Dormancy breaking methods and priming techniques to improve seed germination in *Gynandropsis gynandra* (L.) Briq syn *Cleome gynandra* L. (Cleomaceae)

by

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Professor AO Odindo

Date: 20 July 2022

DECLARATION: PLAGIARISM

I, Khungeka Mangena, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) their words have been re-written, but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

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GENERAL ABSTRACT

Gynandropsis gynandra, commonly known as spider plant, is a leafy vegetable that belongs to the Cleomaeae family. Spider plant is used for its medicinal properties, but also as a nutritional supplement, and an animal (e.g., cows) feed. Farmers experience low and uneven germination when planting this species that studies ascribed to physiological dormancy which leads to low and uneven germination. The study was conducted to understand mechanisms involved in breaking dormancy in spider plant seeds. The objectives were (a) to determine the effects of packaging materials and storage period on seed germination, and (b) to determine the effects of priming agents and duration on seed germination of *G. gynandra*. These objectives were achieved through two experiments based on six accessions of *G. gynandra* originated from West Africa, East Africa, and Asia. In the first experiment, the seeds were stored for four months at room temperature of 25℃ in brown paper bags, aluminium foil paper, and black polystyrene bags. After every storage period, the seeds were tested for electrical conductivity (EC), viability using tetrazolium chloride and germination ability to study the effects of storage period and packaging material on seed viability and vigor of *G. gynandra*. In the second experiment, the six accessions were subjected to two priming agents, PEG-4000, and distilled water, and tested for germination. Final germination percentage (FGP), mean germination time (MGT), mean germination rate (MGR), coefficient of the velocity of germination (CVG), and radicle length (RL) were recorded. Data analysis was done using Genestat version 20th edition (VSN International, United Kingdom) at a 5% level of significance. The study showed that *G. gynandra* fresh seeds displayed physiological dormancy which can be broken by storing seeds for at least two months depending on the genotype in aluminium foil paper. In this study, seed priming with PEG-4000 and distilled water had no effect on seed germination of *G. gynandra*.

Keywords: Accessions, Priming, Storage

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DEDICATION

I would like to dedicate this work to my siblings for their support, my late mother for her prayers until she had to lay to rest, and to my life partner for his support and endless love.

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CHAPTER 1 – GENERAL INTRODUCTION

1.1 Background to the study

Gynandropsis gynandra (L.) Briq., commonly known as spider plant, is a leafy vegetable that belongs to the Cleomaceae family. This species has a branched growth habit and can grow up to 1.5m. The stem is characterized by glandular hairs and parallel lines and the plant becomes woody with age (Sogbohossou et al., 2019). Spider plant has palmately compound leaves with three to five leaflets and white, pink, or lilac petals. It produces small, round seeds, with a rough seed coat, resembling a snail shell enclosed in a linear capsule (Chweya and Mnzava, 1997; Sowunmi and Afolayan, 2015). The species is semi-wild and is believed to have originated in Africa and Asia (Shilla et al., 2016) and spread to drier parts of tropical and sub-tropical regions worldwide (Nemahunguni et al., 2019). It is most likely found on disturbed soils such as arable lands and wasteland with annual plants and grasslands (Chweya and Mnzava, 1997). The spider plant is tolerant to drought and plays an important role in securing food within the households during dry seasons (Ekpong, 2009; Redhead, 1990). In South Africa, the spider plant is found in the Northern Limpopo, KwaZulu-Natal, North-West, Mpumalanga, Gauteng, and Free-State provinces (Sowunmi and Afolayan, 2015). The species is a rich source of micro and macronutrients such as provitamin A, vitamin C and minerals such as calcium (Ca), iron (Fe), magnesium (Mg), and proteins (Chweya and Mnzava, 1997; Opole et al., 1995), therefore, it is used as a supplement in malnutrition management (Dushimimana et al., 2018) and household food security (Onyango et al., 2013).

Although the spider plant is of great importance as a food source, there is not much research done on them because it is regarded as a wild species and there are several factors that limit their

cultivation and use (Sogbohossou et al., 2018). The yields are low, this may be due to several factors such as pest and disease infestation, poor agronomic practices, for example, monocropping, and poor and non-uniform seed germination (Houdegbe et al., 2018).

Low and uneven germination is a major problem in spider plants (Abukutsa-Onyango, 2005; Chweya and Mnzava, 1997; Shilla et al., 2016). The species can only be propagated by seeds, therefore, seed quality is a key element for successful crop production (Ochuodho, 2005). Studies have shown that freshly harvested spider plant seeds have low germination, as low as 37 to 46%. (Ekpong, 2009; Muasya et al., 2009). The reason for low germination is believed to be due to dormancy (Kamotho, 2004). The cause of physiological dormancy in freshly harvested seeds could be due to an immature embryo during seed development, a hard seed coat, or induced secondary dormancy (Ochuodho and Modi, 2005).

To address the problem of low germination in *G. gynandra* seeds, farmers plant more seeds than necessary to obtain high yields (Muasya et al., 2009). Ochuodho (2005) determined that freshly harvested seeds of *G. gynandra* have an after-harvest rest period called after ripening period that extends to the fifth month after harvest and active germination starts six months after harvest, and in three months, it increases to 88%. However, the storage period also depends on the storage conditions applied (Kamotho, 2004). Seeds stored for long storage periods were found to be more viable due to the high levels of gibberellic acid (Sowunmi and Afolayan, 2015). Long-term seed storage promotes the biosynthesis of gibberellic acid which promotes germination, hence the enhanced germination (Finkelstein et al., 2008).

In addition to storing seeds, studies have been conducted where pre-sowing treatments, including seed priming, were applied to break dormancy (Abdoli, 2014; Khaninejad et al., 2012). Seed

priming is defined as a process whereby seeds are soaked to a pre-sowing treatment for a specific period where partial hydration is allowed but the radicle does not emerge (Ibrahim, 2016). The water supplied to the seed is enough to start physiological processes but prevents a seed from germinating; the seed becomes primed. After the priming period, seeds are brought back to their initial moisture content by re-drying (Ibrahim, 2016).

Seed priming has been reported to have a significant effect on seedling characteristics such as radicle protrusion, germination percentage, and seed vigor (Canak et al., 2016). Seeds primed using polyethylene glycol (PEG) and gibberellic acid show improved germination (Abdoli, 2014; Ekpong, 2009; Muasya et al., 2009; Puttha et al., 2014a; Quintero C et al., 2018). This is due to their ability to promote germination (GA) and to induce drought stress (PEG) which improves germination.

1.2 Problem statement

Studies have reported that spider plant seeds have uneven and low germination success, thus highlighting the problem of seed quality concerning genetic differences, and physiological quality (germination capacity and seed vigor). The genetic component involves variations of two or more genetic lines, while differences between seed lots of a single genetic line include the physiological component. Seed quality (germination and vigor) is maximum at physiological maturity during seed development when seeds reach maximum dry matter accumulation. Seed moisture content is high at physiological maturity and undergoes a period of water loss (acquisition of desiccation tolerance) in preparation for germination or dormancy. However, the uneven and low germination observed in newly harvested spider plant seeds could probably be attributed to dormancy and /or low seed vigor and may lead to low seedling emergence and low establishment, which in turn

result in low crop yield. Low vigor may be caused by extreme environmental conditions during seed development, storage conditions, or seed aging. Mostly, seeds developed under moisture stress, nutrient deficiency, and extreme temperatures often result in seeds with low vigor. Preharvest environment conditions characterized by high humidity and warm temperatures can also cause a loss in seed viability and vigor. There are studies done on dormancy breaking methods and seed priming techniques to improve germination and vigor of spider plants and among different treatments studied to break dormancy, the application of gibberellic acid improves germination (Gupta et al., 2019; Muasya et al., 2009; Zharare, 2012a). Ekpong (2009) found that pre-heating at 40 °C for 1–5 days was the most effective method compared to the application of GA and KNO_3 leaching, pre-chilling, and soaking. There are not many studies have been done on seed priming in *G. gynandra*. A study was conducted to investigate the effect of osmotic priming on the germination of *G. gynandra* seeds by applying different concentrations of Potassium nitrate (Thuo, 2003). The results showed no significant difference in the germination of primed and non-primed seeds. However, there has been no study reporting on the interaction effect of seed storage periods and seed priming techniques on spider plant germination. Research is needed to understand the nature and mechanisms involved when newly harvested *G. gynandra* seeds are subjected to different storage materials for storage and seed priming techniques.

1.3 Justification

This study will contribute information and understanding of seed dormancy breaking mechanisms focusing on seed storage period and material, and the seed priming techniques in *G. gynandra* to address and improve the underlying issues of low and uneven germination, which leads to low productivity. The outcomes of the study will also be of great importance to farmers with regards

to improving seedling performance and crop stands of *G. gynandra* and understanding how the different varieties of this species perform.

1.4 Aims and Objectives

This study aims to gain insights into the mechanisms involved in breaking dormancy in *G. gynandra* seeds through testing dormancy breaking methods and different seed priming techniques.

Objectives

(i) To determine the effect of seed storage material and storage period on seed germination in *G. gynandra* accessions from West Africa, East Africa, and Asia

(ii) To determine the effect of seed priming techniques and duration on seed germination in *G. gynandra* accessions from West Africa, East Africa, and Asia

1.5 Hypothesis

Germination of *G. gynandra* seeds is directly influenced by seed storage period and material. Due to their differences in ecological regions (West Africa, East Africa, and Asia) to which they are adapted, seed priming techniques and priming durations in *G. gynandra* are genotype dependent.

1.6 Outline of the dissertation

The dissertation is organized based on paper format and compromises of five chapters linked to the objectives. It is proceeded by an introduction chapter which provides a background, problem statement, justification and aims and objective.

Chapter 1

Presented the introduction to the study, background of the study, justification, aims and objectives, and the hypotheses of the study. It highlighted the importance of studying seed dormancy and dormancy breaking mechanisms in *G. gynandra*.

Chapter 2

This chapter reviewed previous studies on the use of seed storage and seed priming techniques to improve seed germination of *G. gynandra* by breaking seed dormancy.

Chapter 3

This experimental chapter gives reports on the laboratory experiment results on the effect of seed packaging materials and seed storage period on seed germination of six accessions of *G. gynandra*.

Chapter 4

This experimental chapter represented the results of the laboratory experiment on the effects of seed priming techniques and duration on seed germination and on six different accessions of *G. gynandra.*

Chapter 5

This chapter gives a general overview discussion highlighting the major findings, conclusion, and recommendations of this dissertation.

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CHAPTER 2 – LITERATURE REVIEW

2.1 Abstract

Gynandropsis gynandra is a leafy vegetable grown by subsistence farmers in sub-Saharan Africa. The species has small hard-coated seeds that look like a snail shell and the seeds can be found from farmers, in local markets, and seed companies such as Silverhill Seeds and Books in South Africa. Over the years, studies have reported poor and uneven germination in the species which could be a result of dormancy. This is a problem for farmers as it results in poor plant establishment and low yields. Also, different seed lots have shown varying germination rates due to differences in seed origins, growing conditions of the parent plant, and storage conditions. Although this is also dependent on the storage conditions of the seeds, studies have reported that seed germination in orthodox seeds increase with increasing storage period. Reports also show that seed pre-treatments including seed priming increase the germination capacity of *G. gynandra*. These include hydropriming, osmopriming, and halopriming. However, the existing results are still contradictory as other treatments show no effect or negative effect on germination. Further studies on understanding the physiology of dormancy, germination; and ways to overcome dormancy within the species are recommended. Therefore, using a wide range of accessions to get the full context of the issues associated with the poor and uneven seed germination within *G. gynandra* species is recommended.

Keywords*: G. gynandra*, germination, dormancy, seed priming.

2.2 Introduction

Gynandropsis gynandra (L.) Briq. (syn. *Cleome gynandra* L.), usually known as spider plant, is a leafy vegetable that belongs to the Cleomaceae family. This family consists of approximately 300 species in 10 genera (Byng et al., 2016). The species is reported to have originated from Sub-Saharan Africa and South East Asia and is distributed to tropical and sub-tropical regions (Chweya and Mnzava, 1997; Nemahunguni et al., 2019). It is commonly found on disturbed soils such as arable lands and wastelands with other annual plants and grasslands (Chweya and Mnzava, 1997). The spider plant is tolerant to drought and plays a substantial role in food security within the households during dry seasons (Ekpong, 2009; Redhead, 1990). In South Africa, the spider plant is found in Limpopo, KwaZulu-Natal, Mpumalanga, Free State, Gauteng and North West provinces (Sowunmi, 2015).

Figure 2.1: *Gynandropsis gynandra*, also known as Spider Plant (Van Rensburg et al., 2007).

This species has a branched growth habit and can grow up to 1.5 m. The stem is characterized by glandular hairs and parallel lines and the plant becomes woody with age (Sogbohossou et al., 2019). Spider plant has palmately compound leaves with leaflets ranging from three to five and white, pink, or lilac petals. It produces small, round seeds, with a rough seed coat, resembling a snail shell enclosed in a linear capsule (Chweya and Mnzava, 1997; Sowunmi and Afolayan, 2015).

Spider plant is of economic, nutritional, ecological, and cultural value. In South Africa, it is a significant part of the food consumed by many local communities (Nemahunguni et al., 2019). The leaves are used in stew preparation and served as a pot herb or a side dish. In Thailand and Malaysia, the leaves are fermented with rice water to make pickles called Paksian-dong (Redhead, 1990), in India, leaves are used as a flavoring agent in sauces (Nemahunguni et al., 2019). The spider plant is also high in micro and macronutrients such as vitamins, iron calcium, and proteins (Chweya and Mnzava, 1997). Therefore, it is used as a supplement in malnutrition management (Dushimimana et al., 2018) and household food security (Onyango et al., 2013). The seeds contain about 29.6% of polyunsaturated oils (Mnzava, 1990).

Additionally, apart from being important in contributing to food security, spider plant also has several medicinal properties (Chweya and Mnzava, 1997; Mishra et al., 2011). In traditional practice of medicine, the leaves and seeds are used to treat stomach aches and headaches. The spider plant also restores blood; pregnant women consume it during and after pregnancy and men consume it after circumcision (Chweya and Mnzava, 1997; Opole et al., 1995). According to Chweya and Mnzava (1997), the shoots and leaves are also used as forage by game animals, camels, and bovines. The leaves produce compounds that act as a plant protectant as it has antifeedants, repellent, and insecticidal properties. It is therefore intercropped with other vegetables to repel insects (Shippers, 2002). Spider plant also plays a significant role in the economy of developing countries such as Uganda, Malawi, Zimbabwe, Tanzania, Kenya, and Zambia, as the young shoots and leaves are sold in the rural markets by growers and gatherers (Chweya and Mnzava, 1997).

Studies have reported that one major challenge experienced by spider plant farmers is the low and uneven germination (Onyango et al., 2013), which has been associated with seed dormancy

exhibited by the crop (Chweya and Mnzava, 1997; Ochuodho and Modi, 2005). This results in low yields and unavailability of enough seeds for the next planting season, which represent an important constraint as the species is mainly propagated by seeds. In Tanzania and Kenya, some seed companies have begun to sell spider plant seeds (Muasya et al., 2009). In South Africa, seeds can be found from the Vegetable and Ornamental Plant Institute of the Agricultural Research Council at Roodeplaat (Vopi) (Motsa et al., 2015). An enormous seed collection of the species is accessible at the World Vegetable Center (AVRDC), and through seed kits supported by diverse projects, the organization makes some seeds accessible to agriculturalists.

Generally, drying seeds and storing them for long-term may results in a significant reduced germination, or a total loss of viability (Pradhan and Badola, 2012). If the seeds are not dried properly, the high moisture content might reduce the seed viability by promoting fungal growth. (Romanas, 1991). However, this is highly dependent on the storage duration and method adopted (Romanas, 1991). Before seeds are stored, several factors are looked at that influence the seed longevity during storage. These factors include seed moisture content, temperature, relative humidity, and the nature of the seeds, among others (Onyekwelu and Fayose, 2007; Pradhan and Badola, 2008). Spider plant seeds are orthodox seeds, this means that these seeds' moisture contents can be reduced by drying without losing viability, and they can be stored for up to 12 months (Muasya et al., 2012b).

Storing seeds and priming seeds has been proven to improve seed germination and seedling performance of *G. gynandra*. Seeds stored for at least three months show an improved germination success compared to freshly harvested seeds. The rest period is believed to extend up to five months (Kamotho et al., 2014; Ochuodho, 2005; Yepes, 1978; Zharare, 2012b). During the early phases of seed germination, seed priming triggers the normal metabolic activities before the

protrusion of the radicle. The primed seeds results in improved, quicker, and even germination due to the activation of enzymes, reduction of imbibition time, metabolic reparation during imbibition, the building of metabolites that promote germination, and the adjustment of osmotic solution (Hussain et al., 2019). Seed priming has been reported to have a substantial influence on seedling characteristics such as radicle length, germination percentage, and subsequent seed vigor. Seeds primed using polyethylene glycol (PEG) performed the best among the other treatments (Abdoli, 2014; Khaninejad et al., 2012). PEG is a polyether derived compound from petroleum with various functions, from industrial manufacturing to medicine (Chauhan et al., 2019

). In plants, it can be used to induce and regulate plant water shortage in experimental hydroponics culture. Gibberellic acid is a plant hormone that encourages plant growth and development, it also promotes seed germination (Gupta and Chakrabarty, 2013). When used as a seed priming agent in dormant seeds, this plant growth hormone also stimulates germination (Ekpong, 2009; Muasya et al., 2009; Puttha et al., 2014b). This chapter presents the existing knowledge of seed dormancy, seed germination, and conditions required to overcome dormancy in *G. gynandra* seeds. It also highlights gaps and contradictions that are still existing under the species regarding dormancy and germination physiology.

2.3 Seed structure and diversity in Cleome

There are about 320 accessions of *G. gynandra* recorded worldwide at the Global Gateway to Genetic Resources. A study was conducted to comprehend the variety within and among accessions and to identify inter-trait relationships. The results showed morphological differentiation within accessions, particularly within accessions from Africa; and that the accessions are not uniform (Wu et al., 2018).

Gynandropsis gynandra seeds are generally brown or black. Depending on the accession, the seeds are round or fairly-round and pointed at the apical region where the radicle is located. The hilum, a small scar marking its former place of attachment**,** is located at the center of the seed. The exterior of the seed is rough with small rounded or oscillated depressions and ridges on the whole surface of the seed (Blalogoe et al., 2020).

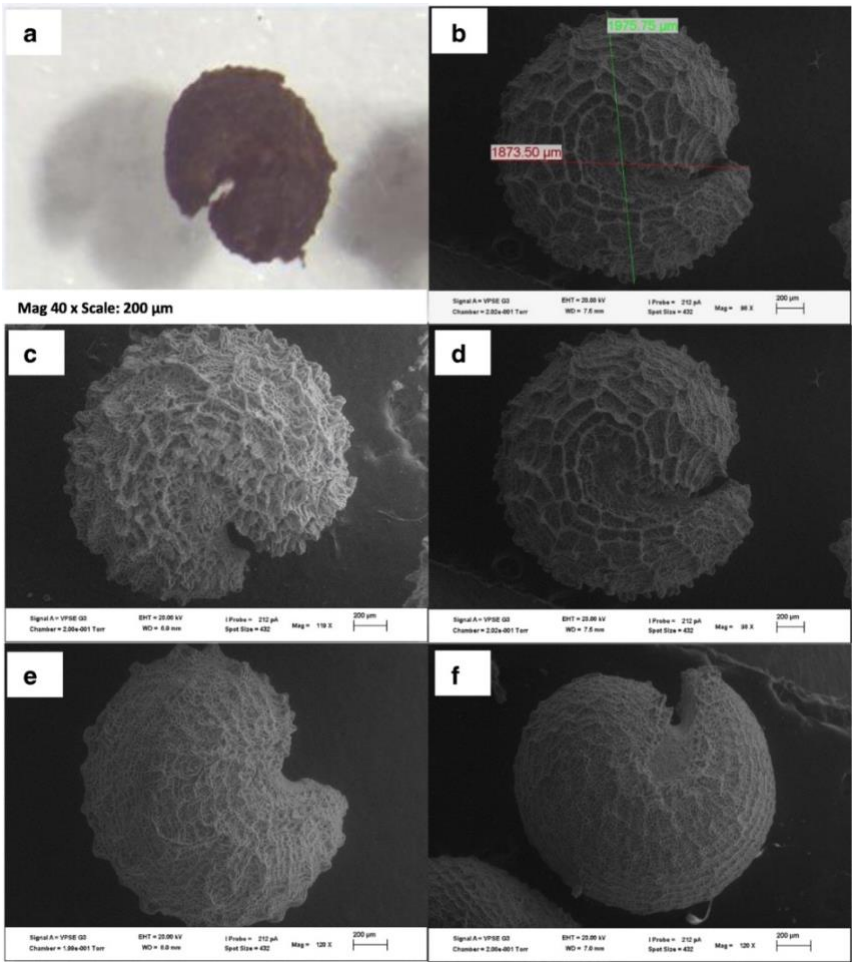

Figure 2.2: Light microscopy showing seed color (a) and seed shape (c, d, e, and f). Fairly round shape with very rough seed surface (c); Round shape with very rough seed surface (d); Round shape with slightly rough seed surface(e); Round shape with slightly rough seed surface (f). Source: (Blalogoe et al., 2020)

2.4 Definition of terms

2.4.1 Seed germination

There are several definitions of seed germination depending on the perspective. In this study, germination is defined according to the seed physiologists as when the radicle has emerged through the seed coat under favorable conditions (Bewley et al., 2012b). These conditions include water, light, and temperature. Depending on the species under study, the radicle should reach a particular length before the seed is deemed germinated (Bewley et al., 2012b). The germination process is initiated when the dry seed takes up water, known as imbibition, and ends with the elongation of the embryonic axis (Bewley, 1997b). However, in many species, an active embryo still fails to emerge from a seed due to the hardness of the covering tissues, which is referred to as coat-imposed dormancy (Bewley et al., 2012b), or due to an immature embryo.

2.4.2 Seed dormancy

Gao and Ayele, (2014) define seed dormancy as the mechanism underlying the inability of a viable seed to germinate under favorable environmental conditions. Seed dormancy and germination are adaptive characteristics of higher plants and are affected by many genetic material and ecological factors such as light, temperature, and seed storage period (Bentsink and Koornneef, 2008). Seed dormancy enable seeds to overcome periods that are not favorable for seedling establishment and is thus important in the plant ecology and agricultural industry (Bentsink and Koornneef, 2008). Therefore, entering a dormant state is an adaptive trait for seeds to maximize germination over time. Seed dormancy also prevents pre-harvest germination (Bewley, 1997b). The seed dormancy

level is controlled by various factors such as light, temperature, and seed storage period. Induction and release of seed dormancy are controlled by plant growth regulators such as abscisic acid and gibberellic acid, respectively.

2.5 Seed development and maturation

The seed development process is comprised of two main stages, embryo development, and seed maturation. Seed development commences with double fertilization, ends with physiological maturity, and can be separated into four stages (Bewley et al., 2012b). Soon after fertilization, the zygote is generally inactive, however the primary endosperm rapidly undergoes mitosis to develop the endosperm tissue, this is classified under phases I and II of the seed development process. In phase III, nutrient reserves accumulate in the endosperm resulting in a dry mass increase. This phase is followed by a rapid seed moisture decrease known as maturation drying, phase IV (Bewley, 1997b). After the ovule has been fertilized, there is a period of seed structure development due to cell division, elongation, and histodifferentiation in which seed structure primordia are constructed and future embryo parts can be envisioned. In this phase, there is a considerable increase in seed size developing the embryonic cells that receive assimilation from the mother plant with high and constant seed moisture content. When changes in cell membrane structure occur at the end of seed maturation stage, there is a substantial reduction of seed moisture content as well as an increase in the synthesis of the enzymes in preparation for an effective seed germination. This period of transition between seed maturation and germination is usually not shown in recalcitrant seeds.

Seed technologists have clarified the maturation process and determined the changes that occur during the seed development process. During this process, the following changes occur:

Seed moisture content: It is the overall amount of water in the seed and is usually expressed as a percentage on a dry weight basis or wet weight basis. During fertilization, for both monocots and dicots, an ovule has a moisture content of approximately 80% (fresh weight basis). This value declines during seed maturation although it continues to be high during most of the maturation stage because water transfers nutrients to the developing seeds from the parent plant (Baroux et al., 2002). Initially, the dehydration stage of seed maturation is slow and is fastened when the seeds reach maximum dry weight; during that time, the seeds possess moisture content of 35% for orthodox monocot seeds to 55% for orthodox dicot seeds, produced in dry fruits. The seeds moisture content continues to decrease until hygroscopic equilibrium is obtained. After hygroscopic equilibrium is reached, the changes in moisture content are associated with changes in relative humidity. Nevertheless, seeds produced in fleshy fruits have a lower decrease in moisture content than seeds produced in dry fruits. Recalcitrant seeds usually possess moisture content of over 60% fresh weight basis due to no changes in desiccation at the end of the maturation stage during seed development (Baroux et al., 2002).

Seed size: Compared to the final seed size, the fertilized ovule is a small structure, this is due to the cell division and expansion that occurs during the seed development process. Seed size partly determines the quantity of seeds that can be produced and the fate of the seed ensuing seedlings, including dispersal, predation, germination, emergence, and seedling performance. Depending on the shade tolerance of each species, crops found in shaded environments generally produce larger seeds. In low-light environmental conditions, species with larger seeds have seedlings with high rate of survival. They have high reserves of proteins and lipids and their more advanced development can facilitate growth (Salisbury, 1974). To compete with small amounts of sunlight that is available in shaded environment, the first shoots of the crops grow taller while leaves grow

broader quickly. This is due to the high amounts of metabolic reserves of larger seeds (Leishman et al., 2000). Nevertheless, plants producing larger seeds tend to produce fewer seeds per fruit or flower (Bruun and Ten Brink, 2008). Additionally, small plant cannot bear large seeds due to seed weight and might result in the death of the plant, which may explain the correlation between plant size and seed size (Aarssen and Eriksson, 2005).

Small seeds are predominantly found in in dry environmental conditions and deserts (Mazer, 1989). Small seeds also have an ability to be stored in dry environments for long periods without losing viability. An example of this is the species under study, *G. gynandra*.

Seed dry weight: After fertilization, the weight of the developing seeds starts to increase due to the accumulation of nutrients and water uptake. During this stage, the seed fill is slow due to cell division and cell elongation. Afterwards, the seed dry mass accumulation increases until the seeds reach their maximum dry weight (Baroux et al., 2002).

Germination: Few days after fertilization, the seeds of several grown species can germinate. At this state, germination refers to the protrusion of the primary root, nor the formation of a normal seedling because histodifferentiation has not been completed and the accumulation of reserves is still initial. Consequently, this germination does not lead to the production of vigorous seedlings (Bruun and Ten Brink, 2008). Hypothetically, it is possible to consider that the percentage of the seeds that can germinate increases during seed maturation phase reaching a maximum when the seeds attain maximum dry weight. This is only found in species with no dormancy due to the imbalance in the germination promoters or inhibiters brought during the reserve accumulation period that may directly affect seed ability to germinate.

Vigor: Seed vigor is defined by International Seed Testing Association as the sum of the seed properties which determines the seed activity and performance level of a seed lot during germination and seedling emergence. Seeds that perform well are referred to as 'high vigor' seeds (Perry, 1978). The changes in seed vigor are usually parallel to the nutrient mobility from the parent plant to the developing seeds. This means that during maturation, the proportion of vigorous seeds increases reaching maximum or close to or at the same time the seeds reach maximum dry weight (Baroux et al., 2002).

2.6 Seed germination and crop performance

Crop yield and quality is influenced by a very important process known as seed germination. Therefore, maximum seed germination is very important for crop production; and a great significance for crop yield improvement and quality is understanding the molecular and physiological aspects of seed dormancy and germination (Tuan et al., 2019). Uneven or poor germination and subsequently uneven seedling growth can lead to a huge financial losses by decreasing crop yield (Ghiyasi et al., 2008). Seed germination may directly or indirectly influence crop yield. The indirect effects include percentage emergence and time from sowing the seeds to seedling emergence. These influence yields by varying the density of the plant population, spatial arrangement, and crop duration. The reports were on the effects on total emergence, rate of emergence, and the uniformity of emergence (Ekpong., 2009, Ghiyasi et al., 2008). All these factors can potentially influence dry matter accumulation by the plant or plant community and therefore possibly affect yield. The total emergence determines plant density, and there is a strong correlation between plant density and yield (Shao et al., 2018).

2.7 Seed dormancy and dormancy classification in *Gynandropsis gynandra*

Several dormancy classification schemes have been published (Baskin and Baskin, 2014; Harper, 1957; Nikolaeva, 1977). Among those, Harper's classification has been the one used regularly as it accommodates the diversity of the types of dormancy known to occur in seeds, irrespective of evolutionary position, life form, or biogeography of the taxon that produced them (Baskin et al., 1998; Nikolaeva, 1999). This classification states that there are two types of dormancy: endogenous and exogenous dormancy (Table 2.1). The dormancy that occurs due to some chemical changes within the embryo of the seed is termed endogenous dormancy. A seed or other germination unit cannot germinate because of an endogenous dormancy due to an insufficiently developed embryo, or specific seasonal cues have not occurred. Exogenous dormancy is caused by conditions outside of the embryo of the seed. For example, when the seed coat is too hard for moisture to infiltrate, effectively preventing germination. Some factors include endosperm or fruit walls or any structures that cover the embryo to prevent germination to occur.

Table 2.3: Classification scheme of organic seed dormancy types, causes, and how they can be broken. (Blalogoe et al., 2020)

Type	Cause	Broken by
Exogenous Dormancy (A)		
Physical	Seed (fruit) coat impermeable to water	Opening of specialized structures
Chemical	Germination inhibitors in fruit coat	Leaching
Mechanical	Woody/hard structure restrict growth	Warm and/or cold stratification
Endogenous Dormancy		
Physiological (C)	Physiological inhibiting mechanism (PIM)	Warm and/or cold stratification
Morphological (B)	Underdeveloped embryo	Appropriate conditions for embryo growth/germination
Morphophysiological (B-C)	PIM of germination and underdeveloped embryo	Warm and/or cold stratification
Exogenous X Endogenous (Combinational)		
(A-B-C)		

Source: Baskin and Baskin (2004); Baskin and Baskin (2014)

The *Cleome* genus has been grouped among the vegetable and flower genera that have primary non-deep endogenous physiological seeds dormancy (Geneve, 1998). In this category Geneve (1998) specifically, has placed *Cleome* species under the class that requires light as one of the essential factors for germination to occur. Seeds of *G. gynandra* have an after-harvest rest period known as latency, specifically for the fresh harvest, that extends to the fifth month after seed collection. An increase in germination is observed from the third month after seeds are stored, and active germination is observed after six months (Ochuodho, 2005; Yepes, 1978; Zharare, 2012a).

Seed germination in spider plants was reported to be due to the hard seed coat, immature embryos, or induced secondary dormancy (Ochuodho and Modi, 2005). Nevertheless, Ekpong (2009) showed that seed dormancy of *G. gynandra* may not be attached to the seed coat as a physical barrier to water absorption but due to the leaching of germination inhibitors on the seed coat. Seeds permeable to water could still not germinate until after soaking for 12 hours (Ekpong, 2009).

Furthermore, when seeds were soaked, a slight decrease in seed germination was observed. Seeds imbibe water two hours after being soaked and continue imbibing for up to 48 hours (Blalogoe et al., 2020). The slight decrease in germination when soaking time increases might be due to water trapped in the tissue between the embryo and seed coat having created an oxygen barrier, a situation also reported in *Datura ferox* and *D. stramonium* seeds (Reisman-Berman et al., 1989). Anoxia caused by prolonged seed soaking may result in irreparable injury due to the toxic metabolites accumulation (Norton, 1986). Thus far, studies that have reported that *G. gynandra* seed exhibits dormancy that leads to low seed germination are contrasting the results from preliminary seed germination test carried out at The World Vegetable Center, Eastern, and Southern Africa, Tanzania, and Arusha (Blalogoe et al., 2020).

The fresh seeds from mature green, yellow, and brown pods were harvested in the test and immediately sowed in soil trays in a screen house. The seeds from all the three categories of the pots germinated. The difference noticed was that after sowing, the seeds from brown pods germinated within three days, followed by yellow then green pods which germinated approximately nine days after sowing (Blalogoe et al., 2020). This may be due to the level of physiological maturity within the seeds. Ekpong (2009) noticed an increase in the germination of *G. gynandra* seeds to about 72% after pre-washing seeds in running water for 60 minutes as opposed to 30, 90, and 120 minutes, demonstrating that this treatment was able to overcome seed dormancy in *G. gynandra*, which is believed to be the result of inhibitors on the seed coat. Additionally, pre-heating the seeds at a temperature of 40 °C for 24 hours was able to break the seed dormancy. To support this, Bewley and Black (1982) noted an enhanced degradation of seed tissues at high temperature. Consequently, germination may be promoted due to the supply of

energy to the embryonic axis that may increase, and an easy diffusion in and out of the seeds by such substances as water, oxygen, inhibitors, and carbon dioxide.

Ekpong (2009) reported a pre-chilling test on spider plant seeds for 24 hours was able to release deed dormancy and increased seed germination to approximately 66% compared to chilling for 3, 5, and 7 days. Seed covering structures and inhibitors are the two factors influencing the duration of moist chilling to release embryo seed dormancy (Khan, 1997). Pre-chilling released dormancy as a result of various metabolisms that occur during these treatments, such as increasing the level and endogenous gibberellins responsiveness but considerably reducing the level of abscisic acid (Bewley and Black, 1982).

2.8 Possible causes of poor seed germination in *Gynandropsis gynandra*

There are several factors that affect the low and uneven germination in *G. gynandra*. Reported factors include the conditions at harvest maturity and seed moisture content, the storage conditions (storage duration, temperature, and storage packaging), and the seed state of dormancy. The seeds of spider plant are mature and ready for harvesting when the pods are yellow and the seeds black (Chweya and Mnzava, 1997). During this stage the moisture content of the seed is too high, more than 25% and a drying period was recommended to decrease the moisture content and favor germination (K'Opondo, 2011).

It is indeed reported that seed dried up to a moisture content of 5% had the highest germination percentage which was 77.5% (Kamotho et al., 2014). One study reported that a range of sowing depth of 0.5 cm and 1 cm is acceptable (Sowunmi and Afolayan, 2015), while another recommended a range of sowing depth of 0.15 cm and 0.35 cm (Seeiso and Materechera, 2011), this is due to their small size because increased sowing depth delayed seedling emergence and

decreased seedling relative growth rate. Regular watering promotes germination in *G. gynandra*. In the field, watering bi-weekly during the germination stage showed the highest germination percentage compared to watering daily and once a week (Sowunmi and Afolayan, 2015). This was due to the seed coat imbibing excess water which was detrimental to the emergence of the radicle. However, this may be influenced by the humidity in the germination environment. For better germination, seeds must be dried and stored after being harvested.

Poor germination in *G. gynandra* is also explained by the observed dormancy in freshly harvested seeds of the species. The highest germination percentage increased as the storage time increased in a seed germination study by Kamotho et al. (2014), freshly harvested seeds showing 14.5% of germination while seeds stored for six months showed 95% seed germination. Similar results were observed in which seeds of this species were stored for five months at a temperature of 15°C which showed germination percentage of more than 90% (Ekpong, 2009). Ochuodho and Modi (2005) observed that seeds germination improved after seeds had been stored for three months.

Gynandropsis gynandra, like several other freshly harvested herbaceous plants seeds require postharvest ripening before dormancy is released (Chweya and Mnzava, 1997; Geneve, 1998; Kamotho et al., 2014). However, during the after-ripening period, the preliminary germination observations do now show an improvement of the rate of germination (Shilla et al., 2016). Furthermore, Ochuodho and Modi (2007); Motsa et al. (2015) used the seeds of *G. gynandra* stored for 3 months and 1 year, respectively, but found the initial germination to be low, but it increased after the application of some mechanisms to break dormancy.

2.9 Requirements for germination in *Gynandropsis gynandra*

For a successful seed germination, certain environmental conditions must be met including optimum temperature, light, oxygen, and soil moisture content. Nevertheless, these conditions are species-specific. Several studies have been carried out to determine the optimum conditions for seed germination in *G. gynandra*. Literature shows that the most studied is the effect of light on seed germination of *G. gynandra* (K'Opondo, 2011; Muasya et al., 2009; Ochuodho, 2005; Ochuodho and Modi, 2005; Sowunmi and Afolayan, 2015; Zharare, 2012b). The results showed a negative response of germination rate reduction in *G. gynandra* when seeds have been exposed to continuous light beyond 12 hours. This indicated that this species is negatively photoblastic. Therefore, the optimum conditions for seed germination in *G. gynandra* would be continuous dark and alternating dark and light for a daylight of 8 hours. According to Geneve (1998), such seeds are termed photo dormant and occur in *G. gynandra* because of their small seed size of less than one milligram (Bewley et al., 2012b). In the wild, spider plant seeds generally spend time covered by plant debris in the soil before the rainy season starts. This might explain the adaptation of *G. gynandra* as a requirement for a successful germination. One approach that farmers could use is to initially cover the nursery bed with black polystyrene bag, then remove it once the seedlings have emerged and transplant the seedlings into the field to adapt with environmental conditions. Warm temperatures would be ideal for germination of *G. gynandra* due to its tropical origin. Several studies have investigated the effect of temperature on the germination of *G. gynandra* seeds (K'Opondo, 2011; Muasya et al., 2009; Ochuodho, 2005; Ochuodho and Modi, 2005; Sowunmi and Afolayan, 2015; Zharare, 2012b). The favorable temperature for seed germination percentage higher than 50% from these studies ranged from 25°C to 40°C.. Nevertheless, Zharare (2012) found alternating 4°C/27°C for 16/8 hours as the optimal temperature for seed germination

of *G. gynandra*, which is a new finding in the species, as given the tropical origin of the species, it was expected to perform better under warm conditions since it is tropical.

2.10 Dormancy breaking mechanisms in *Gynandropsis gynandra*

2.10.1 Seed storage

To meet the global food insecurity crisis, seeds of wild species are collected and kept in dry storage, but often there is a shortage of seeds for this reason. Therefore, more research is focused on maximizing the use of available seeds to release dormancy before sowing. In the field, sowing seeds that are no longer dormant compared to sowing dormant seeds increases restoration success. The most cost-effective seed dormancy breaking mechanism of the available dormancy breaking treatments is dry storage (Baskin and Baskin, 2020). Seeds including member of the Cleomaceae family can undergo a rest period after ripening have nondeep physiological dormancy. Ekpong (2009) reported that when freshly harvested seeds of *G. gynandra* were stored at 15℃ and room temperature (30- 33 8℃) for five months, germination of more than 90% was observed. He suggested that the two storage conditions can break seed dormancy in *G. gynandra*. Ochuodho (2005) observed similar results where seed dormancy of this species was broken by a temperature of 30-38℃ after being stored for three months at 15.8℃. Nevertheless, Kamotho et al. (2007) suggested storing seeds at -20℃ for long storage with low seed moisture content of two to five percent. This challenges farmers as there is no way to achieve such levels of moisture contents and temperature conditions. Other studies have reported that *G. gynandra*, as it is the case for many freshly harvested seeds of herbaceous plants, needs a post-harvest ripening period before dormancy is broken (Chweya and Mnzava, 1997; Geneve, 1998; Kamotho et al., 2014). Similar results have also been found in a close sister group of *G. gynandra*, *Arabidopsis thaliana* (Ali-

Rachedi et al., 2004). The farmers usually collect seeds and store them for the next planting season to avoid the dormancy period. This is achieved by collecting the yellow capsules before they are ripe and drying them in a controlled manner so that seeds can be reserved. However, commercial seed companies and farmers are struggling to store the seeds because of the predictable seed deterioration during storage which results in low seed vigor and decreased number of viable seeds (Mutegi et al., 2001), which supports a more extensive study be undertaken to determine proper seed storage conditions. Apart from seed dormancy, harvesting period, production practices, processing, packaging and storage conditions of *G. gynandra* are other factors which may cause seeds to be not readily available to farmers for propagation (Kamotho et al., 2014; Ngoze, 2005). The traditional method of storing seeds is in pots and gourds. Kamotho et al. (2014) showed that high seed quality of *G. gynandra* is achieved when seeds are harvested at the yellow pod maturity stage, sun-dried to 5% moisture content, and stored for up to six months.

In some cases, seed dormancy manifests itself through embryo immaturity which occurs when the meristematic tissues of the embryo are not fully differentiated when the seed is dispersed, this is classified as morphological dormancy (Baskin and Baskin, 2020). Understanding environmental conditions that promotes seed germination is important for understanding commercial considerations, and it is crucial to obtain germination information in the field and the stage at which the seedling best adapt in a given (Baskin and Baskin, 2020; Dresch et al., 2014).

2.10.2 Seed priming and other pre-treatments for breaking dormancy in *Gynandropsis gynandra*

Seed priming is the activation of metabolic activities that are required for germination to occur through a controlled and monitored hydration, however, it interrupts the radicle from emerging

(Raj and Raj., 2019). Seed priming promotes seed performance**,** ensures uniform germination and seedling emergence, and better establishment**,** improves crop yield in various environments**,** increases environmental stress tolerance, has a better weed suppression effect, and helps to overcome dormancy. There are different seed priming procedures, these include hydropriming, osmopriming, halopriming, solid matrix priming, biopriming and hormonal priming (Lutts et al., 2016; Raj and Raj., 2019).

Table 2.3: Different seed priming methods and their characteristics.

Priming method	Priming agents	Characteristics
Hydropriming	Water	-Simplest, cheap, and eco-friendly. -Useful in dry farming
Halopriming	Inorganic salt solutions (NaCl, KCl, etc)	-Simple and cost-effective agro-technique that ensures better synchrony of emergence and crop stand under several environmental conditions.
Osmopriming,	Osmotic solutions contain chemicals such as mannitol, polyethylene glycol (PEG), sorbitol, glycerol, etc.	- Due to the low water potential of osmotic solutions, seed uptake water slowly which permits seed imbibition and activation of early stages of germination but hinders radicle protrusion.

		- Polyethylene glycol is the common chemical used.
Solid matrix priming	Seeds are mixed and incubated with a wet solid water carrier	-An alternative to osmopriming due to the high cost of osmotic agents and technical problems with aeration in osmopriming. -Materials used as matrices should have high water holding capacity, low matrix potential, low solubility in water, large surface area, nontoxic to seeds, and can stick to the surface of the seed.
Biopriming	Treatment combination of the inoculation of seed with beneficial microorganisms and regulation of seed hydration.	-Microorganisms have the potential to proliferate, colonize, and produce plant growth regulators.
Hormonal priming	Abscisic acid, auxins, gibberellins, kinetin, ethylene, polyamines, and salicylic acid (SA).	-Gibberellic acid improves the photosynthetic activity, antioxidant system, seedling emergence and growth of white clover (Galhaut et al., 2014), and increases salt tolerance and grain yield in spring wheat (Iqbal and Ashraf, 2013).

		-Polyamine priming imparts drought tolerance in rice (Farooq et al., 2006).

Source: (Lutts et al., 2016; Raj and Raj., 2019)

Currently, there are several pretreatment methods that have been used to break dormancy of *G. gynandra* seeds. Yet the results are still contradictory and inconclusive (Shilla et al., 2016). A study by Ekpong (2009) showed that heating freshly harvested seeds of *G. gynandra* at 40°C for one to five days was the most effective method with up to 90% germination capacity compared to other dormancy breaking methods applied such as heating, soaking, leaching, potassium nitrate (*KNO3*), and Gibberellic acid (*GA3*).

Similarly, washing seeds under running water at room temperature for few minutes; soaking in tap water for a few hours before the germination test; and pre-chilling by moistening and maintaining at a cold temperature for a few days before the germination were observed to increase seed germination percentage up to 74%. Nevertheless, Ekpong (2009) observed the least germination percentage in seeds treated with gibberellic acid (34%) and potassium nitrate (16%). However, the results by Muasya et al. (2009) show that gibberellic acid was the most effective treatment to break dormancy in *G. gynandra* seeds when investigating the effect of different pretreatments which included Light, chilling, gibberellic acid, potassium nitrate and leaching.

They also found a significant improvement of germination when applying stratification in seeds at a temperature of 5°C for two weeks and when germination occurred in the dark, whereas potassium nitrate significantly decreased the rate of germination.

Meanwhile, Ochuodho, 2005, investigated the effect of different pre-germination treatments (Scarification, hydration, chilling, and germination in the presence of potassium nitrate and

gibberellic acid) on seeds from different origins that were previously stored for one year and two years from different origins. The results obtained from that study indicated that *G. gynandra* seed dormancy was effectively broken by both preheating at a temperature of 40°C for 15 days and scarification. One study used different levels of *KNO3* (1%, 1.5%, and 2%) to determine its effect on seed germination of *G. gynandra,* the results indicated no significant difference in the germination of primed seeds and non-primed. Also, priming seeds with 2% potassium nitrate negatively affected seed germination. Regardless of the previously mentioned successes, different seed lots have been reported to show different germination (Ochuodho, 2005).

It was also noticed that seed lots from South Africa showed a low germination rate and low overall percentage of germination compared to seed lots from Kenya. Even though there is no clear cause of such difference, it is hypothesized that the difference in seed germination rates could be caused by the different environmental conditions during seed development as the two seed lots are from different regions (Shilla et al., 2016). The effect of gibberellic acid (*GA3*), potassium nitrate (*KNO3*), potassium sulphate (*K2 SO4*), and smoke water on seed germination was studied and it was found that *GA3* was the most effective pre-treatment to break dormancy in *G. gynandra* seeds with *KNO3* and *K2 SO4* reported as ineffective to break dormancy. This indicates that hormonal priming in *G. gynandra* seeds yields better results compared to other chemicals.

2.11 Conclusion

In summary, based on previous studies, freshly harvested seeds of *G. gynandra* exhibit dormancy which can be broken through the application of various seed priming techniques and/or after seeds have been stored for at least three months. Other means of breaking dormancy may also be required

even after seeds have been stored due to low germination that is observed in some studies after storing seeds. Currently, the level of dormancy depends on the harvesting time. Dormancy is high on seeds harvested during the green-pod stage. Additionally, germination has shown to improve when seeds are germinated under dark conditions and alternating dark and light. Many seed priming treatments have shown to increase the rate of seed germination; however, the results vary from one study to another. The improved germination could be due to a decrease in dormancy level as seed storage period increases to over three months. There is also a possibility that these are requirements for germination of *G gynandra* seeds which are not necessarily dormant. It is not clear from these studies that these conditions are the requirements for germination or for breaking seed dormancy in this species. The nature of seed dormancy and the dormancy breaking mechanisms are also not well-understood. More research is recommended on the physiology of seed dormancy and seed germination with a more diverse and large number of accessions to clarify the narrow scientific information available on low seed germination in *G. gynandra*.

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CHAPTER 3: THE EFFECTS OF SEED STORAGE PERIOD AND PACKAGING MATERIAL ON SEED GERMINATION OF *GYNANDROPSIS GYNANDRA* (L)

3.1 Abstract

Studies have reported low and uneven germination in *G. gynandra* seeds which may be attributed to dormancy and /or low seed vigor. This may lead to low seedling emergence and low establishment, which in turn result in low crop yield. The objective of this study was to assess the effect of packaging materials and storage period on seed germination and vigor of six accessions

of *G. gynandra*. Freshly harvested seed samples from three origins (Asia, West Africa, and East-Southern Africa) were dried up to 5% moisture content using the oven-drying method and stored using three different packaging materials (brown paper bag, aluminium foil, and black polyethylene bag) for four months at room temperature of 25℃ and sampled at monthly intervals to test for seed germination and vigor. The experimental design was 6 accessions x 3 packaging materials x 5 storage periods using a Completely Randomized Design with three replicates of 90 treatments (270 experimental units). Data sets (electrical conductivity, viability status, germination, and radicle length) were subjected to Analysis of Variance (ANOVA) at 5% level of significance, and means were compared using the Least Significance Difference. The response of the six accessions highly differed by storage period and storage material for quality parameters including TZ, electrical conductivity, germination, and radicle length. Accession B06 had the highest germination percentage for the first two months (82 and 78% respectively). Among other accessions, accession B03 seeds had the highest final germination percentage and coefficient of velocity of germination (88.89 and 652.5 respectively), with low mean germination time and mean germination rate (1.613 and 0.000505 day^{-1}, respectively). Overall, seed germination increased as the storage period increased beyond 2 months. Seeds stored in aluminium foil paper showed high seed germination (60%), indicating seed dormancy elevation compared to seeds stored in brown paper bags and black polyethylene bags.

Key words: Accessions, Germination, *Gynandropsis gynandra*, Packaging material, Storage period, Seed viability.

3.2 Introduction

Gynandropsis gynandra (L.) Briq., commonly known as spider plant, is a leafy vegetable that is widely consumed in most African countries (Chweya and Mnzava, 1997). In Eastern, Southern, and some West African countries, this species is sold in rural and urban markets, but some communities still harvest the species from natural habitats. Due to the increasing human population and the benefits of this species, the demand has increased yet production remains low (Omondi et al., 2017; Onyango et al., 2013). Spider plant is only propagated by seeds (Chweya and Mnzava, 1997), thus there is a necessity to investigate factors affecting seed germination and quality in *G. gynandra*. The seeds of major crops have been extensively researched regarding seed and crop performance; however, species like spider plant are considered neglected and have received very little attention (Chataika et al., 2020). The so-called neglected species play an important role in food and nutrition security and income generation of the rural communities (Onyango et al., 2013).

A seed is a main input in crop production and its quality affects germination, vigor, seedling emergence, and consequently plant population and yield (Caverzan et al., 2018). The basic requirements for seeds to germinate are water, oxygen, and a suitable temperature. Newly ripe seeds that are unable to germinate when these conditions are optimal are said to be in a state of dormancy (Bewley and Black, 1982). The dormancy level and period are extremely variable. The terms seed germination and dormancy have been misunderstood, for example, many times seeds that have not germinated were incorrectly indicated to as dormant (Baskin and Baskin, 2014). There are several dormancy breaking methods that require varying conditions. Storing seeds for a specified period under certain storage conditions is one of the methods used to break dormancy and has been successfully used in physic nut (*Jatropha curcas)* (Lozano-Isla et al., 2018), tomato (*Lycopersicon esculentum Mill*.) (Dias et al., 2006) and barley (*Hordeum vulgare)* (Ajouri et al., 2004).

However, seeds deteriorate during storage, which leads to a decrease in vigor and percentage germination, leading to poor seedling development and low crop yields. During storage, the most important factors that influence seed viability are temperature and seed moisture content (Thirusendura Selvi and Saraswathy, 2017). Generally, storing the seeds under low-temperature conditions and low moisture content can maintain viability, germination, and vigor. Seed viability and vigor decreases the longer the seeds are stored. The electrical conductivity of seed leachates also increased with prolonged storage under unfavorable conditions (Rao et al., 2006). Seed packaging material and storage duration showed a significant effect on viability and seedling vigor in onion seeds (*Allium cepa* L.) (Rao et al., 2006).

Low and uneven germination has been reported in *G. gynandra* (Chweya and Mnzava, 1997). This species have orthodox seeds (Kamotho et al., 2014) and may be stored for longer periods without losing viability. Various studies have been conducted to improve the germination capacity of the species (Kamotho et al., 2014; Muasya et al., 2012a; Ochuodho, 2005). Germination studies on *G. gynandra* focused more on the effect of breaking dormancy methods. The methods used included the effect of light, storage period, heating, soaking, gibberellic acid, and potassium nitrate on the germination capacity of the species. Although the results of the different studies are conclusive, there are still contradictions (Shilla et al., 2016). For example, the type of dormancy that exists in this species. Seeds of spider plants and the whole genus of Cleome exhibit non-deep endogenous physiological dormancy which prevents germination after pod harvest (Ramphele et al., 2020; Shilla et al., 2016). The present study aimed to determine the response to storage of six accessions of *G. gynandra* from different regions. The objective of this study was to investigate the germination capacity of *G. gynandra* seeds stored for up to four months using different packaging materials.

3.3 Materials and methods

3.3.1 Study materials

Six accessions from three different geographic regions, viz. Western Africa, Eastern-Southern Africa, and Asia were used in the study (Table 1). Self-pollinated plants were harvested in January 2021 at maturity stage from accessions planted for three months (October 2020) before the commencement of this experiment at the seed laboratory of KwaZulu-Natal in South Africa.

Table 3.1: List and geographic origin of genotypes used in this study.

Genotypes	**Parental accession names**	**Institutions**	**Country of origin**	**Region**
B01	HBY/2307b	Kenya Resource Centre for Indigenous Knowledge (KENRIK)	Kenya	East-Southern Africa
B02	ODS-15-013	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi (UAC)	Benin	West Africa

B03	ODS-15-061	Laboratory of Genetics, Biotechnology and Seed Science (GBioS), University of Abomey-Calavi (UAC)	Togo	West Africa
B04	TOT5799	World Vegetable Center	Thailand	Asia
B05	TOT7505	World Vegetable Center	Lao People's Democratic Republic	Asia
B06		World Vegetable Center	South Africa	East-Southern Africa

The different regions were selected because *G. gynandra* seeds are diverse and morphological differences were detected between seeds from Asia and those from Africa (Wu et al., 2018). The seeds were carefully separated from capsules by hand and were dried using the oven at 103°C for 17 hours and silicon-desiccant in an air-tight glass desiccator to reduce the seed moisture content to about 5%. These seeds were packed in three different packaging materials; brown paper bag, aluminium foil paper, and black polystyrene bags, and kept at room temperature of 25 ℃.

Figure 3.1: Packaging materials used to store *G. gynandra* seeds. Brown paper bag (a), aluminium foil paper (b), and black polystyrene bag (c).

3.3.2 Experimental site, treatment structure, and experimental design and layout

The study was carried out at the Seed Science Laboratory in the School of Agricultural, Earth, and Environmental Sciences of the University of KwaZulu-Natal, Pietermaritzburg (29.6196° S, 30.3960° E). The experiment had three factors: accessions with six levels (B01, B02, B03. B04, B04, B05, B06), storage periods with five levels (0, 1, 2, 3, and 4 months), and packaging materials with three levels (aluminium foil paper, black polystyrene bag, and brown paper bag, Refer to figure 3.1), giving a 6 x 5 x 3 factorial treatment structure, laid out in a completely randomized design (CRD) with three replications per treatment (90 treatments) giving a total of 270 experimental units.

3.3.3. Determination of moisture content

The moisture content was determined using the oven method described by the International Seed Testing Association (ISTA) (17h at 103°C in the oven) and the seeds were weighed using a digital balance Ohaus® Pioneer™ Plus analytical. The moisture content was calculated using the following formula:

$$MC = \frac{Loss\ of\ weight}{Initial\ weight} x\ 100 = \frac{m1-m2}{Initial\ weight\ (m1)} x100 \quad (1)$$

m1 is the weight in grams of the seed before drying and m2 is the weight in grams of the seeds after drying.

3.3.4 Electrical conductivity determination

The seed electrical conductivity was determined according to the procedures described by ISTA (2012). The electrical conductivity is an indicator of seed viability It provides a rapid and reliable results, and the technique is not destructive, and the seeds can be used after the conductivity test for seedlings production.Ten seeds of each genotype were replicated three times and soaked in 40 ml of distilled water to allow the probe to be completely submerged in the water solution and kept at room temperature of 25℃. After 24h, the electrical conductivity of the seed leachate was determined using OHAUS Starter 3100C Conductivity Meter. The mean values were expressed in µS cm^{-1}.

3.3.5 Viability Testing

The tetrazolium (TZ) viability test as described by the International Seed Testing Association ISTA book (ISTA., 2012) was performed to ensure that the seeds were viable before conducting a germination test. The tetrazolium test is a quick test used to estimate seed viability and vigor based on color alterations of viable seed tissues in contact with the 2,3,5 triphenyl tetrazolium chloride solution, therefore reflecting the degree of activity of the dehydrogenase enzyme system closely related to seed respiration and viability (Blalogoe et al., 2020; Marcos, 2015). After soaking in the TZ solution, viable seed embryos of spider plant were stained red.

Due to the number of available seeds, three replicates of 10 seeds were used for each treatment. Seeds were imbibed in distilled water for 24 h at room temperature and cut into halves before being immersed in 1.0% of 2,3,5 triphenyl tetrazolium chloride solution. The solution was prepared by dissolving 1 gram of TZ powder in 100 ml of distilled water. After 24 h of incubation, the number of viable and non-viable seeds were identified by the embryo staining and counted under a light microscope at the Plant Pathology Laboratory of the University of KwaZulu-Natal.

3.3.6 Germination test

From the germination data, the following germination parameters were assessed:

After every storage period, seeds were taken out of the packaging materials. Three replicates of 25 seeds per treatment were subjected to a germination test by placing them on top of Whatman filter paper in a petri dish of 9cm in diameter. The seeds were moistened with 7 ml of distilled water

and kept at 30°C in a germination chamber (Labcon, L.T.I.E, South Africa). Water was added as needed. Germination counts were taken every day for 14 days. A seed was deemed germinated when the radicle had protruded with a length of approximately 2mm. On day 9 or germination, the radicle length of the germinated seeds was measured. Germination percentages were determined at monthly intervals for four consecutive months using three replicates of 25 seeds per treatment. The germination test method was carried out according to the ISTA (ISTA., 2012).

Final germination percentage (FGP) = $\frac{Total\ number\ of\ seeds\ germinated}{initial\ number\ of\ seeds\ used} \times 100$ (2)

Mean germination Time (MGT) = $\frac{\Sigma nidi}{\Sigma N}$ (3)

Where ni = the number of germinated seeds at day i, di = incubation period in days, and N = the number of germinated seeds in the test (Baskin and Baskin, 2014). The units for MGT are days.

Mean Germination Rate, (MGR) = $\frac{\sum(\mathbf{n} \times \mathbf{d})}{\boldsymbol{N}}$ (4)

Where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = the total number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981). The units for MGR are d^{-1} .

Coefficient of velocity of germination, (CVG) = $\sum_{i=1}^{k} n_i\, t_i$ (5)

n= the number of seeds germinated every day and t= the number of days from seeding corresponding to n (Al-Ansari and Ksiksi, 2016). CVG has no units.

3.3.7 Data Analysis

Data collected in this study (EC, viability status, germination, and radicle length) was subjected to analysis of variance (ANOVA) using GenStat 20th edition (VSN International, United Kingdom) at the 5% level of significance. The difference among treatment means (accessions, storage periods, storage materials) were compared using the Least Significant Difference (L.S.D.) test at the level of 5 % of significance.

3.4 Results

3.4.1 Viability

The three-way interaction effect of accession x storage periods x storage material on the viability of *G. gynandra* seeds was significant ($p<0.001$) (Table 3.2). The percentage viability values in the non-stored seeds were high for accessions B01 (70%) and B06 (77%). In comparison with non-stored seeds, the control, there was a slight decrease in seeds viability for all the accessions except for accession B02. The effect of the seed storage period on spider plant seeds viability was different for the six accessions. Seed storage material had no significant effect ($p<0.05$) on the viability of *G. gynandra* seeds.

Table 3.2: Results of viability test (in percentages) on *G. gynandra* seeds stored at room temperature for four months in aluminium foil paper, brown paper bag, and black polyethylene bag.

Accession	Months	Aluminium foil paper (%)	Brown paper bag (%)	Black polyethylene bag (%)
B01	0	70	70	70
	1	53	53	37
	2	65	70	83
	3	63	83	73
	4	68	85	77
B02	0	58	58	58
	1	73	55	82
	2	32	40	23
	3	33	23	30
	4	37	28	33
B03	0	58	58	58
	1	53	55	67
	2	52	73	53
	3	53	27	40
	4	57	30	43
B04	0	63	63	63

	1	37	50	15
	2	67	50	72
	3	30	20	27
	4	33	25	32
B05	0	63	63	63
	1	55	35	23
	2	77	70	65
	3	63	17	43
	4	68	20	45
B06	0	77	77	77
	1	55	70	57
	2	85	77	85
	3	77	93	77
	4	77	97	80

accessions*: p<0001. storage period: p<0001. Storage material: p=0.163 (ns).

accessions*storage material*storage period: p= p<0001. Ns= non-significant

3.4.2 Germination

The effects of packaging material, storage period, and all the interaction effects on seed germination of spider plant seeds were significant ($p<0.001$). After freshly harvested seeds of spider plant were stored at room temperature 25℃ for 4 months in different storage materials, it was found that dormancy in seeds of this species could be broken within 2-3 months depending on the genotype with maximum germination of 90% for accession B06 stored in brown paper bags (Fig.3.2).

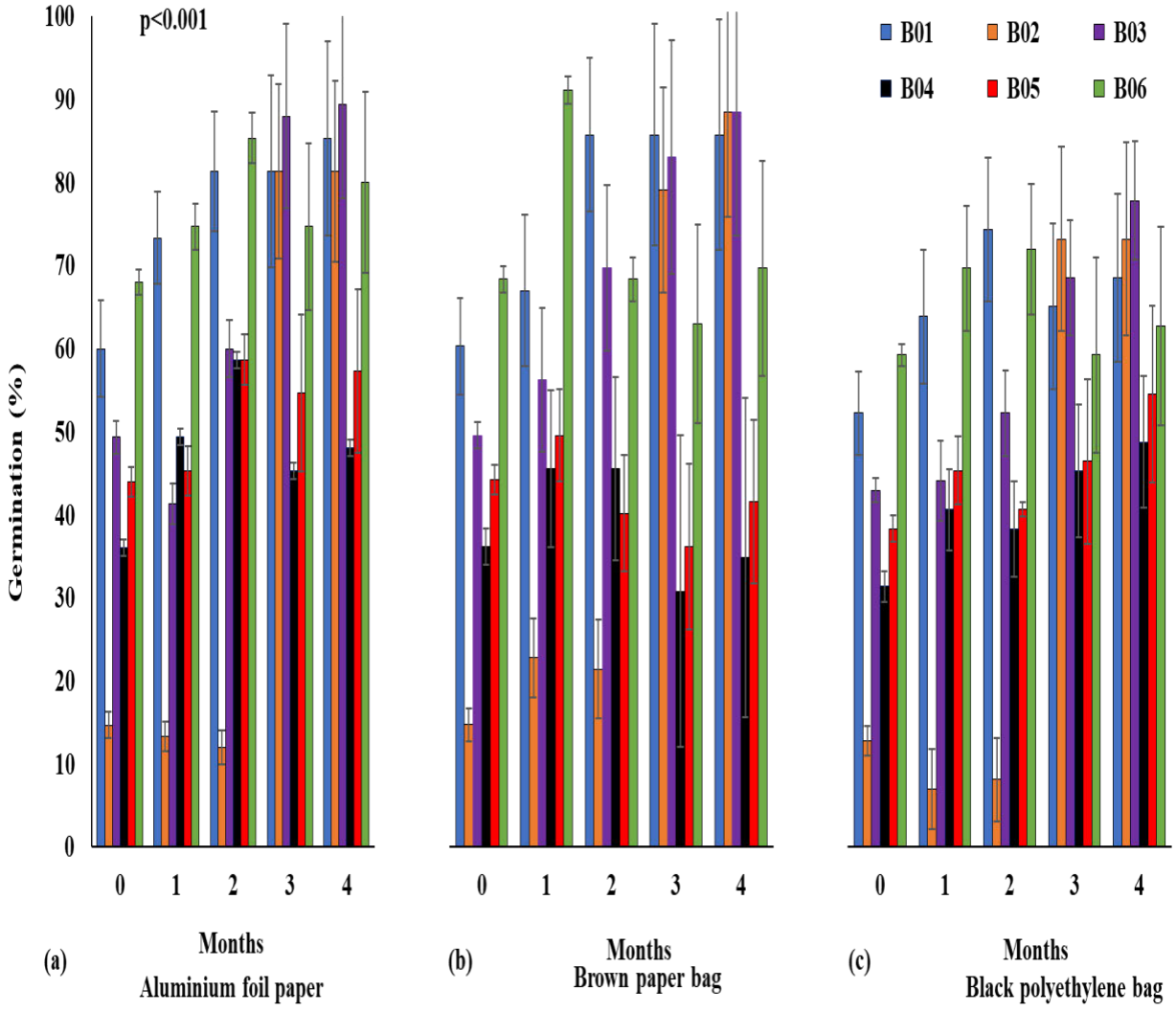

Figure 3.2: Variation in final germination percentage in *G. gynandra* seeds stored at room

temperature of 25℃ for four months in aluminium foil paper, brown paper bag, and black polyethylene bag.

Accessions B02 and B03 showed a significant germination increase in month three of storage across the storage materials while germination of accession B01 remained high even in the early months of storage (months 0, 1, and 2). The least germination percentage was observed in accession B02 in months zero, one, and two of storage. However, it started to rapidly increase in month 3 ranging from 8-22% to a range of 78-84%. After three- and four-months storage, accession B04 had the least germination percentage across the storage materials.

Table 3.3: Germination parameters of the different accessions at month four of storage where the effects of packaging materials are kept constant.

Accession	FGP (%)	CVG	MGT (days)	MGR (day^{-1})
B01	83.11	432.1	2.187	0.000617
B02	84.44	574	1.741	0.000547
B03	88.89	652.5	1.613	0.000505
B04	46.22	381.2	2.497	0.001277
B05	53.78	438	2.195	0.001013
B06	73.78	416.8	2.341	0.000727

FGP= final germination percentage, CVG= coefficient of velocity of germination, MGT= mean germination time, MGR= mean germination rate.

3.4.3 Electrical Conductivity

The seed electrical conductivity results of spider plant accessions are presented in Figure 3.3

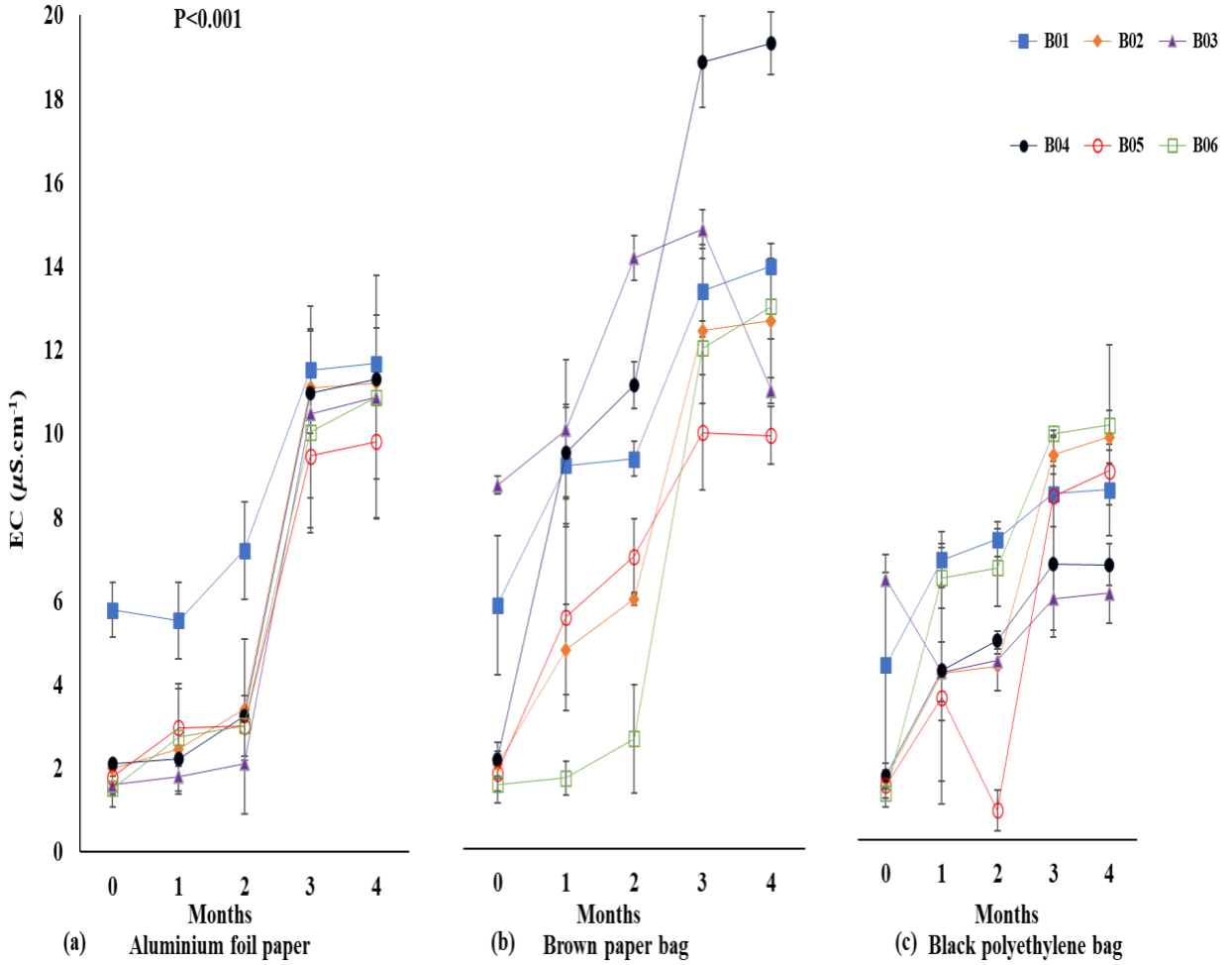

Figure 3.3: Electrical conductivity of *G. gynandra* seeds from different regions, stored at room temperature of 25℃ for four months in aluminium foil paper (a), brown paper bag (b), and black polyethylene bag (c).

The EC results showed a highly significant difference (P<0.001) among different accessions, storage periods, and storage materials, however, the interaction effect of accessions x months x

storage materials showed no significance ($p = 0.95$). EC was highly influenced by storage period and storage material ($p<0.001$). The initial seed electrical conductivity varied from 1.6-5.7 to 10.6-12.5 µS cm1 g -1 for all the accessions across the storage materials. Accession B01 stored in aluminium foil paper showed the highest value (5.78 µS.cm-1) of initial electrical conductivity while accession B04 stored in a brown paper bag showed the highest final electrical conductivity of 18.67 µS.cm-1in month four of storage. Generally, the electrical conductivity test results in seeds of *G. gynandra* were higher in a brown paper bag than in other packaging materials, this could be because the brown paper bag was easily accessible to air from the outside environment. A high electrolyte leakage is a sign of the loss of the cell membrane integrity, this indicates that seeds stored in brown paper bag were less vigorous than seeds stored in aluminium foil and black polyethylene bag. The lowest EC value was observed in B05 stored for month two of storage in a black polyethylene bag.

3.4.4 Radicle growth

The results show that there is a significant difference in seed radicle length of storage material and storage period ($p<0.05$). The highest radicle length was observed in accession B02 after one month of storage in a brown paper bag (10.8 mm).

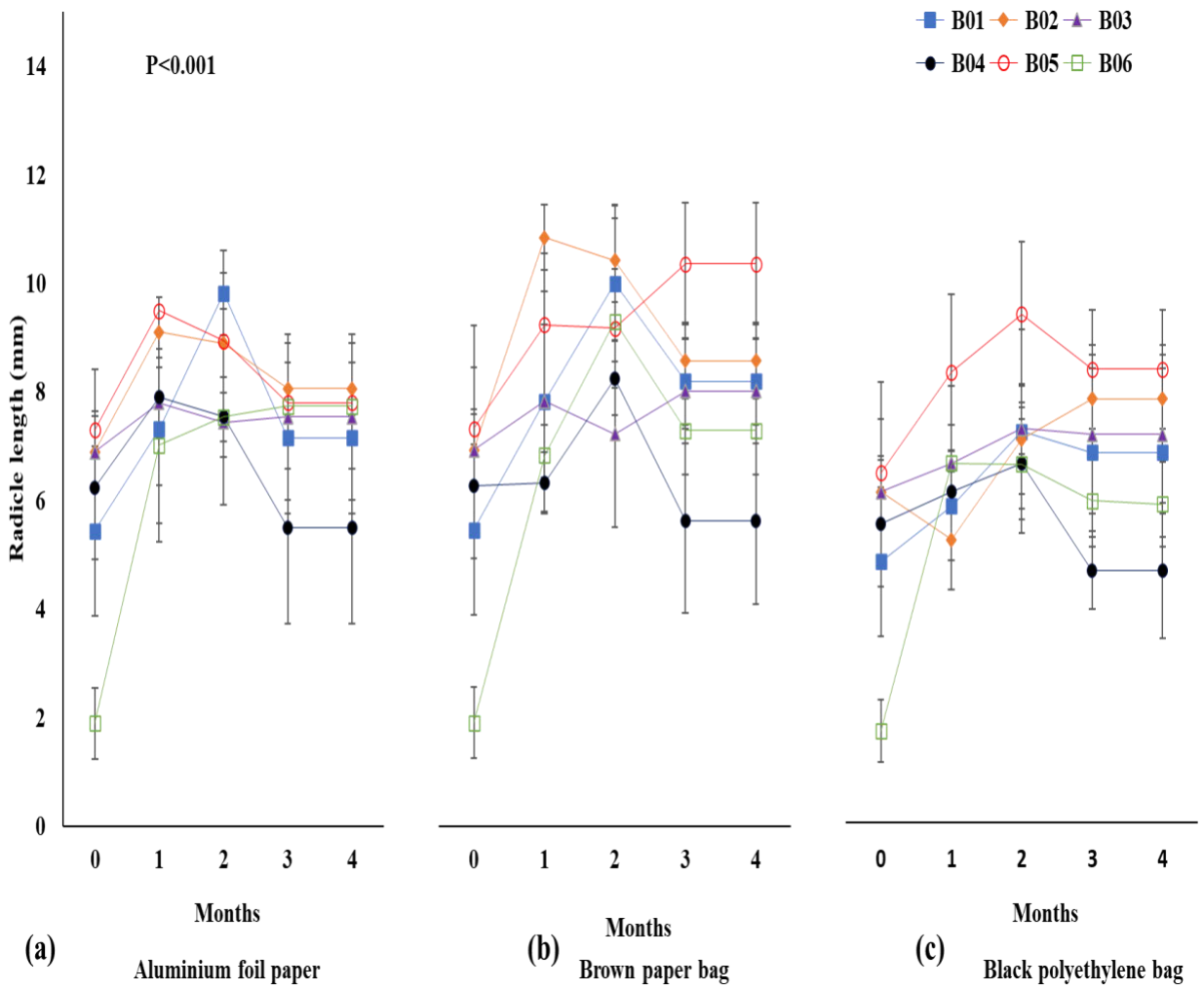

Figure 3.4: Radicle length of *G. gynandra* seeds stored at room temperature for four months in aluminium foil paper, brown paper bag, and black polyethylene bag.

Accession B06 has the shortest radicle length at month zero, however, there was a significant increase from 1.9 mm in month zero to a maximum length of 9.26 mm. Accession B04 had the lowest radicle length at months three and four compared to the other genotypes which decreased from 8.22 mm in month 2 of storage to 5 mm at storage period of four months. Amongst the genotypes, accession B05 has the highest radicle length (9.33 mm), and the highest radicle length was observed in seeds stored in a brown paper bag (8.27 mm) throughout the different accessions.

Table 3.4: Analysis of variance showing mean squares and significant tests of seed viability tests of different accessions of *G. gynandra* stored at room temperature of 25℃ for four months in different storage materials.

Source of variation	d.f.	EC	TZ	Germination	Radicle length
Accessions	5	41.91**	8551.9**	106855.3**	60.77**
Storage duration	4	677.49**	2228.3**	36850.1**	17.32**
Storage material	3	129.82**	335.7	4114.5**	10.72*
Accessions x Storage duration	20	5.52	1472.5**	32475.8**	4.09*
Accessions x Storage material	15	33.40**	7278.9*	2986.9**	4.342*
Storage duration x Storage material	5	18.08	236.2	2819.1**	2.464
Accessions x Storage duration x Storage material	25	5.01	11005.3**	34398.9**	90.76

EC= electrical conductivity, % = percentage, TZ= Tetrazolium Chloride test mean germination time, x = interaction. CV= coefficient of variation, **p<0.001, *p<005, no star= no significance.

3.5 Discussion

The availability of methods that allow discrimination among seed lots for components that determine the physiological performance is essential to developing seed quality control programs and reducing post-harvest and commercialization problems. Tests that produce quick and

consistent information are needed to allow prompt decisions. Hence the electrical conductivity and tetrazolium tests (Santos et al., 2007). The three storage materials used in the present study had no significant effects (P>0.05) on seed viability (Table 3.2). Kamotho et al., 2014 also found no significant difference in packaging materials on the quality of spider plant seeds (Kamotho et al., 2014).

Increasing the storage period from 0 to 4 months significantly increased the mean electrical conductivity (EC) of the seed from a range of 1.6- 5.7 to 10.6-12.5 µS cm1 g -1 seed across the genotypes and storage materials. Overall, tested seeds indicate the rapid deterioration of cell membranes over time. This resulted in an increased seed permeability to water during the imbibition and subsequently increased the electrical conductivity (Ataíde et al., 2016). Due to the deterioration of seeds' cell membrane over time, imbibition during germination tests easily occurred, resulting in early resumption of metabolic activities within the seeds, and thus increasing germination percentages over time (Ferraz et al., 2019). Freshly harvested seeds of *G. gynandra* were dormant as the lowest EC. Similar results were found in seeds of *Enterolobium contortisiliquum* (pacara earpod tree) where prolonging the storage period increased seed electrical conductivity (Ferraz et al., 2019). Several investigators found a high positive correlation between electrical conductivity and seed vigor (El-Borai et al., 1993).

After four months of storage using brown paper bags, spider plant seeds for accession B03 had a low electrical conductivity value (8.17 µS cm1 g -1 seed) and highest germination percentages among all the storage materials (89, 88, 89% for aluminium foil paper, brown paper bag and polyethylene bag respectively). This indicates that seeds with low electrical conductivity have high seed quality (Ramos et al., 2012). Therefore, the cell membranes of seeds of accession B03 had a high cell membrane integrity, this is indicated by low electrolytes leakage.

The accessions under study were from different regions and had a significant effect on germination parameters. Seed sources also play a significant role as a factor. This was observed in the findings of Uniyal et al. (2000), who noted the importance of seed source in the germination of *Grewia oppositifolia* (Uniyal et al., 2000). The germination parameters of *G. gynandra* seeds collected in different agro-ecological areas differed from one another (Uniyal et al., 2000). This indicates that the source of seeds and thus the genotype plays a crucial role in the seed response when submitted to a treatment (Lopez Essou et al., 2017). The standard final seed germination is 85% was met by spider plant seeds accession B01 accessions B02, and B03 at four months of storage (Figure 3.2). However, Wu et al, 2018 found that seeds of *G. gynandra* from Africa are bigger. Therefore, this is in contrast with the results found by Blalogoe et al, 2020, that small seeds had better germination compared to bigger seeds (Blalogoe et al., 2020; Wu et al., 2018) because seeds from Asia (B04 and B05) did not meet the standard final seed germination.

After seeds of the spider plants were kept at room temperature of 25 ℃ for 4 months, it was observed that freshly harvested seeds are dormant. This is shown in the TZ results showing high viability in non-stored seeds, however, the germination tests show low germination percentage compared to the results shown by the viability test. The germination percentage increased after two and three months of storage depending on the genotype. However, other genotypes showed a decrease in germination percentage after one and two months of being stored. Therefore, dormancy in spider plant seeds could be overcome within 2-3 months depending on the genotype in all the packaging materials. Similar results were found in a study by Ekpong, (2009) that *G. gynandra* seeds overcome dormancy after three to five months of storage (Ekpong, 2009) or storing seeds at room temperature ranging from 30-33℃ and 15℃. After four months of storage, B03 had the highest final germination of highest germination percentages 89, 88, and 89% for aluminium foil

paper, brown paper bag, and polyethylene bag, respectively. It showed that this species has an after-ripening period which is usually found in many freshly harvested seeds of herbaceous plants (Geneve, 1998) including spider plant (Chweya and Mnzava, 1997). Although the germination results showed that seeds stored in aluminium foil paper had better germination among the storage materials, the analysis showed that the effect of storage materials on seed viability was non-significant. An after-ripening period is where mature, freshly harvested seeds are kept in dry storage at room temperature to release dormancy, thus promoting seed germination (Bewley, 1997a; Finch-Savage and Leubner-Metzger, 2006). Similar results were found in other crops such as tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), and thale cress (*Arabidopsis thaliana*) (Ali-Rachedi et al., 2004; Groot and Karssen, 1992; Leubner-Metzger, 2007). In the wild, this is generally not a problem, however, the spider plant is being cultivated for human consumption as well. It is a problem when it is produced for commercial purposes.

3.6 Conclusion

Gynandropsis gynandra accessions, collected from different regions, responded differently to the different storage periods and storage materials. The seeds of accession B03 had a continuous increase in germination as the storage period increased. Also, the effect of packaging material on EC and radicle length was significantly different. It is thus concluded that there is a significant difference in the response of spider plant seeds when stored for different storage periods in different storage materials. In addition, the results found in the study by Blalogoe et al., 2020 shows in this study; seeds from Africa yielded better results compared to seeds from Asia, which are in contrast with some studies.

3.7 References

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CHAPTER 4: SEED PRIMING TECHNIQUES TO IMPROVE SEED GERMINATION IN *GYNANDROPSIS GYNANDRA* L.

4.1 Abstract

Gynandropsis gynandra is a leafy vegetable that belongs to the botanical family Cleomaceae. The species exhibits low and uneven germination which could be due to seed dormancy when planted immediately after harvest. The study aimed to investigate the effects of seed priming on the germination of freshly harvested and stored *G. gynandra* seeds. Six accessions of *G. gynandra* seeds which were previously stored for four months were soaked for 0, 6, and 12 hours in 500ppm of polyethylene glycol-4000 (PEG-4000) and distilled water. The experimental design was 6 accessions x 2 priming agents x 3 priming durations using a Completely Randomized Design with three replicates of 36 treatments (108 experimental units) Primed and non-primed seeds were left to germinate at 30°C in a germination chamber (Labcon, L.T.I.E, South Africa) for 14 days. On day 9, radicle length was measured. Seed germination and radicle length were measured. Final germination percentage, mean germination time, mean germination rate, coefficient of velocity of germination, and radicle length were determined and subjected to descriptive statistics and Analysis of Variance (ANOVA). Means were compared using Least Significance Difference (LSD) at a 5% level of significance. The results showed that priming with PEG and water had no benefit on final germination percentage (FGP), mean germination time (MGT), mean germination rate (MGR), coefficient of velocity of germination (CVG), and radicle length (RL). However, increasing priming duration had a negative effect on FGP, CVG, and RL of *G. gynandra* seeds while MGT and MGR increased. For all the accessions, seed priming had a significant effect on radicle length. Seeds primed for 6 hours had the highest radicle length (8.15 mm) compared to 0 and 12 priming durations. Overall, priming *G. gynandra* seeds decreased germination percentage and increased radicle length. It can therefore be concluded that this study showed an unclear indication that priming of *G. gynandra* seeds with PEG-4000 and distilled water had any effect on improving germination.

Keywords: Accessions, Germination, *Gynandropsis Gynandra*, Priming duration, Priming solution

4.2 Introduction

Gynandropsis gynandra (L), commonly known as spider plant, belongs to the family Cleomaceae. It is a valuable indigenous vegetable in Kenya, Benin, and South Africa which is being used as a green vegetable (Blalogoe et al., 2020). Spider plant is commonly found in arable land in altitudes of at least 1800 meters above sea level. This plant species is highly considered as a rich source of vitamin A and C, proteins, and minerals (Chweya and Mnzava, 1997) and is therefore used as a supplement in malnutrition management (Dushimimana et al., 2018) and household food security (Onyango et al., 2013). Spider plant is also used in traditional medicine and acts as a plant protectant when intercropped with other species due to its insecticidal, antifeedant, and repellent characteristics. Spider plant is propagated by seeds mostly at the beginning of the short rains (Chweya and Mnzava, 1997). To maximize yield potential in crops, the use of high-quality, high vigor seeds is important. While rapid and uniform field emergence is two important requirements for increased yield, quality, and ultimately profits in the production of crops, the seeds of some cultivated crop species can have a low and delayed seedling emergence in the field (Yongqing, 1996). To improve germination and seedling establishment of such vegetables and field crops, several pre-sowing treatments such as seed priming have been applied to decrease the time it takes for seedlings to emerge (Fay et al., 1994).

These include alternate wetting and drying, pre-germination, and controlled hydration through an osmoticum such as polyethylene glycol (PEG). This method of controlled hydration is called priming or osmo-conditioning (Khan et al., 1990). Seed priming is a pre-sowing, controlled

hydration treatment in which seeds are exposed to an outside solution with low water potential that stimulates the resumption of metabolic activities but preventing radicle protrusion (Bradford, 1986; Khan, 1992). Priming seeds usually results in improved rate of seed germination and uniform germination and seedling emergence in seed beds under low temperature (Pill and Finch-Savage, 1988; Stoffella et al., 1988), matric stress (Akers et al., 1987), salinity (Pill et al., 1991) and heat (Wurr and Fellows, 1984).

Previous studies on seed priming reported that this treatment results in an improved seed germination, rate of germination and vigor index (Hu et al., 2005; Salah et al., 2015). Polyethylene glycol (PEG) has been used frequently in plant water deficit studies to induce dehydration by reducing water potential (Sen and Alikamanoglu, 2013). It is observed that using PEG as a priming treatment reduces the time it takes for seeds to emerge, resulting in an increased seed germination (Dursun and Ekinci, 2010), and increases salinity tolerance (Munir and Aftab, 2009). Furthermore, the use of PEG as a priming agent increases chilling tolerance in plants (Dong et al., 2013).

The majority of seed priming studies had been conducted on other field crops and vegetables with little work on seed priming of *G. gynandra*. Hence, this study was conducted to assess the effect of PEG-4000 and treatment durations (0, 6 and 12 hours) on the germination of *G. gynandra* using different accessions from different regions (Table 3.1).

4.3 Materials and methods

4.3.1 Study materials

Six accessions (B01, B02, B03, B04, B05, and B06) from three different geographic regions, viz., Western Africa, Eastern-Southern Africa, and Asia were used in the study (Table 3.1). Seeds from

these accessions were harvested in January 2021 after being planted for three months before the experiment commenced at Seed Science Laboratories, School of Agricultural, Earth & Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg Campus.

The different regions were selected because *G. gynandra* seeds are diverse (Table 3.1), and morphological differences were detected between seeds from Asia and those from Africa (Wu et al., 2018).. Seeds were carefully separated from capsules by hand and were oven-dried at 103°C for 17 hours and silicon-desiccant in an air-tight glass desiccator to reduce the seed moisture content to about 5%. These seeds were packed in three different packaging materials; brown paper bag, aluminium foil paper and black polystyrene bags, and kept at room temperature of 25℃. Germination percentages were determined at monthly intervals for four consecutive months using three replicates of 25 seeds. The germination test method was carried out according to the International Seed Testing Association, `Zurich (ISTA., 2012).

4.3.2 Experimental site, treatment structure, and experimental design and layout

The study was conducted at the Seed Science Laboratory in the School of Agricultural, Earth, and Environmental Sciences of the University of KwaZulu-Natal, Pietermaritzburg (29.6196° S, 30.3960° E). The experiment had three factors: accessions with four levels (B01, B02, B03. B04, B04, B05, B06), priming agent with two levels (distilled water and PEG-4000), and priming duration with three levels (0, 6. 12), giving a 6 x 2 x 3 factorial treatment structure, laid out in a completely randomized design (CRD) with three replications per treatment giving a total of 108 experimental units.

4.3.3 Determination of moisture content

The oven method was used to determine the seed moisture content described by the International Seed Testing Association (ISTA) (17h at 103°C in the oven) and the seeds were weighed using a digital balance Ohaus® Pioneer™ Plus analytical balance Model PA114C, AC/DC input 230 V AC, universal plug set. The moisture content was calculated on a dry mass basis using equation 1 (Blalogoe et al., 2020):

$$MC = \frac{Loss\ of\ weight}{Initial\ weight} x\ 100 = \frac{m1-m2}{Initial\ weight\ (m1)} x100 \quad (1)$$

m1 is the weight in grams of the seed before drying and m2 is the weight in grams of the seeds after drying.

4.3.4 Priming treatments

After each storage period, *G. gynandra* seeds were subjected to two priming treatments.

Hydropriming: Seeds were submerged in distilled water for 0, 6, and 12 h giving a 6 x 2 x 3 factorial treatment structure and dried back to their initial weight before transfer to the germination test process.

Osmotic priming: Seeds were submerged in 500ppm solution of Polyethylene glycol-4000 (PEG-4000) for 6 and 12 h and dried back to their initial weight before transfer to the germination test process.

4.3.5 Germination test

After the priming durations, the seeds were airdried back to the initial weight for 6-8 hours, three replicates of 25 seeds per treatment were subjected to a germination test by placing them on top of Whatman filter paper in a petri dish of 90 mm in diameter. The seeds were moistened with approximately 7ml of distilled water and kept at 30°C in a germination chamber (Labcon, L.T.I.E, South Africa). Germination counts were taken every day for 14 days and the seeds were deemed germinated when the radicle had protruded with a length of approximately 2mm. Water was added as needed. On day 9 of germination, five germinated seedlings per treatment were taken out and radicle length was measured.

From the germination data, the following germination parameters were assessed.

Final germination percentage (FGP) = $\frac{Total\ number\ of\ seeds\ germinated}{initial\ number\ of\ seeds\ used} \times 100$ (2)

Mean germination Time (MGT) = $\frac{\Sigma nidi}{\Sigma N}$ (3)

Where ni = the number of germinated seeds at day i, di = incubation period in days, and N = the number of germinated seeds in a test (Baskin and Baskin, 2014). The units for MGT are days.

The germination potential of seeds can also be determined using the mean germination time. Mean germination time is the measure of the rate and time spread of germination (Bewley et al., 2012a). It measures the time it takes for seeds to germinate.

Mean germination rate, (MGR) = $\frac{\sum(n \times d)}{N}$ (4)

Where n = the number of seeds germinated on each day, d = the number of days from the beginning of the test, and N = the total number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981). The units for MGR are t^{-1}.

The mean germination rate is calculated as the reciprocal of the mean germination time

Coefficient of velocity of germination, (CVG) = $\sum_{i=1}^{k} n_i \, t_i$ (5)

where n= the number of seeds germinated every day and t=the number of days from seeding corresponding to n (Al-Ansari and Ksiksi, 2016). CVG has no units.

4.3.6 Data Analysis

Germination parameters and radicle length data were subjected to analysis of variance (ANOVA) using GenStat 20th edition (VSN International, United Kingdom) at the 5% level of significance. The difference among treatment means (accessions, priming agents, priming duration) were compared using the test of Least Significant Difference (L.S.D.) test at the 5% level of significance.

4.4 Results

4.4.1 (I) Final Germination Percentage

The three-way interaction (accession x priming agent x primed duration) and priming agents had no significant effect on the final germination percentage of *G. gynandra* seeds ($p > 0.05$). However, the effect of priming duration on germination was significantly different ($p < 0.001$).

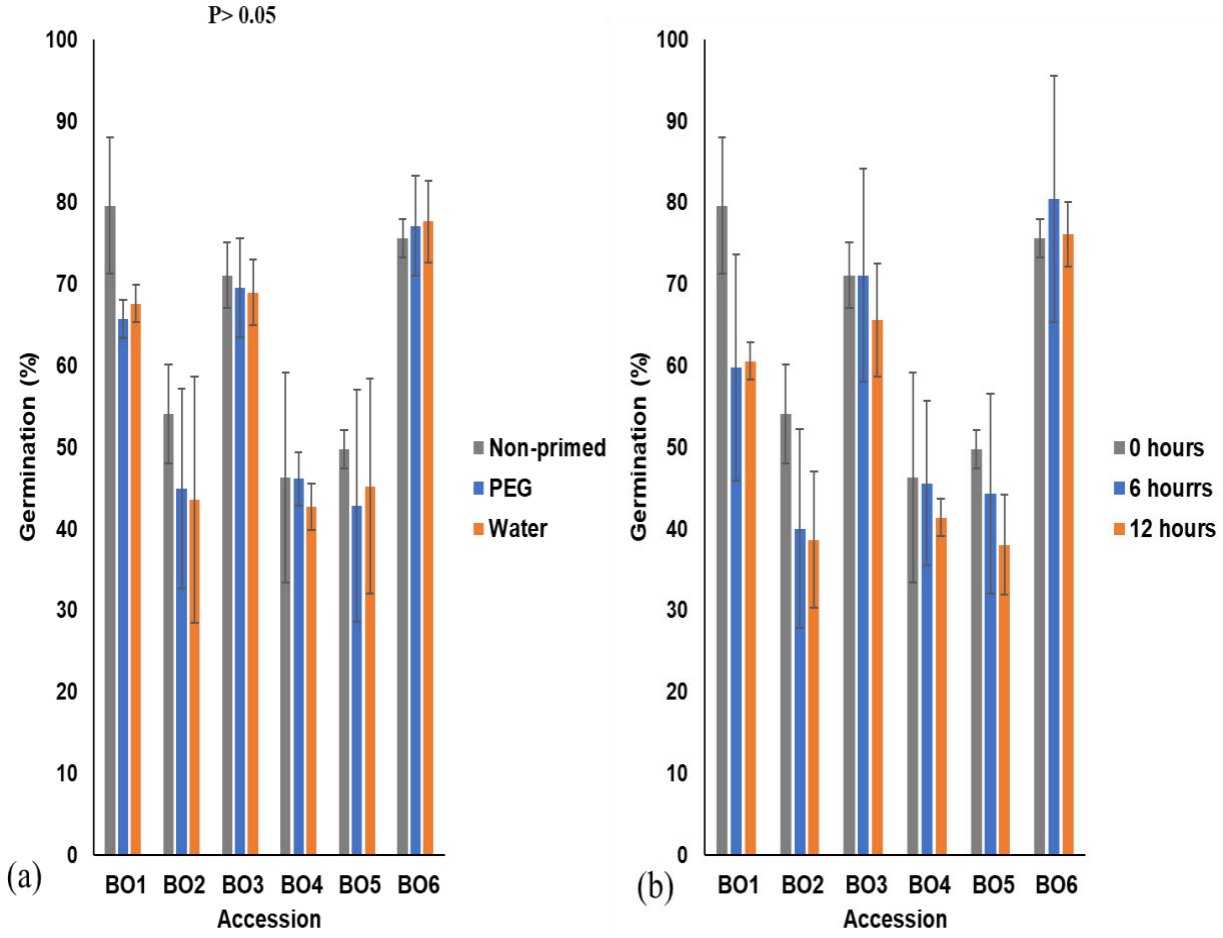

Figure 4.1: Variations in the germination of *G. gynandra* seeds primed with PEG-4000 and distilled water (a) for different priming durations (b) at room temperature of 25 ℃.

Accessions B01, B03, and B06 showed the highest germination percentages ranging from (65-79%). Compared with the control group (non-primed seeds), no priming agent was able to increase the seed germination of *G. gynandra*, nevertheless, priming seeds of accession B06 for 6 hours

had a slight increase in germination. Seeds that were not primed showed the highest germination percentage for accessions B01, B02, B03, B04, and B05, priming was not effective when seeds were soaked for 12 hours.

(II) Mean Germination Time (MGT)

When all other factors are constant (accession, storage period, and storage material), seeds primed with PEG show the highest MGT (1.86 days). Accession B01 had the highest MGT when primed with distilled water (2.20 days) and accession B03 primed with distilled water had the lowest MGT of 1.51 days (Table 4.2). Mean germination time increases as priming duration increases. The highest MGT was observed at 12 hours of priming duration. And the lowest at 6 hours.

Table 4.2: Mean Germination Time (days) of *G. gynandra* seeds primed using distilled water and PEG at room temperature of 25℃ for 0, 6, and 12 hours.

	Priming agent		Priming duration (hours)		
Accession	PEG	Water	0	6	12
B01	2.13	**2.20**	1.87	2.31	**2.31**
B02	1.79	1.78	1.58	1.81	1.95

B03	1.55	**1.51**	1.53	1.52	1.54
B04	2.05	2.02	2.11	1.90	2.11
B05	1.99	2.07	1.94	2.14	2.01
B06	1.64	1.56	1.81	1.50	**1.49**

(III) Mean Germination Rate (MGR)

MGR varied significantly among accessions and priming durations including the control represented by 0 hours priming duration ($p<0.001$). For the control, the maximum mean germination rate was 0.0021 day^{-1} for accession B02, it also reached the maximum MGR with PEG as a priming agent. MGR increased as priming duration increased, and seeds primed with PEG showed the highest MGR compared to seeds primed with distilled water (Table 4.3).

Table 4.3: Mean germination rate (day^{-1}) of *G. gynandra* seeds primed at room temperature of 25℃ using PEG and distilled water for 0, 6, and 12 hours.

Accession	Priming agent		Priming duration		
	PEG	Water	0	6	12
B01	0.001139	0.000998	**0.000524**	0.001346	0.001336
B02	**0.001983**	0.001998	**0.002055**	0.001911	0.002006

B03	0.000835	0.000827	0.000861	0.000744	0.000888
B04	0.001272	0.001416	0.001184	0.001286	0.001562
B05	0.001282	0.001223	0.001049	0.001263	0.001445
B06	0.000614	**0.000597**	0.000644	0.000568	0.000604

(VI) Coefficient of Velocity of Germination

The coefficient of velocity of germination (CVG) indicates the speed of germination. Its value is correlated to the number of germinated seeds, and inversely correlated to the germination time (Talska et al., 2020). Therefore, high CVG indicates a high number of seeds that germinated. There were no significant differences among the priming agents and priming duration with respect to CVG. However, a considerable decrease was noted in CVG as the priming duration increased. The highest CVG (666.3) was observed in seeds of accession B06 primed with distilled water while the lowest (487.1) was observed in accession B05 under the same treatment.

Table 4.4: Coefficient of velocity of germination of *G gynandra* seeds primed using distilled water and PEG for 0, 6, and 12 hours.

	Priming agent		Priming duration		
Accession	PEG	Water	0	6	12
B01	510	493.8	573.5	469.2	**463**
B02	634	631.7	685.6	621	592

B03	625.4	648.1	605.1	655.2	649.9
B04	491.1	513.4	493.3	531	482.5
B05	512.8	**487.1**	514.4	475.4	510.2
B06	627.3	**666.3**	567.4	685	**688.1**

4.4.2 Radicle length

The results showed that there was no significant effect of priming agents on the radicle length of *G. gynandra.* Priming duration had a significant effect on the radicle length of *G. gynandra*. Accession B05 primed with PEG had the highest average radicle length of 9.65 mm and the highest average radicle length when seeds are primed for 6 hours (Figure 4.3). Overall, priming seeds with either PEG or distilled water increased the radicle length.

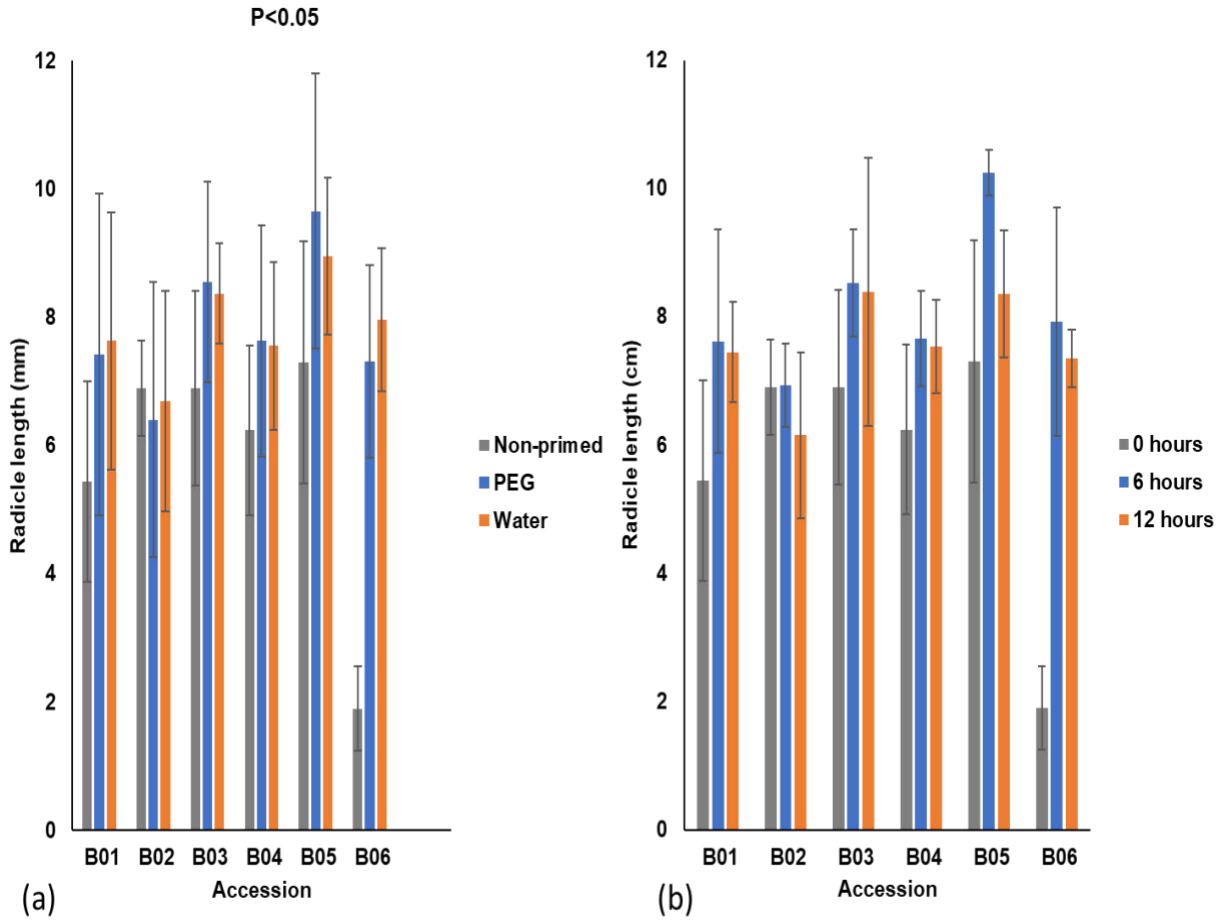

Figure 4.3: Radicle length of *G. gynandra* seeds primed using PEG and distilled water (a) for 0, 6, and 12 hours (b) at room temperature of 25℃.

Across the accessions, seed priming increased radicle length. The priming duration showed that seeds primed for 6 hours have the highest radicle length compared to the control and seeds primed with PEG.

4.5 Discussion

For seed germination to occur reserves mobilization that accumulated during the maturation stage of seed development are required (Penfield, 2017). Degradation of those reserves and resumption of metabolic activities bring the necessary energy to the growing young plants. This mobilization is the significance of the hydrolytic activities that release the nutrients stocked in the reserve, and the mechanisms of their transportation toward the embryo (Mihoub et al., 2005). Depending on the species, the majority of these reserves can naturally be carbohydrate, lipidic, or protein (Khemiri et al., 2004). However, many crop species need specific conditions to germinate. Dormant seeds do not germinate even when germination conditions are met. Seed dormancy can be caused by physiological seed traits or by external conditions, and sometimes there is more than one type of dormancy existing in one seed (Baskin and Baskin, 2014; Baskin et al., 1998; Bewley, 1997a).

Gynandropsis gynandra is one of the dormant species and its regeneration is low due to the dormancy that exists within this species. Studies have been conducted to break the dormancy of this species using pre-treatments such as seed priming techniques, however, the response of seeds to pre-treatments is species-specific (Uniyal et al., 2000).

In the present study, previously-stored seeds of *G. gynandra* from three different regions (Asia, East-Southern Africa, and West Africa) were subjected to priming solutions PEG-4000 and distilled water for 6 and 12 hours before being tested for germination (Figure 4.1). The results showed that priming agents had no significant effect on seed germination of *G. gynandra*, this means that there was no difference in osmo- and hydro-priming concerning germination However, priming seeds for different priming duration significantly affected seed germination across the

accessions. Priming duration of six hours had higher germination percentages compared to the duration of 12 hours. Although priming agents had no significant effect on seed germination of *G. gynandra*, primed seeds showed a slight decrease in germination for all the accessions except accession B06. In contrast, compared to the unprimed seeds, priming seeds increased the radicle length. Similar results were found in alfalfa seeds (*Medicago sativa*) where inducing drought using PEG-4000 decreased germination percentage and increased root length (Anjum et al., 2017). Seed germination decreased as priming duration increased to 12 hours. This may be a result of over-priming which indicates that over-priming is detrimental to seeds (Murray, 1989) and may result in oxygen shortage and the build-up of inhibitors (Khalil et al., 2001). In seed technology, over-priming is excessive prolonged imbibition after seed metabolic activities has been resumed (Lutts et al., 2016).

Priming seeds with polyethylene glycol-4000 increased the mean germination time. This is a challenge as it increases the number of days it takes for seeds to germinate, which may lead to delayed seedling emergence. Polyethylene glycol has been used to induce water stress in seeds (Elahi and Derkhshan, 2016), however, this depends on the water potential of the PEG solution. *Gynandropsis. gynandra* is a drought-tolerant crop (Ekpong, 2009; Redhead, 1990), hence the increase in mean germination time and rate.

4.6 Conclusion

The objective was to study the effect of different priming treatments and duration on the seed germination of *Gynandropsis gynandra* to determine the most appropriate treatment to improve and increase seed germination and seed vigor. The study revealed the existence of variation in germination percentages, mean germination time, mean germination rate, coefficient of velocity

of germination, and radicle lengths between the treatments. Interactions of Accession × Priming duration was significant. In this study, priming *G. gynandra* seeds with 500ppm of PEG-4000 and distilled water showed no statistical difference in improving seed germination of the species under study. However, seed priming increased the mean germination time which may lead to delayed seedling emergence, germination rate, and radicle length. It was concluded that PEG shows better results compared to distilled water. And the best priming duration in both hydro- and osmo-priming for *G. gynandra* seeds is 6 hours.

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CHAPTER 5: GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Findings and implications

In terms of production area, trends of consumption, and research, traditional leafy vegetables are neglected compared to exotic crops. As a result, there is a decline in the yields of native crops in sub-Saharan Africa. Nonetheless, researchers have reported on the significance of these vegetables. However, poor seed quality, storage conditions, and lack of seed packaging have limited major production (Abukutsa-Onyango, 2005). Farmers struggle to store seeds under sub-optimal temperature conditions, relative humidity, and packaging materials, resulting in loss of seed viability. Thus, leading to poor and delayed seed germination in such vegetables. Due to the low number of viable seeds during harvesting, farmers are not able to produce traditional vegetables all-year-round (Aarssen and Eriksson, 2005; Shilla et al., 2016).

Gynandropsis gynandra is one of the traditional leafy vegetables that is most consumed in sub-Saharan Africa. The aim of the study was to gain insights into the dormancy breaking mechanisms in *G. gynandra* seeds through seed storage and different seed priming techniques.

The important step in the study was to conduct a review (chapter 2) of research conducted on seed germination, seed storage, packaging material, and priming techniques in spider plant During the review, it was found that dormancy in *G. gynandra*. could be broken using certain dormancy breaking methods including storing seeds, soaking, stratification, heating, gibberellic acid,

polyethylene glycol, and KNO3. It was also found that storing *G. gynandra* seeds for at least three months improved germination compared to freshly harvested seeds and exposing seeds to dark conditions promoted germination. In literature, several reports on the effects of seed packaging materials showed that packaging material had no effect on the germination of spider plant seeds.

In addition to this, the review discovered a few contradictory reports regarding the use of pre-treatments by various researchers and the distinguishing proof of the critical moment when the seed of the species enters a dormancy state. The review concluded that further studies on seed dormancy and germination with more various and large number of accessions are required to address the narrow scientific information available regarding low and poor germination in this species.

To this end, the effect of the seed storage period and packaging material on six accessions of *G. gynandr*a from diverse origins including Asia, and West, South, and East Africa was done (chapter 3). The study showed that spider plant seeds are significantly different concerning their response to storage periods and packaging materials on germination percentage, mean germination time, and radicle length.

The West Africa, B03 accession recorded the highest values for percentage germination, and coefficient of velocity of germination for the first two months of storage. It is concluded that seed germination increased as the storage period increased from two months, and seeds stored in aluminium foil paper showed better germination compared to the brown paper bag and black polyethylene bag. Freshly harvested seeds of *G. gynandra* species exhibit physiological dormancy with the degree of dormancy varying from one genotype to another (Blalogoe et al., 2020).

The effect of seed priming with 500ppm of PEG-4000 and distilled water was also highly genotype-dependent, with no dormancy breaking evidence when the priming agents were applied (chapter 4). Compared to primed seeds, the seeds with no priming treatment showed the highest germination percentage across the accessions except for accession B06. The results also show that between the priming durations of 0, 6, and 12 hours; seeds primed for six hours had the highest germination percentage and longer radicle length. The results also supported the hypothesis that the level of dormancy in *G. gynandra* seeds is genotype dependent.

Although not consistent, in general, the study showed that at least one accession from the three regions responded better with respect to seed vigor, germination and radicle length compared to other accessions after four months storage. West Africa being accession B03, accession B05 for Asia region, and accession B06 for East Southern African region.

However, farmers usually store *G. gynandra* and other traditional leafy vegetables for up to three months (Muasya et al., 2009). One of the strategies farmers use to avoid the dormancy period *G. gynandra* seeds have after harvest, is most farmers collect and keep the seeds for the next planting season.

5.2 Recommendations

More studies are suggested to develop genotypes with a low level of dormancy to improve germination within spider plant, thus improving yields. Depending on favorable traits, the genotypes in this study can be used in developing seeds with a low level of dormancy through plant breeding. It is further suggested that studies focus on the comparison of germination and underlying genes involved in the control of seed dormancy. For better crop improvement, a focused study is required to understand the type of physiological dormancy in *G. gynandra*. This

will be an opportunity for seeds companies to offer their customers quality seed with good germination. These genotypes can be suggested to farmers if they fulfill the other desired traits.

5.3 References

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APPENDICES

Appendix 1: Analysis of variance tables for chapter 3

Variate: Seed Vigor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	5	42759.5	8551.9	44.10	<.001
Storage period	4	8913.1	2228.3	11.49	<.001
Storage material	3	1007.2	335.7	1.73	0.163
Accession.Storage period	20	29449.3	1472.5	7.59	<.001
Accession.Storage material	15	7278.9	485.3	2.50	0.002
Storage period.Storage material	5	1180.8	236.2	1.22	0.303
Accession.Storage period.Storage material	25	11005.3	440.2	2.27	0.001
Residual	156	30250.0	193.9		
Total	233	131844.1			

Variate: Germination %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	5	534276.6	106855.3	276.18	<.001
Storage period	3	110550.2	36850.1	95.24	<.001
Storage material	2	8229.1	4114.5	10.63	<.001
Accession.Storage period	15	487136.3	32475.8	83.94	<.001
Accession.Storage material	10	29868.7	2986.9	7.72	<.001
Storage period.Storage material	6	16914.8	2819.1	7.29	<.001
Accession.Storage period.Storage_material	30	34398.9	1146.6	2.96	<.001
Residual	3168	1225723.0	386.9		
Total	3239	2447097.6			

Variate: Electrical conductivity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	5	209.551	41.910	4.71	<.001
Storage period	4	2709.948	677.487	76.08	<.001
Storage.material	3	389.465	129.822	14.58	<.001
Accession.Storage period	20	110.447	5.522	0.62	0.893
Accession.Storage_material	15	501.036	33.402	3.75	<.001

Storage period.Storage_material	5	90.383	18.077	2.03	0.077
Accession.Storage period.Storage_material	25	125.320	5.013	0.56	0.953
Residual	156	1389.164	8.905		
Total	233	5525.315			

Variate: Radicle length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	5	325.716	65.143	31.92	<.001
Storage period	4	179.869	44.967	22.04	<.001
Storage_Material	3	21.453	7.151	3.50	0.016
Accession.Storage period	20	140.467	7.023	3.44	<.001
Accession.Storage_Material	15	43.420	2.895	1.42	0.137
Storage period.Storage_Material	5	14.786	2.957	1.45	0.206
Accession.Storage period.Storage_Material	25	90.757	3.630	1.78	0.014
Residual	312	636.692	2.041		
Total	389	1453.160			

Appendix 2: Analysis of variance tables for chapter 4

Variate: Final Germination Percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	5	2491245.1	498249.0	1557.77	<.001
Storage period	3	852217.0	284072.3	888.15	<.001
Storage material	2	401980.8	200990.4	628.40	<.001
Priming agent	1	338.9	338.9	1.06	0.303
Priming hours	1	27224.4	27224.4	85.12	<.001
Accession.Storage period	15	785985.5	52399.0	163.83	<.001
Accession.Storage material	10	1364585.2	136458.5	426.64	<.001
Storage period.Storage material	6	11238.1	1873.0	5.86	<.001
Accession.Priming_agent	5	18143.1	3628.6	11.34	<.001
Residual	12671	4052778.6	319.8		
Total	12958	10773956.0			

Variate: Radicle length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Accession	5	1055.669	211.134	23.67	<.001
Storage period	3	117.353	39.118	4.39	0.004
Priming_agent	1	0.339	0.339	0.04	0.845
Priming_hours	1	135.363	135.363	15.18	<.001
Storage_material	2	430.187	215.094	24.12	<.001

Accession.Storage period	15	250.781	16.719	1.87	0.022
Accession.Priming_agent	5	64.236	12.847	1.44	0.207
Storage period.Priming_agent	3	11.880	3.960	0.44	0.722
Accession.Priming_hours	5	139.387	27.877	3.13	0.008
Months.Priming_hours	3	26.136	8.712	0.98	0.403
Residual	1152	10274.136	8.919		
Total	1439	17494.322			