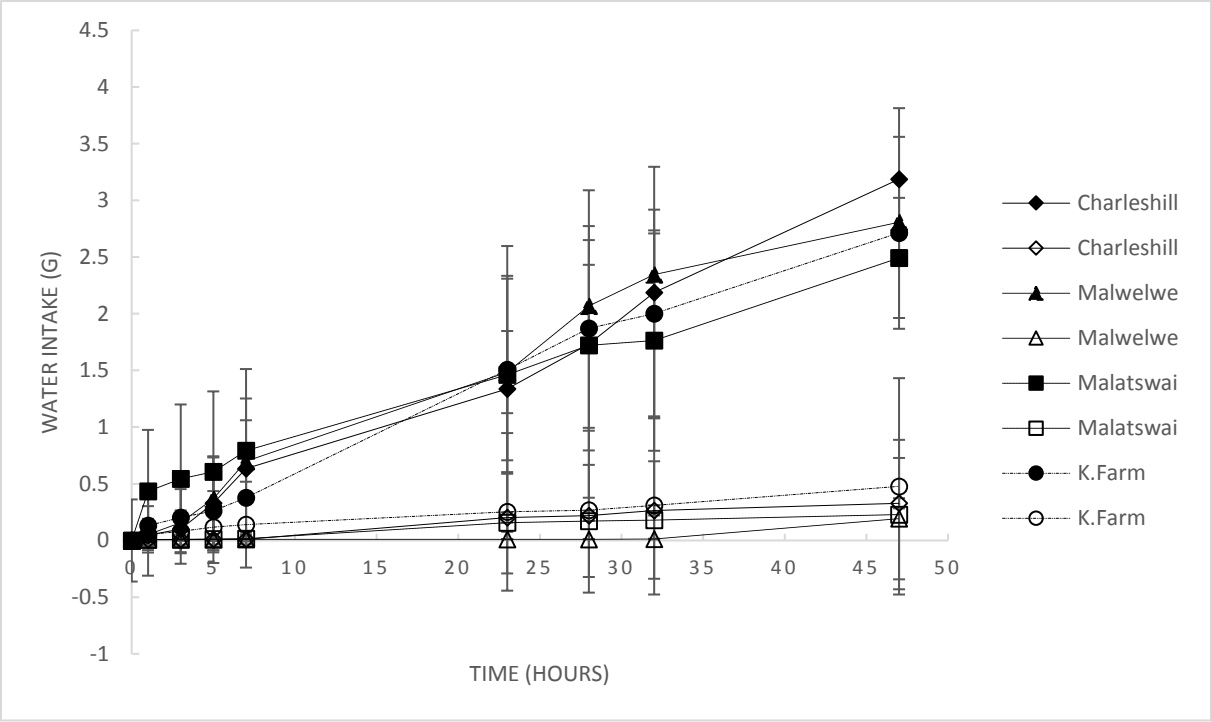

Figure 4. 2: Imbibition rate for scarified and non-scarified (control) seeds of *T. esculentum* from different collection sites at 25°C. Solid symbols are for scarified and open symbols are for non-scarified. Error bars indicate ±SD.

4.4 Imbibition Rate of Scarified and Non-Scarified Seeds from Different Collection Sites

Imbibition curves between scarified seeds and non-scarified seeds from different collection sites were significantly different (Figure 4.2). There was a constant increase in water intake for scarified seeds for all the collection sites with Charleshill site having the highest water intake (3.19 g after 47 hours). Malwelwe and K.Farm recorded slightly lower water intake at 2.8 g and 2.71 g respectively after 47 hours compared to Charleshill while Malatswai had the lowest water intake (2.49 g after 47 hours) for scarified seeds.

The non-scarified seeds did not imbibe water at the same rate compared to scarified seeds for all the collection sites. Among the collection sites, imbibition for non-scarified seeds for K.Farm started after 5 hours, Malatswai and Charleshill after 23 hours and Malwelwe after 47 hours. There was minimal difference in the water intake after 47 hours from the collection sites for non-scarified seeds ranging from 0.19 g to 0.50 g. Non-scarified seeds did not reach the same water content than scarified seeds after 47 hours. The final water content did not exceed to 0.50 g and was significantly lower than the corresponding value in scarified seeds.

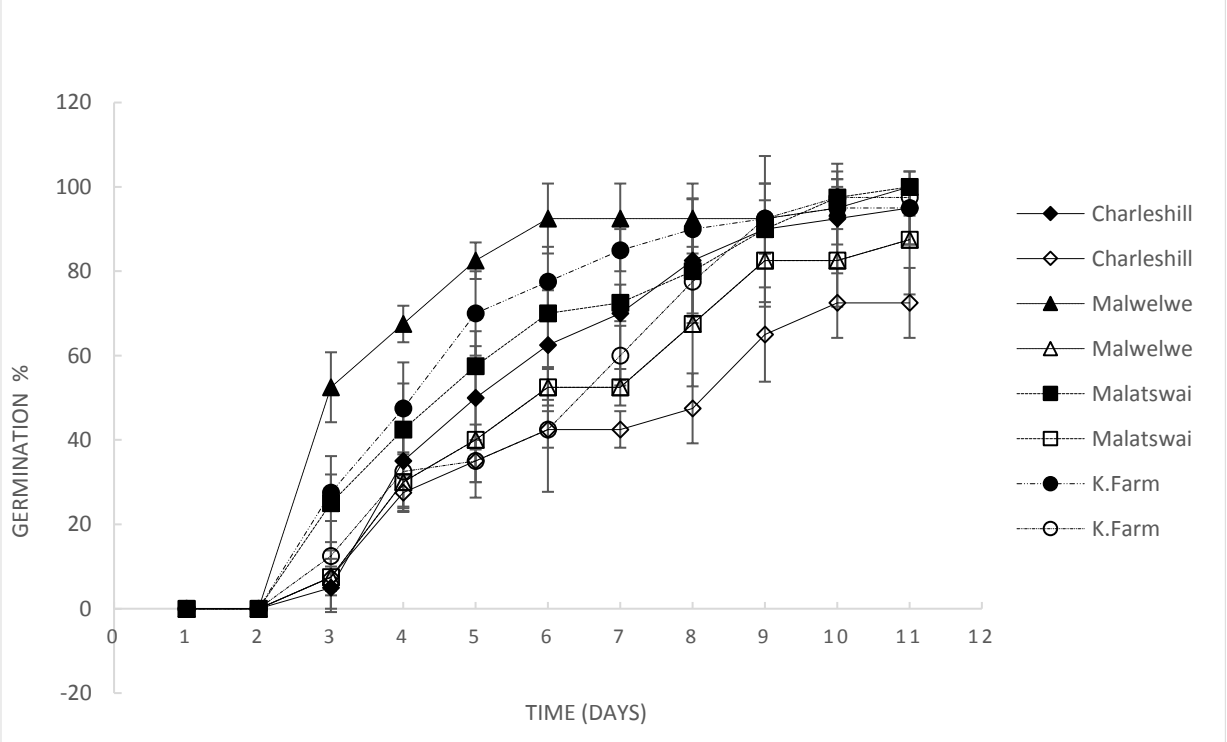

Figure 4. 3: Effects of light treatments on germination of scarified *T. esculentum* seeds from different collection sites at 25°C. Solid symbols are for light treatment and open symbols are for dark treatment. Error bars indicate ±SD.

4.5 Effect of Light on Germination of Scarified *T. esculentum* Seed

Relatively high germination percentages were recorded for all the collection sites for both light and dark treatments (>70%, Figure 4.3). This shows that there was minimal difference between germination percentage under light and dark treatments. The highest germination percentage was observed in the light treatment from Malwelwe and Malatswai (100%) followed by K.Farm (97.5%) in the dark treatment after eleven days post sowing date. Charleshill and K.Farm recorded 95% germination percentage after eleven days post sowing date in the light treatment. Dark germinated seeds from Malatswai and Malwelwe recorded 87.5% germination while Charleshill recorded the lowest germination (72.5%) after eleven days post sowing date. Germinated seeds as observed in the germination percentage experiment showed that minimum number of days taken for seeds to germinate was three days after sowing for all the collection sites.

4.6 Cardinal Temperatures and Thermal Time

There was no significant difference in the base temperature and ceiling temperatures for the collection sites as they ranged from 8.7°C to 10.8°C and 42.7°C to 45.3°C respectively (Table 4.3). There was a significant difference ($p<0.01$) in the optimal temperatures between the collection sites. Seeds from Malatswai had a higher optimal temperature (37.4°C) while seeds from K.Farm had the lowest optimal temperature (32.0°C). There was no significant difference between seeds from Malwelwe and Charleshill as they recorded optimal temperatures of 34.3°C and 36.9°C respectively.

There was no significant difference on the sub-optimal thermal time and the supra-optimal thermal time of all the collection sites (Table 4.3). For sub-optimal thermal time values ranged from 77.4°Cd to 87.1°Cd and for supra-optimal time values ranged from 23.9°Cd to 36.8°Cd for all the collection sites.

Table 4. 3: Three cardinal temperatures (T) and thermal time of seed of *T. esculentum* from different location

	CARDINAL TEMPERATURES			**THERMAL TIME**	
Locations	**Base T**	**Ceiling T**	**Optimum T**	**Sub-optimal range**	**Supra-optimal range**
	(T_b °C)	**(T_c °C)**	**(T_o °C)**	**(Θ_T °Cd)**	**(Θ_{Tsupra} °Cd)**
Charleshill	8.7a	45.1a	36.9ab	87.1a	25.3a
Malwelwe	9.7a	42.7a	34.3bc	77.4a	26.8a
Malatswai	10.8a	45.3a	37.4a	81.5a	23.9a
K.Farm	10.6a	44.4a	32.0c	84.4a	36.8a
P-value	ns	Ns	**	Ns	ns

The alphabets following the figures in columns are for mean comparison and the same alphabet shows that there is no significant difference at ($p \geq 0.05$). ***, **, * Indicate significant difference at $p<0.0001$, $p<0.01$ and $p<0.05$ respectively; and **ns** – not significantly different at $p>0.05$.

4.6.1 Effect of Temperature on Total Germination of Scarified *T. esculentum* Seeds from Different collection sites

The results indicate that temperature influenced total germination of scarified seeds of *T. esculentum*. There was a significant difference ($p<0.0001$) among temperatures on the germination of seeds from within each collection sites (Table 4.4). At 10°C, all the collection sites had their lowest germination percentages. The highest total germination for all collection sites was obtained within the temperature range of 20°C to 35°C. Charleshill and K. Farm had the highest total germination (100%) at 35°C whereas Malwelwe (100%) had the highest at 25°C and Malatswai (90%) attained the highest germination at 20°C. Malwelwe and K. Farm were the only two collection sites where the total germination dropped to around 50% at 40°C while Charleshill seed was the only one that recorded total germination above 50% at 15°C. There was no germination at 45°C for any of the collection's sites.

There was no significant difference in seed germination ($p\geq0.05$) at 15°C, 20°C, 25°C,30°C,40°C and 45°C between the collection sites. There was a significant difference in seed germination ($p<0.01$) when comparing the means of the four collection sites at 10°C with Malwelwe recording the highest total seed germination of 20%, followed by Malatswai (17.5%) and Charleshill (10%). K.Farm recorded the lowest total seed germination of 2.5%. Charleshill and K.Farm did not differ significantly ($p<0.05$) at 35°C with 100% total germination compared to other collection sites. Malwelwe recorded 82.5% total germination while Malatswai was the lowest with 77.5% total germination.

Table 4. 4: The effect of different temperatures on Total Germination Percentage (GP) of *T. esculentum* from different locations

	TG (%)				
Temperatures	**Charleshill**	**Malwelwe**	**Malatswai**	**K. Farm**	P value
10 °C	10.0d (ab)	20.0c (a)	17.5bc (ab)	2.5d (b)	**
15 °C	62.5c (a)	37.5bc (a)	30.0b (a)	30.0c (a)	ns
20 °C	92.5ab (a)	92.5a (a)	90.0a (a)	85.0a (a)	ns
25 °C	95.0a (a)	100a (a)	87.5a (a)	95.0a (a)	ns
30 °C	95.0a (a)	92.5a (a)	85.0a (a)	85.0a (a)	ns
35 °C	100.0a (a)	82.5a (ab)	77.5a (b)	100.0a (a)	*
40 °C	75.0bc (a)	55.0b (a)	70.0a (a)	50.0b (a)	ns
45 °C	0.0d (a)	0.0d (a)	0.0c (a)	0.0d (a)	ns
P-value	***	***	***	***	

The letters following the figures in columns are for mean comparison and the same letter shows that there is no significant difference at ($p \geq 0.05$). Letters not in brackets are mean separation of temperatures and letters in brackets (), are mean separation of locations***, **, * Indicate significant difference at $p<0.0001$, $p<0.01$ and $p<0.05$ respectively; and **ns** – not significantly different at $p>0.05$.

4.6.2 The Effect of Temperatures on Number of Days to 50% Germination of *T. esculentum* from Different Collection Sites

Different temperatures significantly affected 50% germination of *T. esculentum* from all the collection sites ($p<0.0001$, Table 4.5). The number of days to 50% germination (GT_{50}) was not possible to be calculated at 10°C and 45°C for any of the collection sites because the total germination percentage did not reach 50%. Seeds of Charleshill were the only ones that germinated at 15°C, and it was the slowest to reach 50% germination among all temperatures recorded. All the collection sites recorded the fastest GT_{50} at 35°C except for Malwelwe that recorded the fastest GT_{50} at 25°C.

Temperatures 30°C, 35°C and 40°C had no significant difference on GT_{50} between the collection sites (Table 4.5). There was a significant difference ($p<0.05$) at 20°C with Charleshill and K.Farm recording the highest GT_{50} and Malwelwe and Malatswai recording the lowest $GT_{50.}$ There was a significant difference ($p<0.0001$) at 25°C with Charleshill and Malatswai recording the highest GT_{50} and Malwelwe recorded the lowest $GT_{50.}$

Table 4. 5: The effect of different temperatures on number of days to 50% Germination (TG_{50}) of *T. esculentum* from different locations

	TG_{50} (Days)				
Temperatures	**Charleshill**	**Malwelwe**	**Malatswai**	**K. Farm**	P value
10 °C	-	-	-	-	
15 °C	13.2a (a)	-	-	-	***
20 °C	8.0b (a)	6.1a (b)	5.7a (b)	7.2a (a)	*
25 °C	5.4bc (a)	3.3b (c)	6.1a (a)	4.4bc (b)	***
30 °C	5.1c (a)	5.6a (a)	5.8a (a)	5.8ab (a)	ns
35 °C	3.8c (a)	3.6b (a)	3.1b (a)	3.7c (a)	ns
40 °C	4.8c (a)	5.5b (a)	4.0b (a)	5.8ab (a)	ns
45 °C	-	-	-	-	
P-value	***	***	***	***	

The letters following the figures in columns are for mean comparison and the same letter shows that there is no significant difference at ($p \geq 0.05$). Letters not in brackets are mean separation of temperatures and letters in brackets (), are mean separation of locations***, **, * Indicate significant difference at $p<0.0001$, $p<0.01$ and $p<0.05$ respectively; and **ns** – not significantly different at $p>0.05$.

CHAPTER FIVE

DISCUSSION

5.1 Moisture Content and Seed Weight

Seeds from different collection sites differed in moisture content; this may be caused by the environment of those localities. Bower *et al.* (1988) recorded moisture content of 3.9 ± 1.0 on *T. esculentum* seeds collected from the Kalahari Desert (Ghantsi area) which differs from the ones established in this study. The results in this study are in line with Bower *et al.* (1988) and Holse *et al.* (2010) who found that the moisture content of *T. esculentum* is considered to be very low as the dry matter ranges from 94.4% to 98.7%. Variation in moisture content may be due to external factors such as soil composition, climate, harvesting time and maturation state of the beans (Jackson *et al.,* 2010).

According to Jideani *et al.* (2009) the weight of *T. esculentum* seeds is higher compared to other oil seeds and legumes. There was a significant difference ($P<0.0001$) among the collection sites in seed weight of *T. esculentum* (Table 4.2). The effect may be due to seeds being collected during different times of the year, thus leading the seeds to have different weights. The range of seed mass in this study is similar to what Wehmeyer *et al.* (1969) found on light and dark brown *T. esculentum* seeds (between 2-3g). Since *Tylosema* is an edible seed and large seed mass increases the yield per plant, the knowledge about mass of seeds is important because it is an essential parameter for storage, handling and processing of the product (Jideani *et al.,* 2009).

5.2 **Morphology of Seeds**

There was no significant difference ($P\geq0.05$) when comparing the morphology of seeds from the collection sites (Figure 4.1). The seed length, width and thickness are in line with results by

Jideani *et al.* (2009) who was comparing physical properties of *T. esculentum* seeds and described seed length (1.86cm), seed width (1.70cm) and seed thickness (1.31cm) which are slightly smaller seeds than those in this study. However, regarding the variation between populations in both cases (this work and Jideani *et al.* 2009) no significant differences were found in their morphology measurements. This could mean that there is very little diversity in seed shape and size within *T. esculentum* populations in Botswana.

5.3 Imbibition Rate of Scarified and Non-Scarified Seeds from Different Collection sites

T. esculentum seeds are known to have a hard seed coat that is considered to be one of the strategies for survival in the environment. Baskin and Baskin (2004) states that the physically dormant seeds are likely to germinate or rot when the hard testa is compromised and there is enough moisture for seeds to imbibe water. Scarification of seeds had a positive effect on the imbibition of seeds from all the collection sites in this study (Figure 4.2). This is because *Tylosema* seeds have a thick seed coat that is impermeable which is in accordance with most legume seeds that possess physical dormancy. This is supported by Argel and Paton (1999) who states many mature seeds especially legume seeds, are unable to germinate under favourable environmental conditions because they are impermeable to water and gases or they contain a seed coat that constrains the embryo. According to Smykal *et al.* (2014) physical dormancy in legumes includes the development of a water impermeable seed coat that is often caused by the phenolics and suberin-impregnated layers of palisade cells that must be disabled for water to penetrate the seed.

5.4 Effect of Light on Germination of Scarified *Tylosema esculentum* Seeds

The germination rate of seeds from all the collection sites in this study was not influenced by light treatment. Similarly, Silveira and Fernandes (2006) found the same when comparing dark

and light conditions of seed germination in *Melaleuca foliolosa.* This is also in line with other studies that state that light indifference in Fabaceae is widespread (Camargo-Ricalde and Grether, 1998; Melo *et al.,* 1998; Baskin *et al.,* 1998; Hermansen *et al.,* 2000).

5.5 Effect of Temperature on Germination of Scarified *Tylosema esculentum* Seeds from Different Collection Sites

There was no significant difference in the base temperature for the all the collection sites. Even though there was no significant difference in base temperatures, the seeds were collected from four different collection sites where the environment varied in temperature and precipitation thus it may require different conditions for seeds to germinate. There was no significant difference in ceiling temperature for different collection sites. This maybe because the maximum temperatures where the seeds were collected from are in the same range. *Tylosema* seeds seem to be more plastic at lower temperatures, however, the lack of variance observed in ceiling temperature suggest less plasticity in the supra-optimal range of temperatures. All four collection sites had similar T_b, T_c and Thermal time (sub-optimal and supra-optimal) range of temperatures. The results differed from the results found by Hu *et al.* (2015) who reported differing ceiling temperatures of collections from different sites. Optimal temperatures showed significant difference in the collection sites. According to Hardegree (2006) there is large variation in cardinal temperatures among and within species. It is supported by Qui *et al.* (2010) who stated that the variations are often related to ecological and geographical factors and thus provide ecological significance for understanding adaptation to local environments. For all the collection sites, high germination percentage was observed in temperatures between 25°C and 35°C. The results in this study are in line with the statement by Cullis *et al.* (2019) who states that *T. esculentum* grows best in the day light of 32 °C (range 28-37°C).

According to Allen *et al.* (2000) the thermal time model has proven to be a useful tool in a comparative study of seed germination in research. The thermal time results in this study shows

that there was no significant difference in sub-optimal temperatures as all populations had very high values (>77 ^{o}Cd) (Table 4.3). Castillo-Lorenzo *et al.* (2019) found different values for thermal time for sub-optimal temperature range when comparing *Helianthus* species. According to Castillo-Lorenzo *et al.* (2019) having a slower germination rate may be a way for seeds to survive under fluctuating environments to allow germination to spread and prevent young seedlings from getting exposed to extreme conditions such as drought.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

Scarification had a positive effect on breaking the physical dormancy of seeds from all the collection sites in which it resulted in full imbibition of seeds as compared to non-scarified seeds. The presence of light did not enhance germination of seeds as it was seen for all the collection sites. Comparison of morphology of seeds shows no significant difference while moisture content and weight of seeds indicate that there were differences among seeds from different collection sites.

For cardinal temperatures there was a difference in optimum temperatures while in the base temperature, ceiling temperature and thermal times there was no difference among the collection sites. In general, scarification on *T. esculentum* seeds had a positive effect on the germination of the seeds from all the collection sites, and the scarified seeds germinated faster and had higher germinated percentages in temperatures ranging between 20°C to 35°C.

6.2 Recommendations

Since the study was only conducted in a one-year trial, another trial in consecutive generations is needed to verify the results considering the harvesting and storage methods of the seeds as that can have an impact in the final results. Field trials are required to assist farmers who are interested in cultivation of *T. esculentum.* Similar studies related to temperature, like seed water potential or salt tolerance should be conducted on seeds of different collections to be able to identify environmental factors that limit the ecology of the species.

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