The physiology of germination and dormancy in seeds of *Gynandropis 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 Plant Breeding, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the project "Enhancing training and research mobility for novel crops breeding in Africa (MoBreed)", funded by the European Union through the "Intra-Africa Academic Mobility Scheme.

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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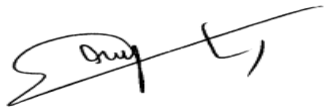

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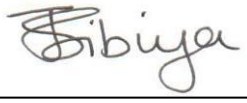

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DECLARATION: PLAGIARISM

I, Jelila Seho Blalogoe, declare that:

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(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;

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GENERAL ABSTRACT

The “spider plant” *Gynandropsis gynandra L.* Briq,” is an important traditional leafy vegetable in many parts of Africa. The species is considered underutilized and has been mainly neglected by research systems. Yields are generally low and this has been attributed to a number of factors including low and non-uniform seed germination. This study sought to gain a deeper understanding of factors influencing germination and dormancy in spider plant seeds. The specific objectives were to, (i) describe and document the phenotypic characteristics and mineral composition of seeds of 29 *G. gynandra* accessions from diverse regions, (ii) determine the pattern of seed germination and dormancy development in seeds of different spider plant accessions and their crosses and (iii) assess the storage potential of spider plant seeds using artificial aging. To achieve these objectives, accessions originating from West Africa, East Africa and Asia were used. In the first experiment, seeds of the accessions from the three regions were subjected to scanning electron microscopy to study seed structure and mineral composition. In the second experiment, seeds from different accessions were planted in pots in a tunnel and data recorded at bi-weekly intervals during development until maturity on the following variables: seed fresh and dry mass, seed moisture content, germination capacity, mean germination time (MGT) and electrical conductivity (EC). In the third experiment, seeds that had been stored for four months and freshly harvested were subjected to the accelerated aging to test for storage potential. The same variables that were in the second experiment were measured in the third experiment in addition to tetrazolium test (TZ). Data analysis was done using R software version 3.5.1. Eight mineral elements were identified in the seeds of spider plant, and the internal and external structure of the seed was revealed. The results showed significant differences among spider plants accessions with regard to shape, size, mineral composition, germination percentage and mean germination with Asian accessions showing a higher germination percentage. The study revealed that spider plant fresh seeds exhibited a physiological dormancy which can be broken by heating at 41°C for 3 days and/or gibberellic acid (0.001%), depending on the genotype. However, the degree of dormancy varied from one genotype to another as follows: weak (% germination >50%), intermediate (% germination>6%<50%) and strong (% germination<6%). Moreover, the study found that a saturated solution of 40% NaCl for 48 h could be used to evaluate the physiological quality in spider plant seeds during storage. It is suggested that further experiments using the diversity observed in the species be conducted to select genotypes with weak dormancy in order to improve the germination capacity in the species.

DEDICATION

I would like to dedicate this work to my parents Raphael and Maimounati Blalogoe for their support and prayers as well as my husband Ardy Obossou and our sons Salim and Amine for their endless love and patience.

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CHAPTER 1
GENERAL INTRODUCTION

1.1 Context/background to the study

Gynandropsis gynandra (L.) Briq. Syn *Cleome gynandra* L., commonly known as “Cleome”, “spider plant” or “Cat’s Whiskers” is a traditional leafy vegetable (TLV) belonging to the family of Cleomaceae. The species is widely distributed throughout the world and is assumed to have originated from Africa (Mishra et al. 2011). The crop is an important leafy vegetable in the rural areas of many countries including Ghana, Kenya, South Africa, Zambia, Zimbabwe and Benin, and is one of the most promising leafy vegetables with good development potential (Smith et al. 2005; van Rensburg Willem et al. 2007; Achigan-Dako et al. 2010; Onyango et al. 2013; Kwarteng et al. 2018). The species is a rich source of vitamins including provitamin A, vitamin C and minerals such as calcium, iron, magnesium and proteins (Opole et al. 1995; Chweya and Mnzava 1997; Jiménez-Aguilar and Grusak 2015).

However, despite being such an important crop species, there are still a number of factors currently limiting its cultivation and use. For example, the species is considered wild and has been largely neglected by researchers (Sogbohossou et al. 2018). Yields are low and this could be explained by several factors such as pest and disease attack, bad agronomic practices and poor and non-uniformity of seed germination (Houdegbe et al. 2018). Low germination capacity, which is also not uniform, is a major problem in this species, which is propagated by seed. Several studies have been done on seed germination of the species, however, the results remain contradictory and inconclusive (Shilla et al. 2016).

Previous studies on the germination of spider plant seem to suggest that fresh seeds at harvest generally have low germination. However, germination has been shown to improve after 3 months of storage (Muasya et al. 2009; Ekpong 2009). Furthermore, germination has increased when seeds were subjected to dark, or alternating dark and light, conditions (Ochuodho and Modi 2005). Several pre-treatments have also been reported to improve the germination rate of the species. The increase in germination could be possibly because of dormancy alleviation over the 3 months. It may also be possible that these conditions are requirements for germination in spider plant seeds, which are not necessarily dormant. From these studies, it is not clear, whether spider plant seeds required those conditions to be able to germinate or break dormancy. The nature of dormancy and the mechanisms of dormancy breaking are also poorly understood.

1.2 Problem statement

The species *Gynandropsis gynandra* has been reported to show low and non-uniform germination as well as significant variation in germination rate. Considerable efforts have been made to understand the physiology of dormancy and germination in this species (Ochuodho and Modi 2006; K'Opondo et al. 2011; Kamotho et al. 2014; Sowunmi and Afolayan 2015). However, contradictions still exist regarding the results of studies on dormancy and germination. For instance, spider plant seeds subjected to dark conditions have been reported to show improved germination (Ochuodho and Modi 2005, Motsa 2015). However, it is not clear whether a period of darkness is a requirement for germination or a dormancy breaking method for spider plant seeds. The lack of clarity is further evident from a number of studies that have reported that freshly harvested seeds stored under ambient conditions for a minimum period of 3 months showed improved germination capacity (Ochuodo 2007, Ekpong 2009). It is probable that freshly harvested spider plant seeds may possibly exhibit some form of dormancy and storing for 3 months allows for dormancy alleviation. The nature and mechanism of dormancy breaking is not well understood. Various authors have reported that the spider plant is able to germinate under a range of conditions, including alternating darkness, continuous dark periods, light and a range of pre-treatments. However, there is a paucity of information on the mechanism by which these treatments are able to break dormancy. Farmers are impacted because low and non-uniform germination means that seedling emergence and establishment can be low and variable, which would subsequently affect plant populations and affect yield. Crop improvement in spider plant would also require that breeders develop varieties with rapid and uniform germination, which is important for commercialization purposes. Research is needed to understand factors influencing the relationship if any between dormancy, low and non-uniform germination in spider plant and environmental conditions during seed development, germination and storage prevailing in areas where they are widely distributed or believed to have originated.

1.3 Justification

This study will contribute knowledge and understanding of the factors underpinning the low and non-uniform germination, which will be useful in improving seed production of spider plant. The knowledge will also be useful to plant breeders in the development of improved *Gynandropsis gynandra* varieties with rapid and uniform germination, which is important for commercialization purposes.

1.4 Aim and objectives

The aim of this study was to gain a deeper understanding of factors influencing the relationship between dormancy, low and non-uniform germination in *Gynandropsis gynandra* and environmental conditions during seed development, germination and storage.

Specifically, the study aimed to:

i) describe and document the phenotypic characteristics and mineral composition of seeds of *G. gynandra* accessions from diverse regions (West Africa, East Africa and Asia),
ii) determine the pattern of seed germination and dormancy development in seeds of different accessions of the spider plant and
iii) assess the storage potential of spider plant seeds using artificial ageing.

1.5 Hypotheses

Based on the above objectives the following hypothesis were tested in this study:

i) *G. gynandra* seed dormancy, composition and germination is strongly genotype dependent.
ii) Different genotypes are adapted to different ecological zones and seeds respond differently to a range of environmental stimuli such as light, darkness, temperature and storage period to break dormancy and be able to germinate.
iii) *Gynandropsis gynandra* seeds are tolerant to accelerating ageing

1.6 Outline of the dissertation

This dissertation is made up of six chapters as shown below:

Chapter 1 provides the introduction, background, justification, objectives, the scope and hypotheses of the study. It highlights the importance of studying seed germination.

Chapter 2 is a literature review of previous studies on seed germination, dormancy and viability in *Gynandropsis gynandra*.

Chapter 3 reports on the laboratory work results on the seed morphology and composition of 29 accessions of *Gynandropsis gynandra*.

Chapter 4 reports on the results of the field experiment and laboratory work on the pattern of seed germination and dormancy development in seeds of five accessions and their crossing of spider plant.

Chapter 5 reports on the laboratory experiment on the seed ageing of four accessions of *Gynandropsis gynandra*

Chapter 6 is a general discussion highlighting the major findings, the conclusion and recommendations.

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CHAPTER 2

GYNANDROPSIS GYNANDRA (L.) BRIQ SYN *CLEOME GYNANDRA L.* SEED GERMINATION AND DORMANCY: LITERATURE REVIEW

Abstract

Gynandropsis gynandra is, like many other African leafy vegetables, grown by subsistence farmers in sub Saharan Africa. The seeds used by farmers come from several sources including farmer's own seeds, seeds from volunteers, neighbours, local markets and most recently seed companies such as Seed Services in Benin. Poor germination was reported in the species and this was attributed to dormancy in the species. In addition, different seed lots have shown different germination rates which was assumed to be due to the provenance of the seeds and the storage conditions. Fresh seed germination is reported to increase with increasing time of storage. Seed pre-treatments were also reported to increase the germination capacity of the species. However, the reported results are in many cases still contradictory. It is hereby recommended that further studies on seed dormancy and germination with more diverse and larger number of accessions be conducted to identify the factors leading to low germination rates in spider plant.

Key words: *G. gynandra*, germination, dormancy, farmers

2.1 Introduction

Spider plant (*Gynandropsis gynandra* (L.) Briq/ *Cleome gynandra* L.) belongs to the botanical family Cleomaceae. The family comprises about 300 species divided into 10 genera (Hall et al. 2002; Hall 2008; Chase et al. 2016). The species is distributed in dry areas of the tropics and sub-tropics and is reported to have originated in sub-Saharan Africa (SSA) and South East Asia (Chweya and Mnzava 1997). In Africa, it is commonly found in Benin, Ghana, Zimbabwe, South Africa, Kenya, Zambia, Uganda, Cameroon, Egypt, Ethiopia, Mozambique, Nigeria, Botswana, and Tanzania (Van Rensburg et al. 2007; Achigan-Dako et al. 2010; Masuka et al. 2012; Kwarteng et al. 2018; Sogbohossou et al. 2018). In South Africa the species is found in the Limpopo, the North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State and the Northern Cape provinces (Van Rensburg et al. 2007).

Gynandropsis gynandra is an erect, annual herbaceous plant up to 1.5 m high in favourable conditions with many branches and sometimes becomes woody with age (Mishra et al. 2011). Several uses of *Gynandropsis gynandra* have been reported in the literature (Opole et al. 1995). The young leaves and shoot, and sometimes the flowers are eaten as pot herb, stew or side dish. In other communities, leaves are used as a flavouring agent in sauces (Chweya and Mnzava 1997; Smith and Eyzaguirre 2005). The leaves are sometimes cooked with other leafy vegetables to reduce the bitterness of the species or boiled briefly, the water discarded, and combined with other ingredients in a stew. The vegetable is a highly recommended meal for pregnant and lactating woman. It is believed that regular consumption of the leaves by pregnant women will ease childbirth by reducing the length of their labour and will help them regain normal health more quickly afterwards (Onyango et al. 2013). In indigenous medicine, the leaves and seeds are used as analgesic, to treat stomach, ache, constipation, conjunctivitis, severe worm infection (Ajaiyeoba 2000; Narendhirakannan et al. 2005). The essential oil extracted from the seeds of *G. gynandra* is occasionally used as an insecticide (Edeoga et al. 2009). The species leaves composition showed that it contains moisture, protein, carbohydrate, fibre, fat, ash, iron and is a rich in vitamin A, B, C and E (Glew et al. 2009; Mibei et al. 2011; Jinazali et al. 2017). Compared with four other African leafy vegetables; *Amaranthus tricolor* L.*, Cucurbita maxima* Duchesne*, Vigna unguiculata* (L.) Walp and *Corchorus tridens* L., spider plant was reported to have a high nutrient content in terms of protein, minerals (iron, calcium, phosphorus and magnesium) and β-carotene (Schönfeldt and Pretorius 2011).

Low and non-uniformity in germination have been reported as one of the main problem encountered by farmers (Onyango et al. 2013). As a result, farmers have difficulties to provide

themselves quality seeds to get uniform seedlings and high yield. Spider plant seeds used to be collected by farmers from volunteer plants, propagated for home consumption and in some cases for sale in local market (Chweya and Mnzava 1997). Other sources of seeds include farmers' saved seeds or borrowed from neighbors or relatives, or buying from local markets (Shilla et al. 2016). Many efforts are going to boost the production of indigenous leafy vegetable including spider plant. Some seed companies in Kenya and Tanzania have started to sell the seed of *G. gynandra* (Muasya et al. 2009). Seeds can be obtained from the Vegetable and Ornamental Plant Institute of the Agricultural Research Council at Roodeplaat (Vopi) in South Africa (Motsa et al. 2015). A large seed collection of the species is available at the World Vegetable Center (AVRDC) and the organization makes some seeds available to farmers through seed kits supported by diverse projects.

Several studies have been conducted to determine the best strategies for improving the germination in the species. The minimum acceptable germination percentage of a seed lot of any crop is 85 % (Abukutsa-Onyango 2003). Seed samples collected from farmer's stores and obtained from research institution showed a germination percentage ranging from 15-92%. It was supposed that the variability observed in germination might be a result of poor seed processing and inherent dormancy. The objective of this review is to analyze the current knowledge and understanding on the germination of spider plant and suggest ways to improve seed quality in the species. The chapter presents the current understanding of dormancy and germination in spider plant seeds, and further discusses conditions used to break dormancy in the species. The review also identifies knowledge gaps that could form the basis for future research. It mainly deals with a comparison of the different results obtained in studies on the germination and dormancy in spider plant with emphasis on areas that require further studies.

2.2 Definition of terms

- **Seed germination**

Seed germination refers to the physiological process culminating in the emergence of the embryo from its enclosing covering, which can include the endosperm, perisperm, testa or pericarp (Bewley et al. 2012). There are many definitions of seed germination and opinions differ on how to determine whether a seed has germinated or not. According to the seed physiologists, germination is defined as the emergence of the radicle through the seed coat (Bewley et al. 2012). For some researchers the radicle must reach a certain length before the seed counts as being germinated (Bewley et al. 2012) . For the seed analyst, germination is the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions. For the farmer, a seed is considered germinated if it emerges from the soil and develops into a normal and vigorous seedling (Bewley et al. 2012). The term seed germination will be used in our thesis in the sense of seed physiologist as the emergence of the radicle.

- **Seed dormancy**

To many people, seed dormancy simply means that a seed did not germinate (Baskin et al. 1998). This definition was said to be incomplete since a non-viable seed might not germinate or the conditions in which the seed were germinated might not be favourable for its germination. Therefore, it is more unanimously recognized that a dormant seed is one that does not have the capacity to germinate although environmental conditions including water, temperature, light and gases are favourable for germination (Koornneef 1994; Vleeshouwers et al. 1995; Bewley 1997; Eira and Caldas 2000; Geneve 2005; Baskin and Baskin 2014)

2.3. Seed development and maturation

The seed development process, from ovule fertilization to physiological maturity, can be divided into four phases (Bewley 1985). Phases I and II comprise cell division and expansion. Reserve accumulation occurs in Phase III as seed dry mass increases. At the end of this phase, seed moisture loss is intensified (Phase IV). Bewley (1985) reported that, after fertilization, there is a period of seed structure formation because of cell division, expansion and differentiation (histo-differentiation) in which seed structure primordia are formed and future embryo parts can be visualized. During this phase, there is a significant increase in seed size forming the embryonic cells that receive assimilates from the parent plant. During this period, seed moisture content remains constant and high. The significant decrease in seed moisture content occurs at the end of maturation when changes in cell membrane

structure organization occurs as well as increases in enzyme synthesis in preparation for successful germination. Recalcitrant seeds usually do not show this transition period between maturation and germination.

There was a significant effort by seed technologists to clarify the maturation process and to define the primary changes occurring during seed development. The following changes occur during seed development:

Seed moisture content: Ovule moisture content at the time of fertilization is approximately 80% (fresh weight basis), both for monocots and dicots. That value decreases during maturation although it remains relatively high throughout most of the maturation period because water is the vehicle for transferring nutrients from the parent plant to the developing seeds (Baroux et al. 2002). The initial phase of dehydration is slow and is accelerated from the time the seeds reach maximum dry weight; at that time, seeds possess 35% to 55% moisture content for orthodox monocot and dicot seeds, respectively, produced in dry fruits. This decrease in moisture content proceeds until hygroscopic equilibrium is attained. From that point on, moisture content changes are associated with variations in relative humidity. However, seeds produced in fleshy fruits have a lower reduction in moisture content than seeds produced in dry fruits. Developing recalcitrant seeds do not show marked changes in desiccation at the end of maturation, possessing moisture contents usually over 60% (fresh weight basis) (Baroux et al. 2002).

Seed size: The fertilized ovule is a small structure with respect to final seed size. Plant species with large seeds have an advantage under low light conditions, when their greater protein and lipid reserves, or their more advanced development, can facilitate growth (McDonald 1994). However, large seeds usually come at the cost of seed number per flower or fruit (Bruun and Ten Brink 2008). In addition, large seeds cannot be physically borne on small plants because of the weight of the seed, which may partly explain the association between plant size and seed size (Grubb et al., 2005).

Seed dry weight: After sexual fusion, the developing seeds begin to increase in weight because of nutrient accumulation and water uptake. Seed fill is initially slow because cell division and elongation are occurring during this stage. Soon after, dry mass accumulation increases until seeds reach their maximum dry weight (Baroux et al. 2002).

Germination: Seeds of various cultivated species are able to germinate a few days after ovule fertilization. In this case, germination refers to protrusion of the primary root, not the formation of a normal seedling because histo-differentiation has not been completed and reserve accumulation is still incipient at this phase. Therefore, this germination does not lead to the

production of vigorous seedlings (Bruun and Ten Brink 2008). Theoretically, it is possible to consider that the percentage of germinable seeds increases during maturation, reaching a maximum around the time when seeds attain maximum dry weight. This is only found in species where dormancy does not occur, because the imbalance in the germination promoters/inhibitors induced during the reserve accumulation period may directly affect seed germinability.

Vigour: Seed vigour changes are usually in parallel with nutrient reserve transfer from the parent plant. This means that the proportion of vigorous seeds increases during maturation, reaching a maximum near to or at the same time as seed maximum dry weight (Baroux et al. 2002).

2.4. Germination and crop performance

Good seed germination is very important for crop production. Uneven or poor germination and subsequently uneven seedling growth can lead to great financial losses by reducing crop yield (TeKrony and Egli 1991). Seed germination may influence crop yield through both indirect and direct effects. The indirect effects include those on percentage emergence and time from sowing to emergence. These effects influence yields by altering plant population density, spatial arrangement, and crop duration. The effects of seed vigour on emergence and stand establishment are well documented (Roberts 1972). Effects have been reported on total emergence, rate of emergence, and the uniformity of emergence. All of these factors can potentially influence dry matter accumulation by the plant or plant community and thus potentially affect yield. Total emergence determines plant density, and there is a strong relationship between plant density and yield (Willey and Heath, 1969). Direct effects on subsequent plant performance are more difficult to discern.

2.5. Classification of seed dormancy

Various schemes for classifying dormancy have been published (Harper 1957; Nikolaeva 1977; Lang et al. 1985; Lang 1987; Baskin and Baskin 2014). Nikolaeva's (1977, 1999) scheme is the most comprehensive classification system of seed dormancy ever published. According to this scheme, there are two kinds of seed dormancy: endogenous and exogenous (Table 2.1). In endogenous dormancy, some characteristics of the embryo prevent germination, while in exogenous dormancy some chemical or characteristics of structures, including endosperm, seed coats or fruit walls, covering the embryo prevent germination.

Table 2.1 : Simplified version of Nikolaeva (1977) classification scheme of organic seed dormancy types

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)

Based on various kind of dormancy in the Nikolaeva scheme, Baskin et al. (1998) and Baskin and Baskin (2014) proposed a dichotomous key to distinguish between kinds of dormancy based on seed (or fruit) coat permeability to water, embryo morphology and whole seed physiological responses to temperature or to a sequence of temperatures (Table 2.2.)

Table 2.2: A dichotomous key to distinguish nondormancy: the dormancy classes morphological, physical and physical + physiological (combinational)

1. Embryo differentiated and fully developed	2
2. Seeds imbibe water	3
3. Root emergence occurs within about 4 wk (usually in a few days)	4
4. After root emergence, shoot emergence occurs within a few days.	NONDORMANT
4. After root emergence, shoot emergence is delayed 3–4 wk or more	PHYSIOLOGICAL EPICOTYL DORMANCY
3. Root emergence requires more than 4 wk	5
5. After root emergence, shoot emergence occurs within a few days.	PHYSIOLOGICAL REGULAR DORMANCY
5. After root emergence, shoot emergence is delayed 3–4 wk or more	PHYSIOLOGICAL EPICOTYL DORMANCY
2. Seeds do not imbibe water	6
6. Scarified seeds become fully imbibed (usually in 1 day) and germinate within about 4 wk (usually in a few days)	PHYSICAL DORMANCY
6. Scarified seeds become fully imbibed (usually within 1 day) but do not germinate within about 4 wk	COMBINATIONAL DORMANCY
1. Embryo undifferentiated or if differentiated it is underdeveloped	7
7. Embryo not differentiated	8
8. After seed dispersal, embryo differentiates and grows in imbibed seed	9
9. Seeds germinate within about 4 wk	MORPHOLOGICAL DORMANCY
9. Seeds do not germinate within about 4 wk.	MORPHOPHYSIOLOGICAL DORMANCY[a]
8. After seed dispersal, embryo never differentiates into a root-shoot axis	10
10. Seeds germinate within about 4 wk	SPECIALIZED MORPHOLOGICAL DORMANCY
10. Seeds do not germinate within about 4 wk	SPECIALIZED MORPHOPHYSIOLOGICAL DORMANCY
7. Embryo differentiated but underdeveloped	11
11. After seeds are placed on a moist substrate, the embryo grows, and seeds germinate within about 4 wk	MORPHOLOGICAL DORMANCY[b]
11. After seeds are placed on a moist substrate, the embryo does not grow, and seeds do not germinate within about 4 wk.	MORPHOPHYSIOLOGICAL DORMANCY[b,c]

Source: Baskin and Baskin (2014)

2.6. Possible causes of poor seed germination in *Gynandropsis gynandra*

Various factors might influence the low germination reported in spider plant such as the production environment (conditions at harvest maturity and seed moisture content), the storage conditions (duration and storage packaging) and the dormancy state of the seed.

The condition under which the mother plant is grown (Ochuodho 2005) and the differences in length of time of the male and female flower production (Tibugari et al. 2012) have great influence on the quality of seeds produced and hence affect germination of seeds. Spider plant seeds are mature and ready for harvesting when the pods are yellow and the seeds black (Chweya and Mnzava 1997). At this stage the seed moisture content is too high (>25%) and

a drying period was recommended by K'Opondo et al. (2011) to reduce the moisture content and favor germination. Kamotho et al. (2013) found that seed dried to 5% moisture content recorded the highest percentage of germination. The seed should not be sown too deep because they are small seeded and the depth of sowing should not exceed 1 cm. Sowunmi and Afolayan (2015) recommended an optimal sowing depth between 0.5 cm and 1 cm while Seeiso and Materechera (2011) recommended an optimum sowing depth between 0.15 cm and 0.35 cm. Watering regularly also promotes spider plant germination. Watering bi-weekly when germinating in the field showed the highest percentage of germination compared to watering daily and once a week (Sowunmi and Afolayan 2015). However, this may increase or decrease depending on the humidity of the germination environment. Once the seed is harvested and dried it must be stored in a dry area for better germination.

Poor germination in spider plant is explained also by dormancy observed in freshly harvested seeds of the species. In a seed germination experiment, Kamotho et al. (2014) recorded the highest germination percentage as storage time increased from fresh harvest (14.5%) and maximum (95%) after six months. Similar results were observed by Ekpong (2009) who reported germination of more than 90% when freshly harvested seeds of spider plant was stored at 15°C and room temperature for 5 months, and Ochuodho and Modi (2005) who observed that seeds germinated better after three months of storage. Chweya and Mnzava (1997); Geneve (1998); Kamotho et al. (2014) indicated that *G. gynandra*, as it is the case for many freshly harvested seeds of herbaceous plants, needs postharvest ripening before dormancy is broken. However, preliminary observations by Shilla et al. (2016) on germination do not support improvement of germination rate during the after-ripening period. Moreover, in their study, Ochuodho and Modi (2007); Motsa et al. (2015) used seed of *Gynandropsis gynandra* stored for 3 months and 1 year, respectively, but found the initial germination to be low, but it increased after applying some dormancy breaking methods. This seems to suggest that dormancy in *Gynandropsis gynandra* is biotype dependent. Zharare (2012) indicated that seed germination in spider plant and Amaranthus species is strongly biotype dependent where differences in seed germination in *G. gynandra* biotypes originating from different environments were assumed to reflect habitat specific selection.

2.7. Conditions/requirements for germination in *Gynandropsis gynandra*

For a seed to germinate, appropriate conditions must be met including optimum temperature, soil moisture content, oxygen and light. However, there are specific requirements depending on the nature of the crop. In the case of *Gynandropsis gynandra* some studies have been carried out to determine the optimum conditions for germination. The effect of light on *Gynandropsis gynandra* germination is the most studied in the literature (Ochuodho et al.

2005; Ochuodho and Modi 2007; Muasya et al. 2009; K'Opondo et al. 2011; Zharare 2012; Sowunmi and Afolayan 2015; Motsa et al. 2015). The results showed that *G. gynandra* responded negatively to continuous light when exposed beyond 12 hours by reduced germination rate. This showed that the species is negatively protoblastic (seeds require darkness for germination). The optimum conditions for germination of the seed would therefore be continuous dark and alternating dark and light for mostly 8 hours of light per day. In the literature, these kinds of seeds are termed photodormant (Geneve 1998) and occurs in spider plant due to its small seed size (<1mg) (Bewley et al. 2012). Under wild conditions, spider plant seeds usually spend time in the soil covered by plant debris before the rainy season. This would probably explain the adaptation of the species to dark conditions as a requirement for germination. One strategy that could be used by farmers in the field is to initially cover the nursery bed with black plastic, then removing it after seed emergence and transplanting the seedlings in the field.

Because of the tropical origin of *Gynandropsis gynandra*, warm temperatures would be ideal for its germination and development. The effect of temperature on the germination capacity of spider plant seed has been investigated in several studies (Ochuodho and Modi 2005; K'Opondo et al. 2011; Zharare 2012; Sowunmi and Afolayan 2015; Motsa et al. 2015). From these studies, the favorable temperature for germination percentage higher than 50% ranged from 25°C to 40°C. The optimum temperature of germination varied from one study to another but was always within this range. However, Zharare (2012) found alternating 4°C/27°C for 16/8 h as optimal temperature for germinating spider seed, contradicting the tropical origin of the species, whereby low temperatures would not promote the germination capacity of the species.

2.8. Pretreatments for breaking dormancy in *Gynandropsis gynandra*

Various pretreatment methods have been used to overcome seed dormancy in *Gynandropsis gynandra,* however, the results remain contradictory and inconclusive (Shilla et al. 2016). Ekpong (2009) indicated that among the different methods of breaking dormancy in fresh seeds of *G. gynandra* such as heating, soaking, leaching, potassium nitrate (KNO_3) and Gibberellic acid (GA3); heating at 40°C for one to five days was the most effective method with up to 90% germination capacity reported. In addition, (i) leaching by washing seed under running water at room temperature for a few minutes); (ii) soaking in tap water for a few hours before the germination test; and (iii) pre-chilling by moistening and maintaining at cold temperature for a number of days before the germination, were observed to increase germination up to 74%. However, the study showed that GA_3 and KNO_3 obtained the lowest germination percentage 34% and 16% respectively (Ekpong 2009). Muasya et al. (2009)

investigated the effect of different pretreatments including potassium nitrate, leaching, light, GA and chilling (cold stratification) and found that GA_3 was the most effective treatment for breaking dormancy in the species. They also found that stratification for two weeks at 5°C and germination in the dark improved germination significantly whereas KNO_3 lowered the germination rate. Ochuodho (2005) meanwhile tested the effect of different pre-germination treatments (chilling, scarification, hydration and germination in the presence of KNO_3 or GA_3) on one-year and two-year seed lots from different origins. These seeds were not freshly harvested and results showed that both a 15 day-pre-heating at 40°C and scarification effectively broke seed dormancy in *G. gynandra*. Despite the above successes, a significant difference in germination between seed lots tested has been reported (Ochuodho 2005). The author noticed that the seed lot from South Africa showed slower rate of germination and lower final germination percentage than lots from Kenya. Although it is not very clear on the cause of such a difference, it is hypothesized that environmental conditions during seed development in the two locations could have influenced the germination rates of seed lots since the two lots of seeds were obtained from different locations (Shilla et al. 2016). Zharare (2012) by testing the effect of GA_3, KNO_3, K_2SO_4 and smoke water on the germination of the species found GA_3 to be the most effective pre-treatment to break dormancy in spider plant with KNO_3 and K_2SO_4 reported as inefficient to break dormancy.

2.9. Summary and conclusion

Based on the different information extracted from the previous studies, it can be summarized that *Gynandropsis gynandra* fresh seeds exhibit dormancy, which could be overcome after a minimum period of storage of three months. Stored seeds have also been reported to have low germination capacity, which is increased by some dormancy breaking methods. It not clear whether only fresh seed of spider plant are dormant or both stored and fresh seed. Moreover, germination has also been shown to increase when seeds are subjected to dark or alternating dark and light conditions. Several pretreatments have also been reported to improve the germination rate of the species with the results varying from one study to another. The increase in germination could be possibly as a result of dormancy alleviation over the 3 months. It may also be possible that these conditions are requirements for germination in *Gynandropsis gynandra* seeds, which are not necessarily dormant. From these studies, it is not clear, whether spider plant seeds required those conditions to be able to germinate or break dormancy. The nature of dormancy and the mechanisms of dormancy breaking are also poorly understood. It is hereby recommended that further studies on seed dormancy and germination with more diverse and large number of accession taken is done to explain the narrow scientific information available with regard to low germination in spider plant.

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CHAPTER 3

PHENOTYPIC CHARACTERIZATION AND MINERAL COMPOSITION OF ASIA, WESTERN AND EASTERN AFRICA SEEDS OF *GYNANDROPSIS GYNANDRA* (L.) BRIQ ACCESSIONS

Abstract

Spider plant (*Gynandropsis gynandra* L.) is an important African leafy vegetable, which has been characterized for leaf yield components and nutritive quality. However, there is little information on variability with respect to seed morphological characteristics and mineral element content. The study tested the hypothesis that spider plant seeds from different geographical areas vary with respect to seed mineral content and morphological traits. Twenty-nine accessions of *Gynandropsis gynandra* from West Africa, East-South Africa and Asia were screened for seed size (area, perimeter, length, and width), 100 seeds weight, mean germination time, percentage germination and mineral elements composition. The scanning electron microscope (SEM), light microscopy and energy dispersive spectroscopy (EDX) solution were used to study the morphology and mineral composition. The accessions differed significantly ($p<0.001$) with respect to seed size (area, perimeter, length, width), 100 seeds weight, mean germination time and percentage germination. Eight (8) mineral elements, including carbon (C), oxygen (O), magnesium (Mg), aluminium (Al), phosphorus (P), sulphur (S), potassium (K) and calcium (Ca) were identified. The hierarchical cluster analysis based on fourteen (14) variables grouped the accessions into three distinct clusters showing the presence of genetic diversity, with the clustering of accessions occurring along regional basis. Asian accessions recorded highest values in terms of percentage of germination and phosphorus content, while western Africa accessions showed highest values in terms of seed size. Materials from different geographical origins could be used as parents for the genetic improvement of germination capacity and yield of the species.

Keywords: seed morphology, mineral elements, germination, characterization

3.1 Introduction

Successful breeding programmes and crop production require good quality seeds for high yield to ensure food and nutritional security for growing populations (Ambika et al. 2014; Khan and Hakeem 2015). Seed germination and seedling emergence are critical phases in the development of plants and this implies that seed vigour is an important trait for the selection of important crop cultivars (Eggert and von Wirén 2013; Marcos Filho 2015). The capacity of a seed to germinate quickly and competitively depends on the genetic and physiological constitution of the seed (Kaydan and Yagmur 2008; Adebisi et al. 2013). In addition, seed size is an important physical indicator of physical seed quality which can affect vegetative growth and is frequently related to yield (Ambika et al. 2014). It is commonly known that because of the larger store of carbohydrate in the seed's endosperm or cotyledons, seedlings from larger seeds have a better start in life and better field performance than smaller seeds (Gunaga and Vasudeva 2011). Morphological measurements including length, width, area, perimeter and weight are important parameters for determining the size and shape of seed (Wyllie-Echeverria et al. 2003). Seed shape and size are important factors that can influence water imbibition, seed moisture content and consequently the germination of seeds, and seed quality (Balkaya and Odabas 2002; Cerdà and Garcıa-Fayos 2002; Mandal et al. 2008). For instance, Gholami et al. (2009) observed an increase in germination as well as greater speed of germination in larger seeds compared with small seed in pinto bean. It was shown that larger seeds of *Amaranthus* possess higher physiological quality (Menaka and Balamurugan 2008) than smaller seeds.

Seed mineral composition also plays an essential role in plant establishment and growth. Seeds contain several macronutrients like phosphorus (P) and micronutrients, including Zinc (Zn), Boron (B), Molybdenum (Mo), Selenium (Se), copper (Cu), cobalt (Co) which are important for seed germination, seedling emergence and seed vigour (Tyler and Zohlen 1998; Zhu and Smith 2001; Eggert and von Wirén 2013). It has been shown that annual pasture legume yields are positively correlated to phosphorus concentration in seeds (Bolland and Paynter 1990).

Breeding for grain yield, seed size, seed mineral content and seed vigour needs a fundamental assessment of the seed metrics, their mineral composition and germination capacity. In *Gynandropsis gynandra*, a dicotyledonous species belonging to the family Cleomaceae and sub family of Cleomoidae (Chweya and Mnzava 1997), several characterization studies have been conducted (Masuka et al. 2012; Wasonga et al. 2015; Kwarteng et al. 2018) using traits of interest including leaf number, number of branches, stem colour and leaf area. The studies reported a large genetic diversity among accessions. The species mineral composition of the

leaves has also been assessed and major minerals reported were potassium, calcium, magnesium, phosphorus, iron, manganese and zinc (Omondi et al. 2017). Although *Gynandropsis gynandra* is a leafy vegetable, the leaf yield is not only dependent of the leaf components but also of seed quality and mineral composition. However, variation of the quality and mineral composition of the seeds of *G. gynandra* is not well documented. Information on the variability with respect to seed morphological traits and mineral composition among accessions from different regions could be useful in depicting the genetic diversity within the species.

The objectives of this study were, therefore, (i) to screen seeds of *G. gynandra* from different geographic regions for their mineral composition and (ii) to assess the genetic diversity among accessions based on seed morphology and mineral composition in relation to seed quality with respect to germination. The study hypothesized that mineral composition and size of the seed of *Gynandropsis gynandra* vary among accessions from different geographical regions, and large seeds with high phosphorus content germinate better than small seeds with low phosphorus content.

3.2 Materials and methods

3.2.1 Plant material

Twenty-nine (29) accessions from three different geographic regions, namely, western Africa, eastern-southern Africa and Asia were used in the study (Table 3.1). Seeds from these accessions were harvested in 2017 from an experimental site at the Faculty of the Agronomic Sciences (FSA) of the University of Abomey Calavi in Benin and stored for 15 months before commencement of this experiment.

Table 3.1: List of accessions included in the study and their geographic origins

Accession	Institution	Origin	Region
TOT1048	AVRDC	Thailand	Asia
TOT3527	AVRDC	Lao People's Democratic Republic	Asia
TOT3536	AVRDC	Lao People's Democratic Republic	Asia
TOT4976	AVRDC	Thailand	Asia
TOT5799	AVRDC	Thailand	Asia
TOT7196	AVRDC	Malaysia	Asia
TOT7198	AVRDC	Malaysia	Asia
TOT7200SC	AVRDC	Malaysia	Asia
TOT7486	AVRDC	Lao People's Democratic Republic	Asia
TOT7505	AVRDC	Lao People's Democratic Republic	Asia
BAR 1807B	KENRIK	Kenya	East Africa
ELG 19/07A	KENRIK	Kenya	East Africa

Accession	Institution	Origin	Region
HBY/2307b	KENRIK	Kenya	East Africa
KF-07	KENRIK	Kenya	East Africa
KSI 2407A	KENRIK	Kenya	East Africa
TOT8887	AVRDC	Uganda	East Africa
TOT8926	AVRDC	Kenya	East Africa
TOT6439	AVRDC	Zambia	South Africa
TOT8931	AVRDC	South Africa	South Africa
ODS-15-013	GBioS	Benin	West Africa
ODS-15-019	GBioS	Benin	West Africa
ODS-15-020	GBioS	Benin	West Africa
ODS-15-044	GBioS	Benin	West Africa
ODS-15-045	GBioS	Togo	West Africa
ODS-15-053	GBioS	Togo	West Africa
ODS-15-061	GBioS	Togo	west Africa
ODS-15-121	GBioS	Ghana	West Africa
ODS-15-100	GBioS	Togo	West Africa
ODS-15-115	GBioS	Ghana	West Africa

GBioS: Laboratory of Genetics, Horticulture and Seed Science, AVRDC: World Vegetable Center, KENRIK: Kenya Resource Centre for Indigenous Knowledge

3.2.2 Seed morphology traits and mineral composition data collection

Morphological and mineral element analyses were performed at the Microscopic Microanalysis Unit (MMU) and the Phytopathology Laboratory of the University of KwaZulu-Natal, College of Agriculture, Engineering and Science. Scanning Electron Microscope (SEM) images were obtained with Zeiss Microscopy (EVO/LS15) of the Microscopic Microanalysis Unit (MMU). Seed samples were mounted on copper stubs on a double-sided adhesive carbon tape and placed at different positions to facilitate observation. SEM images of 87 seeds were used to determine their dimension (images analysis). In addition, fractured (longitudinal and vertical) seeds were used to study the seed anatomy. To complement seed description, the mineral analysis of seeds was done using the Integrated Energy Dispersive Spectroscopy Solution (EDS) of the EVO.

Light Microscopy: seed observations were also made using a Binocular zoom stereo microscope (Carl Zeiss Stemi SV6) equipped with an Electronic Light source (Schott KL 1500) and with a digital microscope camera to complement the internal seed description of the species.

The imaging analysis was performed on individual seeds using the digital image analysis software (AnalysisSIS) at the Microscopic Microanalysis Unit. Before measurement, the system was calibrated to millimeters under x100 magnification and 200(um) scale before the measurements. The variables measured on imported SEM images included:

a) *Seed length (SL):* the distance between 2 points stretching from the base of the embryo axis to the tip of the endosperm of the seed
b) Seed width (SW): the length of the line drawn across the widest section of the seed,
c) *Seed area (A)* and *perimeter (Pe)* were directly obtained after drawing a circle around the seed touching all edges.

In addition, 100 seeds weight was recorded using the average weight of samples of 10 seeds randomly chosen and weighed using a precision balance Ohaus® Pioneer™ Plus analytical balance Model PA114C, AC/DC input 230 V AC, universal plug set measuring up to 4 decimal places. The 10 seeds weighed was a replicate for times for each accession.

Moreover, germination capacity of the seeds was investigated using 10 seeds in petri dishes and placing in an incubator at a temperature of 30°C under dark conditions. Each treatment (accession) was replicated 5 times. The number of newly germinating seeds was counted each day for 7 days and used to calculate Mean Germination Time (1) and the percentage germination.

$$MGT = (\Sigma \mathrm{nidi}) / \Sigma \mathrm{N}) \ (1)$$

Where *ni* = the number of germinated seeds at day *i*, *di* = incubation period in days, and *N* = number of germinated seeds in test. The speed of germination is said to increase while the value of MGT decreases. The data for the shoot length (mm) and root length (mm) were measured five days after germination.

3.2.3 Data analysis

Descriptive analysis was conducted to show trends in morphological traits, germination and mineral elements. Analysis of variance (ANOVA) was carried out on all quantitative variables to describe the variation among accessions. Treatment means were separated using Least Significant Difference (LSD 5%). Pearson correlation analysis was done to show the linear correlation among morphological traits, mineral content and germination parameters. In addition, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed to group the 29 accessions into different clusters using seed morphological traits, germination parameters and mineral elements in the R package “FactoMiner” (Lê et al. 2008). A dendrogram was generated using the function “hclust” of the R package “vegan” (Grum and Atieno 2007) to support the results of the HCA. All the data were analyzed using R software version 3.5.1. (Crawley 2012).

3.3 Results

3.3.1 Morphological characteristics of the seed of *G. gynandra*

3.3.1.1 External and internal seed structures of *G. gynandra* seeds

Gynandropsis gynandra seeds are generally brown or black in color. The seeds are round or fairly round depending of the accession and pointed at the apical region where the radicle is located. The hilum is located at the center of the seed. The surface of the seed is rough with small rounded or oscillated depressions and ridges on the whole surface of the seed. Among the spider plant accessions, there were two major seed types observed based on the seed surface. The first one consisted of accessions with slightly rough seed surface (Figure 3.1) and accessions with very rough seed surface (Figure 3.2). Illustrations of cross and longitudinal sections, based on observations from the Scanning Electron Microscope (SEM) showed the embryo and the seed coat (Figure 3.3) but not the overall organization of the seed. The longitudinal section of seeds observed under a light microscope revealed the overall organization of the spider plant seeds (Figure 3.4). The embryo consisted of a hypocotyl-radicle axis and two cotyledons and the endosperm in the micropylar region.

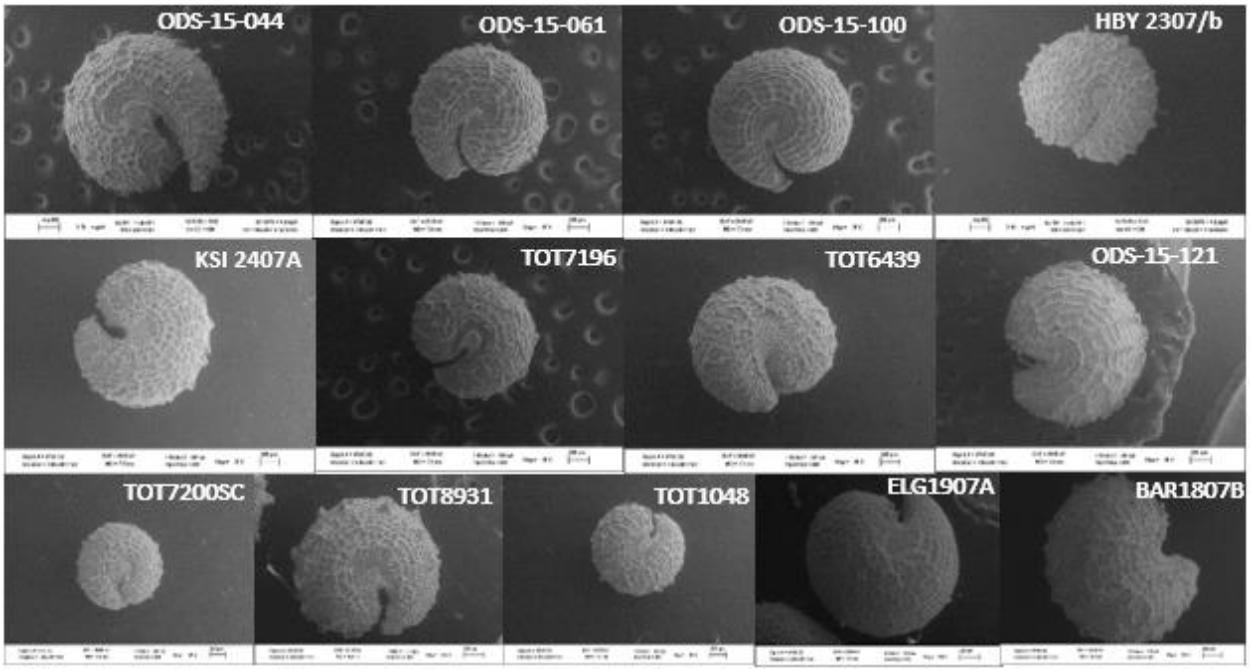

Figure 3.1: SEM of *Gynandropsis gynandra* accessions with slightly rough seed surface

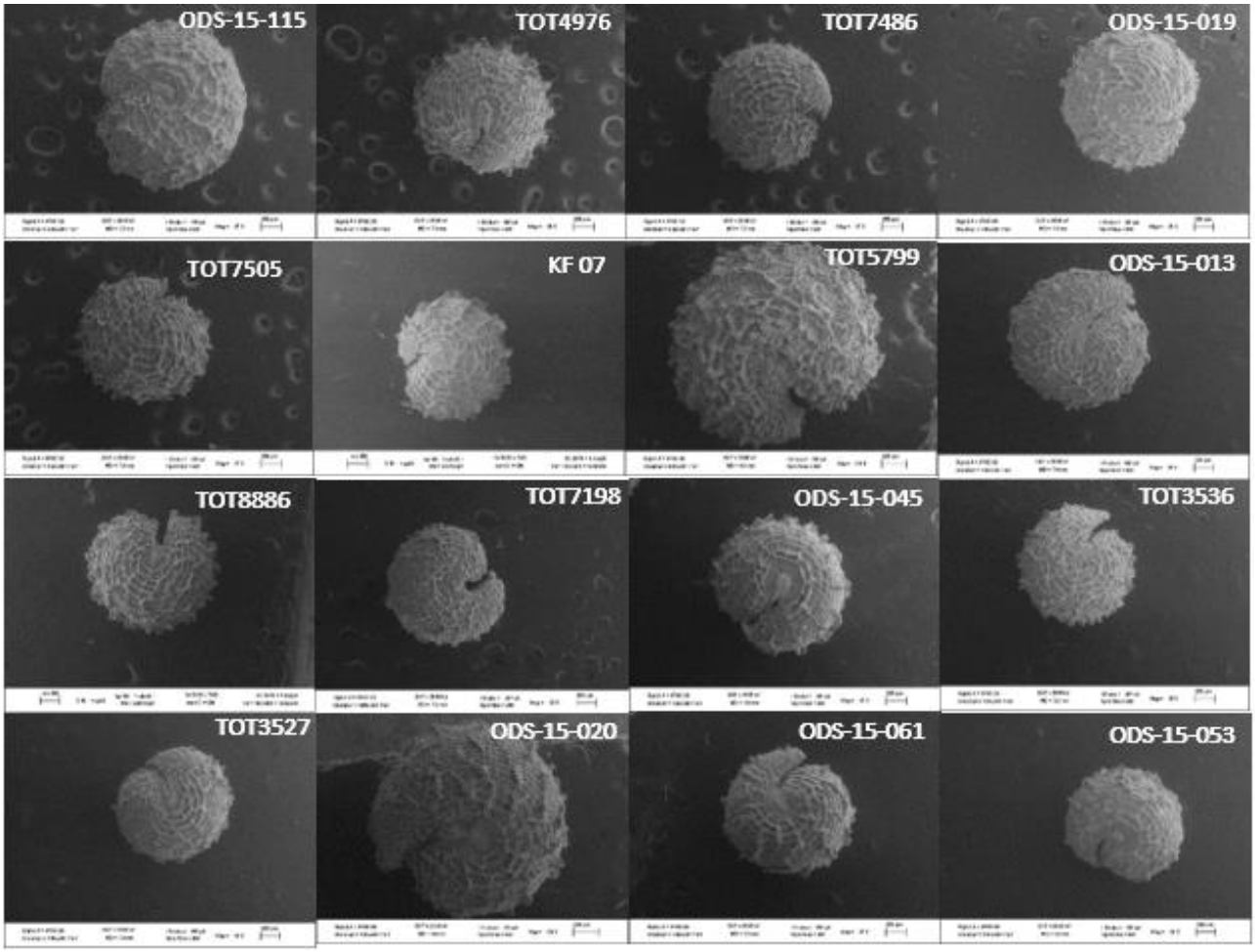

Figure 3.2: SEM of *Gynandropsis gynandra* accessions with very rough seed surface

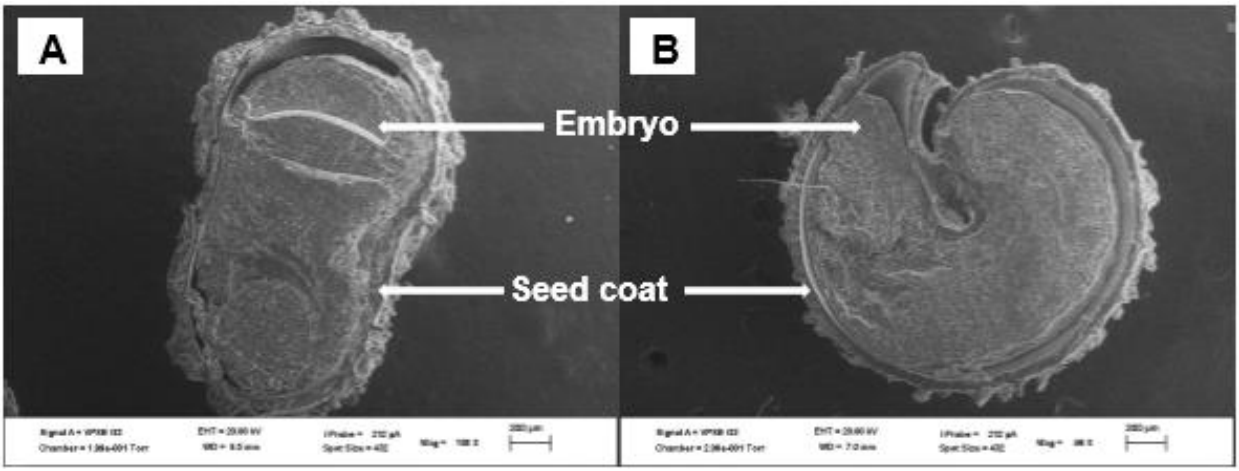

Figure 3.3: *Gynandropsis gynandra* internal seed morphology under SEM **A**: Seed cross section **B**: Seed longitudinal section

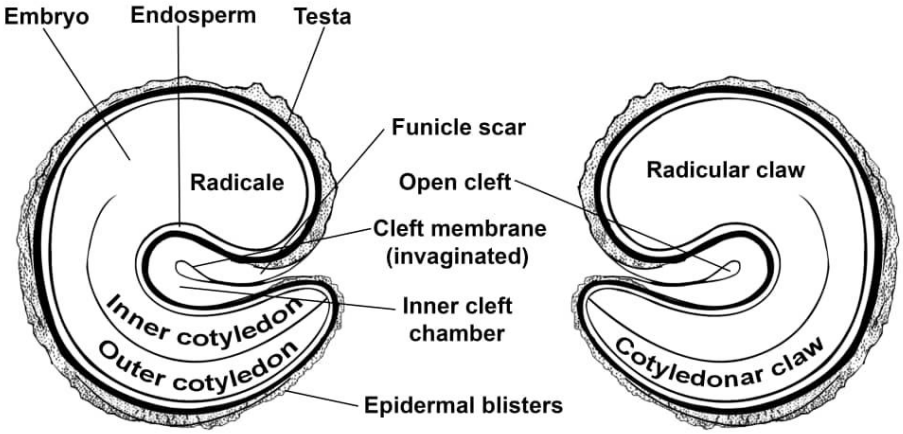

Figure 3.4: Illustration of *Gynandropsis gynandra* seed in longitudinal section as viewed in light microscope: adapted from Iltis et al. (2011).

3.3.2 Variation in quantitative morphological characteristics/traits of *G. gynandra* seeds

The analysis of variance (ANOVA) showed highly significant differences among the spider plant accessions with respect to all morphological seed traits (Table 3.2). The seed area ranged from 95.42 to 218.81 mm² with an average of 149.42 mm². High values were obtained for accessions ODS-15-044, ODS-15-115, TOT6439, ODS-15-020, ODS-15-100, ODS-15-013, ODS-15-021 and BAR1807B. The lowest value was obtained for accession TOT3527. The seed perimeter ranged between 3.87 and 6.19 mm with an average of 4.90 mm. The highest values were obtained for ODS-15-020, TOT8887, ODS-15-044, ODS-15-115, ELG 19/07A, BAR 1807B, ODS-15-013, ODS-15-100, ODS-15-121, while the lowest value was obtained with accession TOT3527. The seed width ranged between 0.93 and 1.74 mm with an average of 1.29 mm. The highest values were obtained in accessions ODS-15-044, ODS-15-115, TOT6439, ODS-15-013, ODS-15-020, ODS-15-100 and the lowest value by accession TOT3527. The seed length ranged from 1.19 to 1.69 mm with an average of 1.43 mm. The accessions BAR1807B, ODS-15-044, ODS-15-020, ODS-15-115, ODS-15-121, TOT6439, TOT8887, ODS-15-100, ODS-15-15-019 showed the highest values while the lowest value was obtained by accession TOT7200SC. The 100-seed weight of spider plant accessions ranged from 6.33 to 17.1 mg with an average of 10.44 mg. The accessions with the highest seed weight were ODS-15-121, ODS-15-111, TOT6439 and KSI2407A. Accession 7198 showed the lowest seed weight.

Table 3.2: Mean of the morphological traits of the seed of *Gynandropsis gynandra* accessions

Accessions	Seed area	Seed perimeter	Seed width	Seed length	100 Seed weight
BAR1807B	179.31	5.48	1.40	1.69	11.17
ELG19/07A	147.93	5.51	1.36	1.46	9.53
HBY/2307b	154.51	4.98	1.33	1.43	13.67
KF07	113.54	4.23	1.11	1.23	7.03
KSI2407A	149.54	4.84	1.35	1.35	14.00
ODS-15-013	182.35	5.43	1.48	1.54	11.67
ODS-15-019	161.05	5.00	1.33	1.49	13.20
ODS-15-020	190.61	**6.19**	1.47	1.65	13.20
ODS-15-044	**218.81**	5.82	**1.74**	**1.69**	13.83
ODS-15-045	145.57	4.76	1.28	1.41	11.40
ODS-15-053	130.09	4.44	1.24	1.36	7.10
ODS-15-061	132.72	4.46	1.33	1.25	11.23
ODS-15-100	186.82	5.27	1.45	4.89	12.13
ODS-15-115	215.01	5.64	1.51	1.67	14.77
ODS-15-121	181.88	5.26	1.37	1.68	**17.10**
TOT1048	116.94	4.22	1.07	1.32	8.43
TOT3527	**95.42**	**3.87**	**0.93**	1.25	8.73
TOT3536	108.23	4.17	1.12	1.24	7.73
TOT4976	142.46	4.65	1.24	1.42	6.87
TOT5799SC	124.57	5.12	1.19	1.34	6.47
TOT6439	200.68	5.49	1.51	1.62	14.67
TOT7196	129.26	4.39	1.22	1.32	6.50
TOT7198	105.37	4.19	1.02	1.33	**6.33**
TOT7200SC	102.80	4.02	1.09	**1.19**	7.30
TOT7486	128.00	4.41	1.20	1.35	8.73
TOT7505	137.40	4.63	1.23	1.40	7.53
TOT8886	154.24	4.85	1.26	1.48	11.63
TOT8887	159.10	6.03	1.32	1.51	13.10
TOT8926	138.93	4.69	1.29	1.38	7.57
Mean	149.42	4.90	1.29	1.43	10.44
SD	33.63	0.62	0.17	0.15	3.13
CV (%)	22.51	12.65	13.18	10.49	29.98
F value (5%)	14.39 ***	14.30 ***	8.75 ***	8.31***	45.22***
LSD (5%)	51.75	0.96	0.33	0.31	2.72

SD= Standard Deviation CV= Coefficient of variation, LSD= Least Significant Difference, ***p<0.001
Values in bold indicate minimum and maximum

3.3.3 Mineral element composition in seeds of *Gynandropsis gynandra*

The screening of the seeds of *G. gynandra* by the Energy Dispersive Spectroscopy Solution showed a total of eight mineral elements, including carbon (C), oxygen (O), magnesium (Mg), aluminium (Al), phosphorus (P), sulphur (S), potassium (K) and calcium (Ca).

Highly significant ($p<0.001$) differences were observed among accessions with respect to all mineral elements, except for aluminium (Al) (Table 3.3). In addition, the coefficient of variation was relatively high (>20%) for magnesium, phosphorus, sulphur, potassium and calcium. The C content in the seeds ranged from 55.92g/100g to 62.82g/100g with an average of 58.50g/100g while O ranged between 33.39 g/100g and 40.11 g/100g with an average of 38.04 g/100g (Table 3.3). The accessions TOT8887, BAR1807B, TOT6439 and TOT8887 showed the highest carbon content while the highest value for oxygen was obtained by accessions ODS-15-013 and ODS-15-061. Moreover, the lowest value for carbon was observed in the accession ODS-15-053 while accession TOT8887 showed the lowest oxygen value. The Mg content of spider plant seeds ranged from 0.11 g/100g to 0.56 g/100g with an average of 0.30 g/100g while the Al content ranged from 0.01 g/100g to 0.70 g/100g with an average of 0.20 g/100g. The highest value for magnesium were recorded for the accession ODS-15-053 while the highest aluminium value was obtained for accession TOT8887. The P and S content in the accessions ranged, respectively, from 0.15 g/100g to 0.59 g/100g and from 0.33 g/100g to 1.17 g/100g with an average of 0.30 g/100g and 0.67 g/100g. The highest phosphorus and sulphur values were both recorded for the accession ODS-15-053. Spider plant K seed content ranged from 0.08 g/100g to 2.58 g/100g while the C content ranged from 0.42 to 1.79 g/100g with an average of 1.05 g/100g for K and 0.97 g/100g for Ca. Accession ODS-15-019, showed the highest K, while ELG 1907A showed the highest Ca content.

Table 3.3: Mean of mineral composition (g) of fifteen months *Gynandropsis gynandra* seeds

Accessions	C	O	Mg	Al	P	S	K	Ca
BAR 1807B	61.60	35.71	0.28	0.33	0.18	0.50	0.19	1.21
ELG 19/07A	59.81	37.13	0.21	0.52	0.19	0.82	**0.08**	1.23
HBY/2307b	58.39	38.74	0.21	0.08	**0.15**	0.42	0.22	**1.79**
KF 07	56.82	39.61	0.44	0.07	0.45	0.60	1.40	0.60
KSI 2407A	58.03	39.37	0.20	0.16	0.20	**0.33**	0.30	1.40
ODS-15-013	**55.92**	**40.11**	0.28	0.14	0.42	0.86	1.24	1.03
ODS-15-019	56.51	38.85	0.21	0.13	0.26	0.65	**2.58**	0.82
ODS-15-020	57.39	38.03	0.27	0.46	0.29	0.70	2.45	**0.42**
ODS-15-044	57.88	38.78	0.22	0.06	0.15	0.69	1.29	0.92
ODS-15-045	57.91	38.09	0.31	0.02	0.36	0.82	1.94	0.55
ODS-15-053	58.57	37.32	**0.56**	0.31	**0.59**	**1.17**	0.99	0.50
ODS-15-061	56.36	40.02	0.39	0.55	0.20	0.60	1.31	0.56
ODS-15-100	59.89	36.37	0.17	0.06	0.23	0.57	2.03	0.71
ODS-15-115	57.05	39.16	0.40	0.14	0.23	0.80	1.32	0.89
ODS-15-121	57.46	38.77	0.25	0.35	0.23	0.50	1.16	1.28
TOT1048	58.91	38.18	0.34	0.18	0.21	0.77	0.83	0.59
TOT3527	57.69	39.39	0.36	0.22	0.32	0.72	0.50	0.80
TOT3536	57.31	39.31	0.43	0.06	0.47	0.81	0.63	0.98
TOT4976	58.84	38.41	0.30	0.12	0.28	0.67	0.30	1.09
TOT5799SC	59.47	37.22	0.31	0.38	0.36	0.79	0.55	0.91
TOT6439	61.59	35.84	**0.11**	0.14	0.16	0.41	0.11	1.63
TOT7196	58.70	37.40	0.34	0.05	0.36	0.93	1.61	0.63
TOT7198	60.11	36.36	0.26	0.11	0.34	0.66	1.65	0.50
TOT7200SC	59.96	37.25	0.38	0.10	0.28	0.75	0.66	0.62
TOT7486	57.36	38.92	0.30	0.19	0.43	0.60	0.99	1.21
TOT7505	58.30	38.02	0.34	0.04	0.30	0.87	1.09	1.05
TOT8886	57.50	38.76	0.28	0.09	0.36	0.59	1.40	1.01
TOT8887	**62.82**	**33.39**	0.18	**0.70**	0.39	0.73	0.66	1.13
TOT8926	58.40	38.61	0.36	**0.01**	0.27	0.70	1.06	0.58
Mean	58.50	38.04	0.30	0.20	0.30	0.69	1.05	0.92
SD	1.64	1.48	0.10	0.18	0.11	0.17	0.67	0.35
CV (%)	2.80	3.89	33.33	90.00	36.67	24.64	63.81	38.04
F value	3.60**	3.44***	4.91***	1.47^{ns}	3.068***	6.15***	5.46**	6.73***
LSD	5.02	4.67	0.25	0.86	0.36	0.41	1.68	0.78

SD= Standard Deviation CV= Coefficient of variation, LSD= Least Significant Difference, *** p<0.001; ** p<0.01; ^{ns}= non-significant, C=Carbone, O= Oxygen, Mg=Magnesium, Al= Aluminium, P= Phosphorus, S= Sulphur, K= Potassium, Ca= Calcium. Values in bold indicate minimum and maximum

3.3.4 Variation in seed germination among *G. gynandra* accessions

The analysis of variance (ANOVA) of spider plant mean germination time and percentage germination showed highly significant differences ($p < .001$) among accessions (Table 3-4). The mean germination times ranged from 3 to 5 days with an average of 4 days. Accession ODS-15-013 showed the lowest mean germination time (2.50 days) while accession TOT7200SC showed the highest value (5 days). The spider plant seeds percentage germination after 7 days varied from 18 to 100%. Five Asian accessions obtained 100% germination including TOT1048, TOT3527, TOT6439, TOT7198 and TOT7505. The lowest germination percentage was observed from the east African accession KF07. Moreover, the coefficient of variation was high for the percentage germination (43.57%), which was indicative of implied diversity among the accessions used in this study.

Table 3.4: Mean of the germination parameters of the seed of *Gynandropsis gynandra*

Accessions	Mean germination time (days)	Germination (%)
BAR1807B	4.10	32.26
ELG19/07A	3.71	35.90
HBY/2307b	4.67	23.08
KF07	4.00	**18.18**
KSI2407A	4.86	41.18
ODS-15-013	**2.50**	28.57
ODS-15-019	4.00	37.04
ODS-15-020	3.69	80.77
ODS-15-044	4.28	90.00
ODS-15-045	4.13	42.11
ODS-15-053	3.14	25.00
ODS-15-061	4.90	58.82
ODS-15-100	4.93	87.50
ODS-15-115	2.88	32.00
ODS-15-121	4.75	66.67
TOT1048	4.65	**100.00**
TOT3527	4.42	**100.00**
TOT3536	4.25	84.21
TOT4976	3.91	77.78
TOT5799SC	2.76	93.75
TOT6439	4.96	**100.00**
TOT7196	4.57	82.22
TOT7198	4.88	**100.00**
TOT7200SC	**5.00**	92.31
TOT7486	3.76	91.30
TOT7505	4.18	**100.00**
TOT8886	3.63	72.73
TOT8887	3.55	73.33
TOT8926	2.88	42.11
Mean	4.07	65.82
SD	0.73	28.68
CV (%)	2.70	43.57
F value	1.961e+28***	2.659e+29***
LSD	3.03E-14	3.25E-13

SD= Standard Deviation CV= Coefficient of variation, LSD= Least Significant Difference, *** p<0.001
Values in bold indicate minimum and maximum

3.3.5 Correlation among morphological traits, germination parameters and mineral elements of *G. gynandra* seeds

The Pearson correlation analysis among germination percentage, mean germination time, morphological traits and mineral elements of spider plant revealed highly significant moderate and negative correlations between seed area (-0.52), seed perimeter (-0.58), seed length (-0.53) and 100 seeds weight (-0.58), seed width (-0.45) with magnesium (Table 3.6). Moderate and negative correlations were observed between 100 seed-weight (-0.53), mean germination time (-0.49) and sulphur (Table 3.6). Likewise, significant moderate and negative correlations were also detected between 100 seed-weight (-0.52), seed area (-0.47), seed width (-0.45), mean germination time (-0.44) and phosphorus (Table 3.6). A significant, weak and negative correlation was observed between phosphorus and seed perimeter (-0.37) (Table 3.6). In contrast, a significant moderate and positive correlation was observed between calcium and 100 seeds weight (0.47) (Table 3.6). Non-significant correlations were detected between mineral elements, morphological traits and percentage germination (Table 3.5).

Table 3.5: Pearson correlation analysis between morphological traits, germination parameters and mineral elements of *Gynandropsis gynandra* seeds

Variables	Mg	P	S	Ca	MGT	%germination
Seed area	**-0.52*** **	**-0.47***	-0.26^{ns}	0.33^{ns}	-0.16^{ns}	-0.20^{ns}
Seed perimeter	**-0.58*** **	**-0.37***	-0.17^{ns}	0.32^{ns}	-0.32^{ns}	-0.19^{ns}
Seed width	-**0.47***	**-0.45***	-0.20^{ns}	0.3^{ns}	-0.17 ^{ns}	-0.26 ^{ns}
Seed length	-**0.53*** **	**-0.43***	-0.24^{ns}	0.34^{ns}	-0.18 ^{ns}	-0.14 ^{ns}
100 Seeds weight	-**0.58*** **	-**0.52*** **	-**0.53*** **	0.47*	0.13 ^{ns}	-0.23 ^{ns}
MGT	-**0.29^{ns}**	**-0.44***	**-0.49***	0.11^{ns}	1.00	0.38 ^{ns}
% germination	-**0.19^{ns}**	-0.06^{ns}	0.00^{ns}	-0.10^{ns}	0.38^{ns}	1.00

***p<0.001, ns=non-significant, Mg=Magnesium, P=Phosphorus, S= Sulphur, Ca= Calcium, MGT= Mean germination time, %germination= germination percentage

3.3.6 Principal component and hierarchical cluster analysis of *G. gynandra* accessions

The principal component analysis using morphological traits, germination percentage, mean germination time and mineral elements of spider plant seeds revealed that the three first components explained 70.69% of the total variation. The first principal component axis (PCA1) explained 37.66% of variations and was highly correlated with magnesium, phosphorus, calcium, seed area, seed perimeter, seed width, seed length and seed weight. The second principal component axis (PCA2) explained 18.73% of the variation and was highly correlated

with carbon, potassium, mean germination time and percentage germination. The third principal component axis (PCA3) explained 14.30% and was highly correlated with oxygen, aluminium and sulphur (Table 3.6).

Table 3.6: Correlation between variables and the three first principal components

Variables	Unit	Principal Component		
		1	2	3
Carbon	g/100g	0.27	**-0.73**	0.55
Oxygen	g/100g	-0.28	0.55	**-0.70**
Magnesium	g/100g	**-0.77**	0.37	0.11
Aluminium	g/100g	0.25	-0.11	**0.55**
Phosphorus	g/100g	-0.66	0.29	0.40
Sulphate	g/100g	-0.53	0.39	**0.58**
Potassium	g/100g	-0.06	**0.59**	-0.06
Calcium	g/100g	**0.58**	-0.36	-0.23
Seed area	mm^2	**0.90**	0.36	0.04
Seed Perimeter	mm	**0.87**	0.25	0.36
Seed width	mm	**0.84**	0.39	0.03
Seed length	mm	**0.88**	0.27	0.17
100 Seeds weight	mg	**0.85**	0.19	-0.24
Mean Germination Time		0.05	-**0.62**	-0.52
Percentage of germination	%	-0.13	-**0.52**	0.03

Significant values are indicated in bold

The Hierarchical cluster analysis (HCA), grouped the 29 spider plants accessions into three major clusters (Figure 3.5). The results of the HCA were supported by the dendrogram (Figure 3.6), which separated all accessions into three clusters (I, II and III). The analysis of the dendrogram revealed that accessions were grouped based on their geographical origin. Cluster I was composed of Asian accessions only, while Cluster III consisted of mainly of West African accessions (6) and one East African and South African each. Cluster II was mainly composed of East African accessions (6), four West African accessions and one Asian accession.

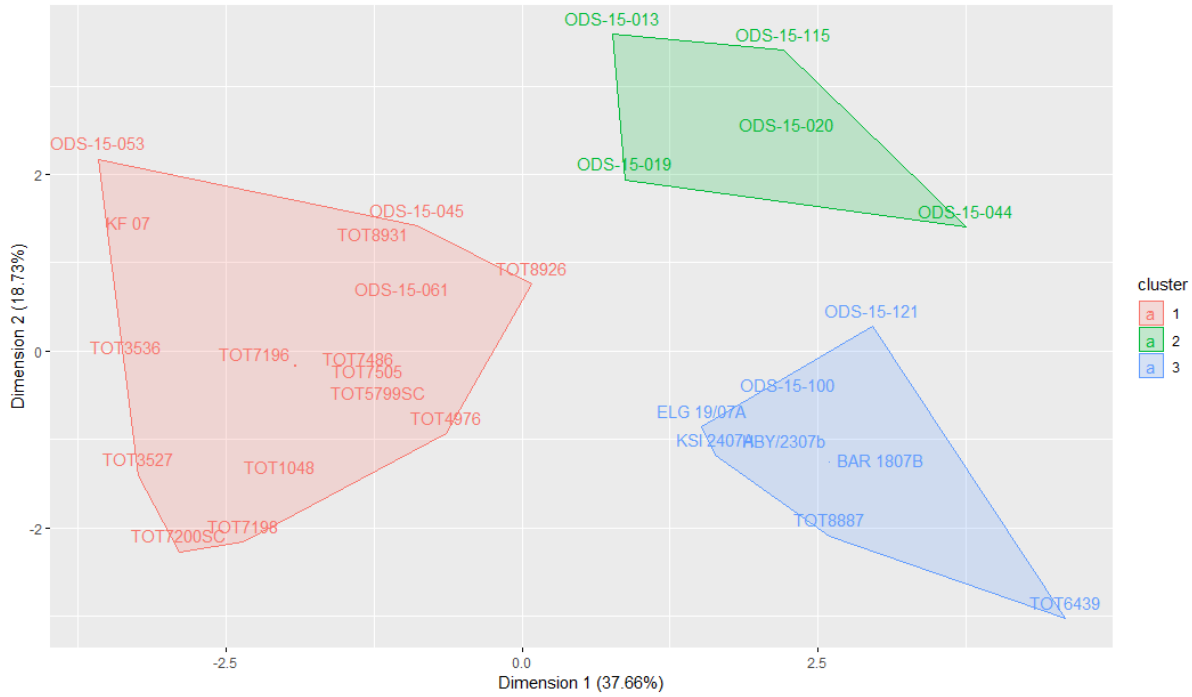

Figure 3.5: Clusters of *Gynandropsis gynandra* accessions

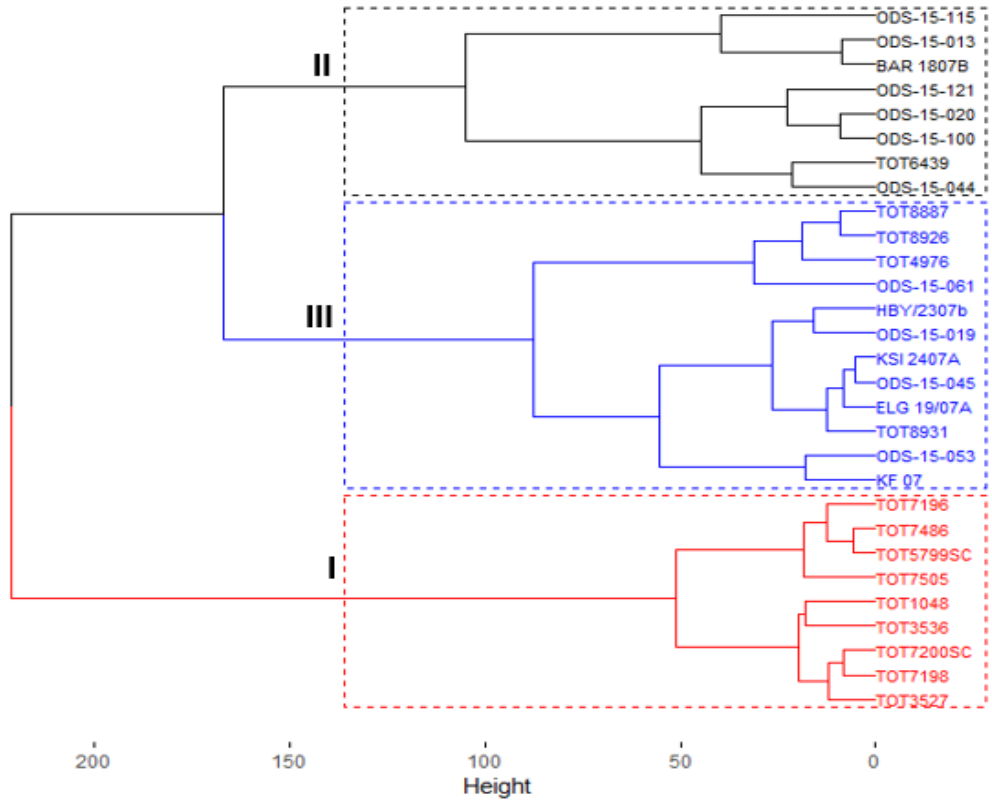

Figure 3.6: Dendrogram of accessions of *Gynandropsis Gynandra*

Furthermore, the clusters were highly separated (overall ANOSIM R= 0.5, Table 3.7) and discriminated by morphological traits (seed area, seed length, seed perimeter, 100 seed weight and seed width), mean germination time and most mineral elements (magnesium, phosphorus, sulphate, calcium, carbon, oxygen, and potassium) (Table 3.8).

Cluster 1 was composed of 55.17% of the spider plant accessions (Table 3.8). The seeds of the accessions of this cluster were characterized by high magnesium (0.36±0.07 g/100g), phosphorus (0.35±0.10 g/100g), and sulphur (0.75±0.15 g/100g) contents. In addition, all accessions of this cluster presented the lowest values for morphological traits including seed area (125.35±16.88), seed perimeter (4.44±0.33), seed width (1.18±0.11), seed length (1.33±0.08) and seed weight. Cluster 2 consisted of 17.24% of spider plant accessions. The seeds had a high oxygen (38.58±1.16) and potassium (1.72±0.61) contents. In contrast to cluster 1, the seeds of accessions in cluster 2 had highest values for all morphological traits including seed area (193.57±23.9), seed perimeter (5.61±0.44), seed width (1.50±0.15), seed length (1.61±0.09) and seed weight (13.33±1.13). In contrast to cluster 1 and cluster 2, cluster 3 grouped 27.58% of accessions with the highest carbon (59.95±1.93) and calcium (1.30±0.33) contents. Cluster 3 was also composed of accessions with highest seed perimeter (5.36±0.37), seed width (1.38±0.07) and mean germination time. No significant differences were observed among clusters regarding the seed germination percentage and seed aluminium content.

Table 3.7: Results of the pair wise analysis of similarity among clusters

Overall test			
Overall R[a]=0.5***			
Clusters	1	2	3
1	0		
2	0.74***	0	
3	0.5***	0.47[ns]	0

[a] Test statistic comparatively measuring the degree of separation of the clusters, ***p<0.001, ns=non-significant

Table 3.8: Description of clusters of *G. gynandra* accessions

Variables	Cluster 1	Cluster 2	Cluster 3	F value
	n=16	n=5	n=8	
Carbon	58.26 ±1.07^{b}	56.95±0.76^{b}	**59.95±1.93^{a}**	8.47**
Oxygen	**38.30 ±1.02^{a}**	**38.99±0.76^{a}**	36.91±2.01^{b}	4.45*
Magnesium	**0.36±0.07^{a}**	0.28±0.08^{b}	0.20±0.05^{c}	13.69***
Aluminium	0.16±0.15	0.19±0.16	0.29±0.23	1.65^{ns}
Phosphorus	**0.35±0.10^{a}**	0.2±0.1^{ab}	0.22±0.08^{b}	5.73**
Sulphate	**0.75±0.15^{a}**	**0.74±0.09^{a}**	0.53±0.17^{b}	6.21* *
Potassium	1.06±0.47^{b}	**1.78±0.67^{a}**	0.59±0.68^{b}	6.69**
Calcium	0.76±0.24^{b}	0.82±0.27^{b}	**1.30±0.33^{a}**	11.23***
Seed area	125.35±16.88^{c}	**193.57±23.9^{a}**	169.97±19.7^{b}	31.39***
Seed perimeter	4.44±0.33^{b}	**5.61±0.44^{a}**	5.36±0.37^{a}	29.49***
Seed width	1.18±0.11^{b}	**1.50±0.15^{a}**	1.38±0.07^{a}	20.3***
Seed length	1.33±0.08^{c}	**1.61±0.09^{a}**	1.54±0.12^{b}	26.64***
100 seeds weight	8.16±1.78^{b}	**13.33±1.13^{a}**	13.17±2.3^{a}	26.8***
Mean germination time	**4.07±0.7a^{b}**	3.47±0.75^{a}	**4.44±0.57^{b}**	3.19***
Percentage of germination	73.78±27.93	53.68±29.28	57.49±28.27	1.45^{ns}

*** p<0.001; ** p<0.01; * p<0.05; ns= non-significant. Values in bold indicate the cluster in which each variable was high

3.4 Discussion

The morphological characteristics of the seeds are relevant for taxonomic identification of plant species and the assessment of the existing genetic diversity within crop species (Adewale et al. 2010; Daryono and Sentori 2015). This study investigated seed morphology, mineral composition and germination of twenty-nine *Gynandropsis gynandra* accessions from West East and Southern Africa, and Asia. Based on the results of this study, morphological traits including seed area, seed perimeter, seed length, seed width and 100 seeds weight were significantly different among accessions of spider plant. This revealed that seed traits could be incorporated into breeding programmes for an effective improvement of *Gynandropsis gynandra* as reported by Mohammed et al. (2016) on Bambara groundnut (*Vigna subterranea* [L.] Verdc.).

The results showed significant differences among the accessions with respect to germination (%) and the mean germination time. In addition, the coefficient of variation was high (43.57%) for germination (%), thus suggesting the existence of genetic diversity among accessions. The Asian accessions showed higher germination (>80%), while the East African and most West

African accessions revealed low germination (<50%). The results suggest that the lower germination observed in the East and West African accessions could be dormancy related as opposed to the Asian accessions, which showed relatively higher germination percentages. Baskin and Baskin (2014) explained that in general the number of plant species that may acquire dormancy tend to increase with geographical distance from the equator and correlates with the occurrence of seasons. Dormancy variation can also be found within plant species. In Arabidopsis species (*Arabidopsis thaliana*) it has been observed that accessions from southern Europe and parts of Asia show a tendency for higher seed dormancy levels compared with accessions from northern Europe (Debieu et al. 2013). In our case, spider plant accessions from Asia show lower dormancy levels compared with accessions from Africa which is in contrast with the explanation given by Baskin and Baskin (2014).

Genetic variation in dormancy has been studied in many species by crossing genotypes with different dormancy levels, followed by the analysis of their progeny and parallel selection for seed dormancy (Lin et al. 1998; Alonso-Blanco et al. 2003; Wang et al. 2018). This finding implies that seed dormancy and related problems in seed germination of *Gynandropsis gynandra* could be improved through crosses among accessions from different regions (e.g. Asian, eastern and western African accessions) and thus improving seed germination.

The study established the presence of eight (8) mineral elements, including carbon (C), oxygen (O), magnesium (Mg), aluminium (Al), phosphorus (P), sulphur (S), potassium (K) and calcium (Ca) in the seeds of *G. gynandra* accessions. One hundred gram (100 g) of dry mature seeds of *G. gynandra* contains on average 58.00 g of carbon, 38.04 g of oxygen, 0.30 g of magnesium, 0.20 g of aluminium, 0.30 g of phosphorus, 0.69 g of sulphate, 1.05 g of potassium and 0.92 g of calcium. These amounts are generally higher compared to those in *Amaranth* grain which contains calcium (0.0783 to 1.0046 g), iron (0.00361 to 0.02251 g), magnesium (0.04431 to 0.09738 g), potassium (0.2678 to 0.4736 g) and zinc (0.00053 to 0.00123 g) (Kachiguma et al. 2015).

The analysis of association patterns among morphological seed traits, mineral elements and germination parameters revealed significant negative correlations between seed size (area, length, perimeter, and width), 100 seeds weight and mean germination time with phosphorus. Seeds with high phosphorus content showed a reduced mean germination time, thus suggesting that P plays an important role during germination. Based on the results in this study, the hypothesis that larger seeds germinate faster/ better than smaller seeds is rejected because no correlation was observed between seed size germination and mean germination time. However, despite the fact that no correlations between seed size and germination were observed, the Asian accessions which had the smallest seed size germinated better than

those from East and West Africa. Similar results were reported in pea (*Pisum sativum* L.) where seed with low 100 seeds weight showed higher germination percentage than those with higher values for 100 seed weight (Peksen et al. 2004).

Phenotypic diversity assessment is important to depict the extend of genetic diversity within crop species for the development and deployment of improved varieties with farmers' desired traits (Govindaraj et al. 2015; Fu 2015). Our results, showed three clusters of accessions highly discriminated by seed parameters including seed area, seed length, seed perimeter, seed width, 100 seeds weight, mean germination time, magnesium, phosphorus, sulphate, calcium, carbon, oxygen, and potassium. The clustering was also explained by the geographical origin of accessions as reported by Wasonga et al. (2015) who used 32 accessions from Kenya and South Africa to investigate the diversity within the species. Cluster 1 grouped accessions from Asia, which were characterized by high oxygen, magnesium, phosphorus, sulphate contents and mean germination with lowest morphology traits parameters including seed area, seed length, seed perimeter, seed width, and 100 seeds weight. Cluster 2 was composed of western and eastern African accessions, characterized by high oxygen, sulphate, potassium and high seed size parameters namely seed area, seed length, seed perimeter, seed width, and 100 seeds weight and lowest mean germination time. Cluster 3 grouped Asian, western Africa, eastern Africa and southern Africa accessions and were characterized by high carbon content, calcium content and mean germination with lowest phosphorus content. The genetic diversity observed among *G. gynandra* accessions for seed metric parameters, seed germination and mineral contents can be used to improve the germination capacity of the species as well as the yield of the species.

3.5 Conclusion

This study has generated useful information with regard to the internal and external seed morphology as well as mineral composition of *Gynandropsis gynandra* seeds. Seed mineral composition except for aluminum differed significantly among different accessions. The relatively high-level of dissimilarity observed among clusters, and especially among accessions from different geographical areas provides a basis for the identification of desirable parents. These parents can be used to create segregating populations for the genetic improvement of the crop. The diversity observed among spider plant accessions using seed attributes was also an indicator that a systematic selection of spider plant accessions into homogenous group of seed could be done for an effective breeding to boost seed quality, crop productivity and nutritional security.

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CHAPTER 4

GERMINATION AND DORMANCY IN SEEDS OF FIVE *GYNANDROPSIS GYNANDRA* (L.) BRIQ GENOTYPES AND THEIR CROSSES

Abstract

Gynandropsis gynandra (spider plant) adoption in production systems is limited by several constraints including poor seed germination and a poor understanding of dormancy traits in the species. Fifteen (15) genotypes of spider plant were used in this study to understand the variation in seed dormancy in the species and identify proper dormancy breaking methods. Firstly, seeds were planted using a randomized complete block design (RCBD) in pots in a tunnel and data were recorded biweekly during the development until maturity on the following variables: fresh seed and dry mass, germination percentage and electrical conductivity (EC). Secondly, freshly harvested mature seeds were subjected to 41°C heating for three days, 0.01% of GA3 and 0.1% of KNO3. The number of germinated seeds was recorded as percentage of germination and the data were subjected to analysis of variance. The results showed that the germination capacity of spider plant seeds varied from one genotype to another. Spider plant seeds matured when the pods were yellow or brown in colour, and the seeds were black or brown depending of the genotype. The species was dormant with the level of dormancy varying from weak (germination percentage between 50% and 80%), intermediate (germination percentage between 6% and 50%) and strong dormancy (germination percentage <6%). The effect of pre-treatments was also highly genotype dependent, with dormancy of one (01) genotype (ELG1907A) broken by GA3, dormancy of nine (09) genotypes (including ODS-15-020) broken by heating and dormancy of three genotypes broken by both GA3 and heating. Potassium nitrate was not able to break spider plant dormancy; in contrast, it inhibited the germination capacity of the species. It is concluded that the spider plant exhibits a physiological dormancy, which is broken by warm stratification and gibberellic acid.

Key words: dormancy, germination, gibberellic acid, *Gynandropsis gynandra*, pre-treatments

4.1 Introduction

Seed is a primary input in crop production and its quality affects germination, vigour, seedling emergence and consequently plant population and yield. Seed germination requires water, oxygen and suitable temperature. Seeds that germinate when these three requirements are satisfied are said to be germination ripe (Thomson 1979). However, when a newly ripe seed fails to germinate under suitable conditions, it is said to be in a state of dormancy. The duration of the state of dormancy is extremely variable. Seeds may also not germinate because of some inherent properties and thus, the lack of germination is a seed rather than an environmental problem (Eira and Caldas 2000). In some cases, an after ripening process occurs at the end, of which the dormancy disappears and the seed germinates freely under suitable conditions for normal growth. The terms seed germination and dormancy have been misunderstood, for example many times seeds that have not germinated were wrongly referred to as dormant (Baskin and Baskin 2014).

The two main categories of seed dormancy are primary and secondary dormancy (Baskin et al. 1998). Primary dormancy is induced during seed maturation of the mother plant (Shilla et al. 2016) and is divided into three groups as follows: (i) exogenous (physical, chemical and mechanical), (ii) endogenous (morphological and physiological) and (iii) combinational (morphophysiological and exo-endodormancy). Secondary dormancy is an adaptation for the prevention of seed germination when environmental conditions are not favourable for seedling growth and refers to imposition of a new dormancy mechanism in seed that have overcome primary dormancy (Geneve 1998). Several methods requiring certain conditions can be used to break seed dormancy. These include scarification, cold stratification, leaching seed, warm stratification, chemicals (GA3, KNO3, etc), chilling stratification, red light, darkness, storage periods and conditions, and sometimes the combination of one or more of those conditions.

Low and non-uniformity in germination has been reported in *Gynandropsis gynandra* L. (Briq) (Chweya and Mnzava 1997). The seeds of the species are orthodox (Kamotho et al. 2014) and may be non-dormant, quiescent or dormant. Several studies have been done to improve the germination capacity of the species (Ochuodho 2005; Muasya et al. 2009; Kamotho et al. 2014). The poor germination of the species was attributed to the fact that the species is dormant without a preliminary study of the dormancy of the species. Germination studies on *Gynandropsis gynandra* focused much more on the effect of breaking dormancy methods on the germination. 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, several contradictions still exist (Shilla et al. 2016). Therefore, it is important to extend the seed germination studies to

dormancy related studies where the dormancy of species will be properly elucidated and to use a larger sample size (number of genotypes) to test the effect of dormancy breaking methods on the germination capacity of the species. The objectives of this study were: (1) to investigate the pattern of seed development in spider plant and identify the critical stage for seed maturity and harvesting, (2) to document the variability in seed dormancy and identify the type of dormancy in *G. gynandra*, and (3) to evaluate different dormancy breaking methods (heating, gibberellic acid and potassium nitrate) that promote fresh seed germination in *G. gynandra*. It was hypothesized that (1) spider plant seeds are dormant and dormancy is genotype dependent, and (2) the effect of preheating to break dormancy in spider plant is also genotype dependant

4.2 Material and methods

4.2.1 Plant material

Fifteen genotypes of *Gynandropsis gynandra* seeds composed of five pure lines and ten hybrids were used in this experiment (Table 4.1). The seeds were obtained from the initial experiment where crossing between five spider plants accession without their reciprocal was made at the experimental site of the University of KwaZulu-Natal under greenhouse conditions. The freshly harvested seeds were stored for 2 months prior to the experiment. The experiment was divided into two parts, namely field and laboratory experiment.

Table 4.1: List of inbred lines and hybrids used in this study

Genotypes	Accessions name		Category
	Female	Male	
TOT8887	TOT8887	TOT8887	Inbred line
TOT5799SC	TOT5799SC	TOT5799SC	Inbred line
ODS-15-020	ODS-15-020	ODS-15-020	Inbred line
ELG1907A	ELG1907A	ELG1907A	Inbred line
BAR1807B	BAR1807B	BAR1807B	Inbred line
TOT8887 X TOT5799SC	TOT8887	TOT5799SC	Hybrid
TOT8887 X ODS-15-020	TOT8887	ODS-15-020	Hybrid
TOT8887X ELG1907A	TOT8887	ELG1907A	Hybrid
TOT8887X BAR1807B	TOT8887	BAR1807B	Hybrid
TOT5799SC X ODS-15-020	TOT5799SC	ODS-15-020	Hybrid
TOT5799SC X ELG1907A	TOT5799SC	ELG1907A	Hybrid
TOT5799SC X BAR1807B	TOT5799SC	ELG1907A	Hybrid
ODS-15-020 X ELG1907A	ODS-15-020	BAR1807B	Hybrid

Genotypes	Accessions name		Category
	Female	Male	
ODS-15-020 X BAR1807B	ODS-15-020	ELG1907A	Hybrid
ELG1907A X BAR1807B	BAR1807B	ELG1907A	Hybrid

(i) Field experiment

The seeds of the 15 spider plant genotypes were sown in a nursery three week before transplanting in pots in the greenhouse. Seedlings of the genotypes were planted in a randomized complete block design (RCBD) with five replications from 15th October to 20th December 2018. One (01) plant was transplanted per pod and five (05) plants were transplanted per genotype. Seeds were harvested at three developmental stages as follows: (R1) two weeks after bud appearance, (R2) four weeks after bud appearance and (R3) six weeks after bud appearance. At each developmental stage, data was collected on pod colour, seed colour, seed moisture content and percentage of germination.

(ii) Laboratory tests

Freshly mature harvested seed (R3) from the field experiment were dried for five days at room temperature and used in the following tests at the Seed Technology laboratory, University of KwaZulu-Natal, Pietermaritzburg.

Electrical conductivity: Twenty seeds were soaked in 40 ml of distilled water for 24 hours in a room set to a constant temperature of 25°C. After 24 hours of soaking, the conductivity of the soak solution was immediately tested with OHAUS Starter 3100C Conductivity Meter and reading were expressed in $\mu S.cm^{-1}$ seed. Four runs of 10 seeds were tested for each treatment.

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 balance Model PA114C, AC/DC input 230 V AC, universal plug set. The moisture content was calculated using the following formula.

$$MC = \frac{Loss\ of\ weight}{initial\ weight} x100 = \frac{(M1 - M2)}{M1} x100$$

M1 is the weight in grams of the seed before drying and M2 is the weight in grams of the seeds after drying.

Imbibition test: To assess the imbibition behavior of spider plant seed the weight of one seed (5 seeds per treatment) replicated four times were taken for each treatment at 0, 2, 4, 6, 8, 10, 12, 24 and 48 hours. For each treatment, two filter papers were put in petri dishes and moistened with 7 ml of distilled water. After each weight measurement, 4 ml of water were added to make sure that water was sufficient in the petri dishes.

Germination test: For each genotype, 100 untreated seeds were divided into four replicates of 25 seeds and were used as control for the germination test. The seeds were placed on top of paper towels moistened with distilled water and kept at 28°C in an incubator. Water was added as needed. Germination counts were taken every day for 14 days.

Pre-germination treatments: The freshly harvested mature and dried seeds in room temperature (25°C) for five days were subjected to various treatments as follows:

- **heating:** Fresh seeds were put in an oven set at 40°C for 3 days and thereafter seeds were placed under ambient conditions for 24 hours before being tested for the germination.

- **potassium nitrate:** seeds were soaked in 0.1% of potassium nitrate for 24 hours and then subjected to the germination test

- **gibberellic acid:** seeds were soaked in a GA3 solution at concentration of 0.01% for twelve hours and washed in distilled water before performing the germination test.

4.2.2 Data analysis

Analysis of variance (ANOVA) was performed on seed moisture content, electrical conductivity and percentage of germination. The Student-Newman-Keuls (SNK) test was conducted on the ANOVA results to separate mean values of pre-treatments using the R package “Agricolae” (de Mendiburu and de Mendiburu 2017). Histograms were constructed to present the distribution of seed and pod colours at different development stages using the R package “ggplot2” (Wickham 2010). A bar line was used to draw the imbibition curves for each genotype using the function “ggarrange” of the R Package “ggpbur” (Kassambara 2017). All data were analysed using R software version 3.5.1 (Crawley 2012).

4.3 Results

4.3.1 Seed and pod colours during *G. gynandra* development

The development of the pod from the flower bud appearance (0 week) to pod maturation (4 to 6 weeks) is presented in Figure 4.1. Pod size increased from the appearance of the flower

bud (0 weeks) to maturation (between 4 and 6 weeks after the appearance of the flower bud). Seed formation actually began two weeks (R1) after flower bud emergence and hand pollination.

Figure 4.1: Image of *Gynandraopsis gynandra* pod development from flower bud appearance to pod maturity.

Pod development was also reflected by a change in pod colour. Two weeks after the flower bud appearance (R1), the pods of all spider plant genotypes used in this study were green (100%). Four weeks after (R2), 69% of pods turned yellow-green while 31% turned yellow. At the maturity stage R3 (6 weeks after flower bud development), 38% of pods turned brown while 62% were yellow (Figure 4.2). Figure 4.3 presents the different shade of pod colour of the species during seed development.

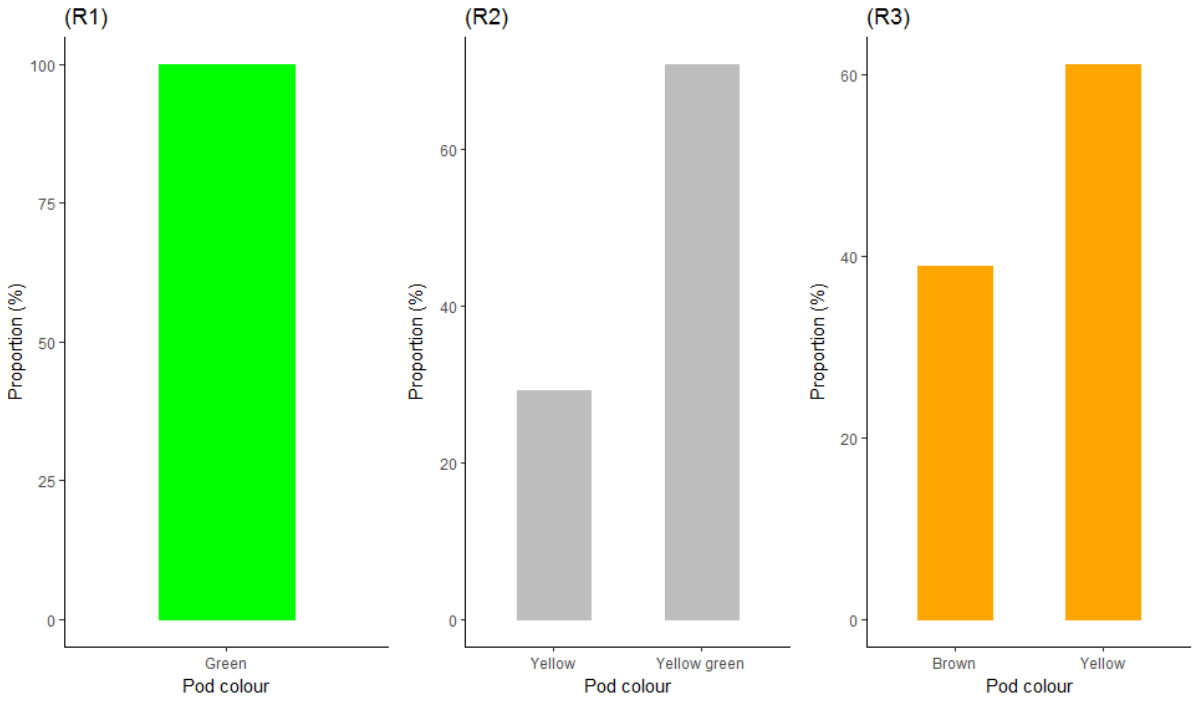

Figure 4.2: *Gynandropsis gynandra* genotypes distribution by pod colour and development stage : R1 = 2 weeks after flower bud appearance; R2 = 4 weeks after flower bud appearance, R3 = 6 weeks after flower bud appearance

Figure 4.3: Images of *Gynandropsis gynandra* pod shade colour during development

Spider plant seed colour is also an important parameter for judging the seed maturity of the species. Two weeks after the appearance of the flower bud, all genotypes showed green colour. Two weeks later (R2), the spider plant seed colour was green black for all the genotypes. At maturity stage, that is, 6 weeks after the appearance of the flower bud, 58% of

the genotypes had a brown seed colour while 42% had a black colour (Figure 4.4). Figure 4.5 presents the different shades of pod colour of the species during seed development.

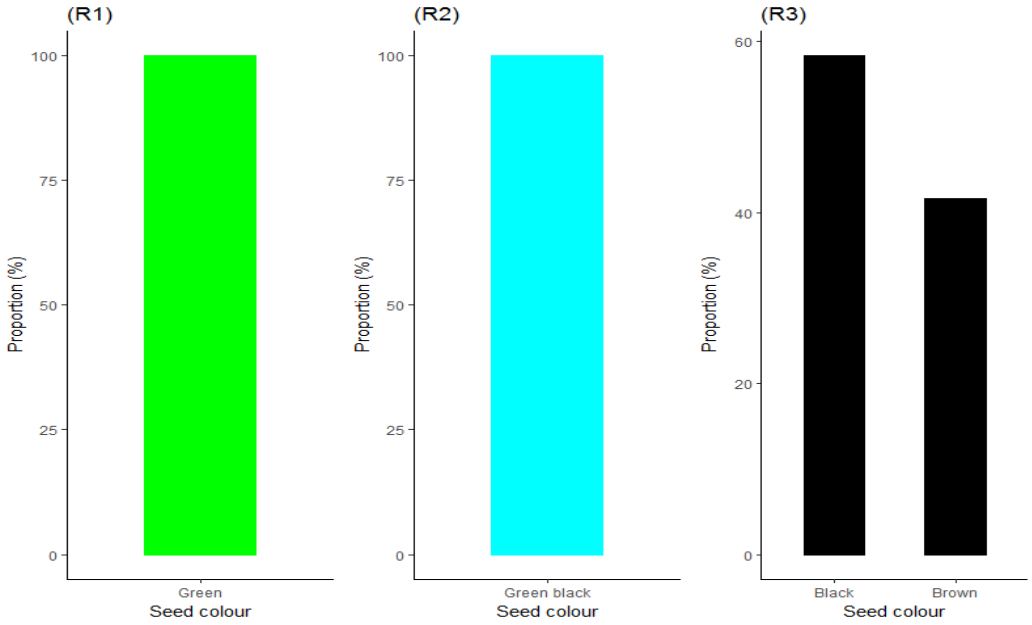

Figure 4.4: *Gynandropsis gynandra* genotypes distribution by seed colour and development stage: R1 = 2 weeks after flower bud appearance; R2 = 4 weeks after flower bud appearance, R3 = 6 weeks after flower bud appearance

Figure 4.5: Images of *Gynandropsis gynandra* seed shade colour during development

4.3.2 Seed moisture content, electrical conductivity and germination percentage in *Gynandropsis gynandra* during seed development

a) Moisture content

Data on spider plant seed moisture content at different development stages (R1, R2 and R3) are summarized in Table 4.2. Seed moisture content decreased with time. Two weeks (R1) after the flower bud appearance the seed moisture content ranged from 74.36±7.54% to 86.38±2.74% with an average of 80.39%. The crosses between accessions TOT8887 and ODS-15-020 showed the lowest value (74.36%), while accession ELG1907A showed the

highest value. At development stage R2 (4 weeks after bud appearance), the seed moisture content ranged from 34.67±6.80% to 58.73±7.38% with an average of 45.68%. Accession BAR1807B had the lowest seed moisture content (34.67%) and accession TOT5799 had the highest moisture content (58.73%). At maturity stage (R3), seed moisture content continued to decrease up to 18.69% on average. At this stage the seed moisture content ranged between 10.47±4.33% to 25.55±5.13% with the lowest value obtained by the cross between TOT5799SC X ODS-15-020 and the highest value obtained by accession ELG1907A. These data revealed that spider plant seeds at maturity stage, still need to be dried to reduce the moisture content to about 5% as recommended by Kamotho et al. (2014) to facilitate germination and storage.

Table 4.2: Mean and standard deviation of *G. gynandra* seed moisture content at different developmental stage

Genotypes	Seed moisture content (%)		
	R1	R2	R3
TOT8887	83.66±5.16	49.66±6.57	23.25±9.36
TOT5799SC	80.32±1.55	58.73±7.38	12.25±4.4
ODS-15-020	81.08±2.28	48.85±12.73	15.22±3.26
ELG1907A	86.38±2.74	55.68±7.30	25.55±5.13
BAR1807B	78.51±17.48	34.67±6.80	21.83±2.74
TOT8887 X TOT5799SC	84.61±8.42	42.04±5.19	14.11±2.30
TOT8887 X ODS-15-020	76.14±5.12	40.8±10.45	12.48±2.02
TOT8887 X ELG1907A	74.36±7.54	43.49±1.76	18.64±5.82
TOT8887 X BAR1807B	78.1±5.22	45.64±8.55	22.8±1.87
TOT5799SC X ODS-15-020	80.34±4.29	39.81±3.66	10.47±4.33
TOT5799SC X ELG1907A	77.77±5.81	45.9±5.81	21.77±9.37
ODS-15-020 X ELG1907A	80.17±10.60	44.53±7.01	20.76±2.29
ELG1907A X BAR1807B	81.84±5.37	43.23±7.78	21.69±071
TOT5799SC X BAR1807B	77.98±6.21	48.67±3.10	24.55±9.80
ODS-15-020 X BAR1807B	84.65±3.07	43.44±4.26	14.98±6.10
Overall mean	80.39	45.68	18.69
CV (%)	4.22	12.83	25.70

CV= Coefficient of variation; R1 = 2 weeks after flower bud appearance; R2 = 4 weeks after flower bud appearance, R3 = 6 weeks after flower bud appearance

b) Electrical conductivity

The results of spider plant seed electrical conductivity during the three development stages are presented in Table 4.3. Electrical conductivity was high two weeks after flower bud appearance (R1) for all genotypes compared to four weeks (R2) and 6 weeks later (R3). The seed electrical conductivity 2 weeks after flower bud appearance (R1) ranged from 22.99±11.65 µS.cm $^{-1}$ to 79.46±28.69 µS.cm $^{-1}$ with an average of 46.89 µS.cm $^{-1}$. The cross

between accessions TOT5799SC X ELG1907A showed the lowest electrical conductivity value and the cross between TOT8887 X BAR1807B the highest value. Electrical conductivity four weeks after flower bud appearance ranged from 7.44±1.84 µS.cm^{-1} to 20.39±26.83 µS.cm $^{-1}$. At this stage (R2), the cross between TOT8887 X BAR1807B with the highest value at the stage R1 showed lower electrical conductivity (7.44±1.84 µS.cm $^{-1}$) than accession ELG1907A which was higher (20.43±6.73 µS.cm-^{1}). Six weeks after flower bud appearance (R3), the seed electrical conductivity ranged from 7.23±0.89 µS.cm $^{-1}$ to 26.92±1.33 µS.cm $^{-1}$. The spider plant seed's electrical conductivity four weeks after flower bud appearance (R2) is lower to the spider plant electrical conductivity six weeks later (R3) for all the genotypes except for accession ELG 1907A (20.43±6.73 µS.cm $^{-1}$ and 13.78±7.22 µS.cm $^{-1}$ respectively) and the cross between TOT5799SC X ELG1907A (10.99±7.82 µS.cm $^{-1}$ and 7.23±0.89 µS.cm^{-1} respectively). The average seed electrical conductivity four weeks (R2) after flower bud appearance was 11.78 µS.cm $^{-1}$, while the average seed electrical conductivity 6 weeks (R3) after flower bud appearance was 19.71 µS.cm $^{-1}$.

Table 4.3: Mean and standard deviation of *G. gynandra* seed electrical conductivity at different development stage

Genotypes	Electrical Conductivity (µS.cm $^{-1}$)		
	R1	**R2**	**R3**
TOT8887	33.56±13.28	11.01±3.10	22.57±4.24
TOT5799SC	44.54±15.42	9.63±2.43	12.20±5.30
ODS-15-020	51.78±21.46	20.39±26.83	25.22±5.29
ELG1907A	56.22±13.54	20.43±6.73	13.78±7.22
BAR1807B	31.93±29.35	9.06±0.47	20.67±1.46
TOT8887 X TOT5799SC	28.65±11.82	9.39±2.25	23.68±1.88
TOT8887 X ODS-15-020	26.48±1.88	10.13±3.28	21.75±4.59
TOT8887 X ELG1907A	73.46±15.93	16.24±14.02	18.91±1.68
TOT8887 X BAR1807B	79.46±28.69	7.44±1.84	18.49±2.37
TOT5799SC X ODS-15-020	69.7±24.90	15.97±7.28	25.08±1.17
TOT5799SC X ELG1907A	22.99±11.65	10.99±7.82	7.23±0.89
ODS-15-020 X ELG1907A	38.37±3.19	8.78±1.04	17.71±1.45
ELG1907A X BAR1807B	53.8±14.55	6.22±0.60	19.53±1.50
TOT5799SC X BAR1807B	50.84±36.86	9.32±2.69	21.97±6.27
ODS-15-020 X BAR1807B	41.6±9.32	11.79±2.93	26.92±1.33
Overall mean	46.89	11.78	19.71
CV (%)	37.33	37.47	27.07

CV= Coefficient of variation; R1 = 2 weeks after flower bud appearance; R2 = 4 weeks after flower bud appearance, R3 = 6 weeks after flower bud appearance

c) Percentage of germination

The germination percentage of spider plant during development is summarized in Table 4.4. No germination (0%) were recorded two weeks after flower bud appearance (R1) for all spider plant genotypes in this study. At development stage 2 and 3 the germination percentage of spider plant seeds varied among genotypes. Two weeks after flower bud appearance (R2), the germination percentage of spider plant seed ranged from 0.00% to 54±17.80% with an average of 14%. The cross between TOT8887 X BAR1807B showed the highest germination percentage (34±17.80%) and accession TOT8887, BAR1807B, cross TOT8887 X TOT5799SC and cross TOT5799SC X BAR1807B showed the lowest value (0%). At the maturity stage (R3), the germination percentage ranged from 0% to 34±9.63% with an average of 11%. A decrease in the percentage of germination was observed for some spider plant genotypes between development stage 2 and 3. These include TOT8887 X ODS-15-020 (21±18.42% R2 and 0.00% R3), TOT8887 X ELG1907A (30±11.45% R2 and 4.00±8.94% R3), TOT8887 X BAR1807B (34±17.80% R2 and 2±2.19% R3) ODS-15-020 X ELG1907A (26.00±16.33% R2 and 0.00% R3) and ELG1907A X BAR1807B (35±5.21% R2 and 8.00±1.79% R3). In contrast, the germination percentage of some genotypes increased between 4 weeks after flower bud appearance and 6 weeks after bud appearance. These include TOT8887 (0.00% R2 and 2±2.19% R3), TOT5799SC (18±9.20% R2 and 62±33.30% R3), ODS-15-020 (8.00±2.83% R2 and 33.60±9.63% R3) and TOT5799SC X ODS-15-020 (0.00% and 10±4.56%).

At the maturity stage 3 (R3), spider plant genotypes were classified into 3 groups according to their percentage germination as follows:

Group 1 with percentage of germination less than 6% included 10 genotypes: TOT8887 (1.60±2.19%) , ELG1907A (3.20±3.35%), BAR180B (0.00%), TOT8887 X TOT5799SC (1.60±2.19%), TOT8887 X ODS-15-020 (0.00%), TOT8887 X ELG1907A (4.00±8.94%), TOT8887 X BAR1807B (1.60±2.19%), ODS-15-020 X ELG1907A (0.00%) , TOT5799SC X BAR1807B (3.20±3.35%) and ODS-15-020 X BAR1807B (4.80±1.79%).

Group 2 with germination percentage between 6% and 50%, included ODS-15-020 (33.60±9.63%), TOT5799SC X ODS-15-020 (42.40±15.39%), TOT5799SC X ELG1907A (10.00.4±4.56%), and ELG1907A X BAR1807 (8.00±1.79%).

Group 3 with germination percentage higher than 50% had only genotype TOT5799SC (62.40 ± 33.30%).

Table 4.4: Mean and standard deviation of *G. gynandra* seed percentage germination at different development stage

Genotypes	Percentage germination (%)		
	R1	R2	R3
TOT8887	0.00	0.00	01.60±02.19
TOT5799SC	0.00	17.60±09.20	**62.40±33.30**
ODS-15-020	0.00	08.00±02.83	**33.60±9.63**
ELG1907A	0.00	08.00±01.79	03.20±03.35
BAR1807B	0.00	0.00	0.00
TOT8887 X TOT5799SC	0.00	02.40±03.58	01.60±02.19
TOT8887 X ODS-15-020	0.00	**20.80±18.42**	0.00
TOT8887 X ELG1907A	0.00	**29.20±11.45**	4.00±8.94
TOT8887 X BAR1807B	0.00	**33.60±17.80**	1.60±2.19
TOT5799SC X ODS-15-020	0.00	24.80±7.15	**42.40±15.39**
TOT5799SC X ELG1907A	0.00	0.00	10.00.4±4.56
ODS-15-020 X ELG1907A	0.00	**26.00±16.33**	0.00
ELG1907A X BAR1807B	0.00	**35.20±5.21**	8.00±1.79
TOT5799SC X BAR1807B	0.00	0.00	3.20±3.35
ODS-15-020 X BAR1807B	0.00	4.80±3.35	4.80±1.79
Mean	0.00	13.55	11.30
CV (%)	-	115.63	162.51

CV= Coefficient of variation; R1 = 2 weeks after flower bud appearance; R2 = 4 weeks after flower bud appearance, R3 = 6 weeks after flower bud appearance

d) Analysis of variance (ANOVA) of *Gynandropsis gynandra* seed moisture content, electrical conductivity and germination percentage

The analysis of variance showed a highly significant difference ($p<0.001$) between spider plant genotypes and developmental stage for moisture content, electrical conductivity and germination percentage (Table 4.5). The interaction between genotypes and development stages was also highly significant ($p< 0.001$).

Table 4.5: Mean and standard deviation of *G. gynandra* seed percentage germination at different development stage

Source of variation	Df	MC		EC		% germination	
		MS	F value	MS	F value	MS	F value
Genotypes	14	164	3.87***	694	4.27***	982	15.25***
Development stage	2	69587	1642.6***	25236	155.36***	4015	62.38***
Genotypes* R stage	28	97	2.28***	512	3.15***	942	14.64***
Residual	172	42		162		64	

*** p<0.001; Genotypes* R stage = interaction between genotypes and development stage; Df = Degree of freedom; MS = Mean Square, MC = Moisture Content, EC = Electrical conductivity

4.3.3 *Gynandropsis gynandra* seed imbibition and embryo morphology

The seed images of *Gynandropsis gynandra* revealed that the embryo is fully developed with the presence of cotyledons and radicle, and relatively small amount of endosperm (Figure 4.6).

Figure 4.6: *Gynandropsis gynandra* embryo seed morphology showing the presence of embryo 6 weeks after bud appearance (R3) (scale 200 µm)

The imbibition curves of the 15 spider plant genotypes data showed a substantial increase in seed mass for all the genotypes (Figure 4.7). All spider plant genotypes started to imbibe water two hours after imbibition. Genotypes ODS-15-020 X TOT8887 and ODS- 15-020 X BAR1807B showed the highest initial mass 1.78 g and 1.66 g respectively with a final mass of 2.88 g and 2.66 g respectively after 48 h of imbibition in distilled water. In contrast, Genotypes TOT5799SC and ELG1907A X BAR1807B had the lowest initial seed mass 1 g and 1.15 g respectively with a final seed mass of 1.75 g and 2.26 g respectively. The average increase in seed mass is equal to 0.99 g for all seeds after 48 h of imbibition with the highest increase in mass obtained by the genotype ELG1907A (1.18 g).

4.3.4 Effect of breaking dormancy methods on *Gynandropsis gynandra* germination percentage

The analysis of variances showed a highly significant difference between the effect of the three dormancy breaking pre-treatments ($p<0.001$). In addition, the different genotypes responded differently to the three breaking dormancy methods (Table 4.6). Heating for 3 days at 41°C improved the germination capacity for the majority of spider genotypes including the self-pollinated accessions. For example, germination capacity for the genotype ODS-15-020 ranged from 23.00±3.83 to 79.00±14.37. The genotypes BAR1807B and the cross TOT8887 X ODS-15-020 ranged from 0.00 to 20.00±3.26 and 7.00±3.83b to 22.00±8.3a respectively.

Similarly, the crosses TOT8887 X ELG1907A, TOT8887 X BAR1807B, TOT5799SC X ODS-15-020 ranged between 16.00±3.26b and 29.00±3.83a, 15.00±8.87b to 48.00±11.77a and 3.00±2.00b to 27.00±5.03a) respectively. The range for the crosses, TOT5799SC X ELG1907A, ELG1907A X BAR1807B, ODS-15-020 X BAR1807B were from 4.00±0.00c to 21.00±3.82a, 6.00±4.00b to 18.00±5.16a and from 11.00±6.00c to 40.00±6.53a, respectively). Gibberellic Acid (GA) was able to improve the germination capacity of the self-pollinated of accession ELG1907A from 0.00 to 68.00±10.33a. No difference was observed between the effect of heating and GA on four spider plant accessions including self-pollinated accessions TOT8887 and the crosses TOT8887 X TOT5799SC, ODS-15-020 X ELG1907A, TOT5799SC X BAR1807B. The potassium nitrate (KNO3) treatment was not able to break the dormancy in spider plant; on the contrary it reduced the germination percentage of spider plant genotypes in this study.

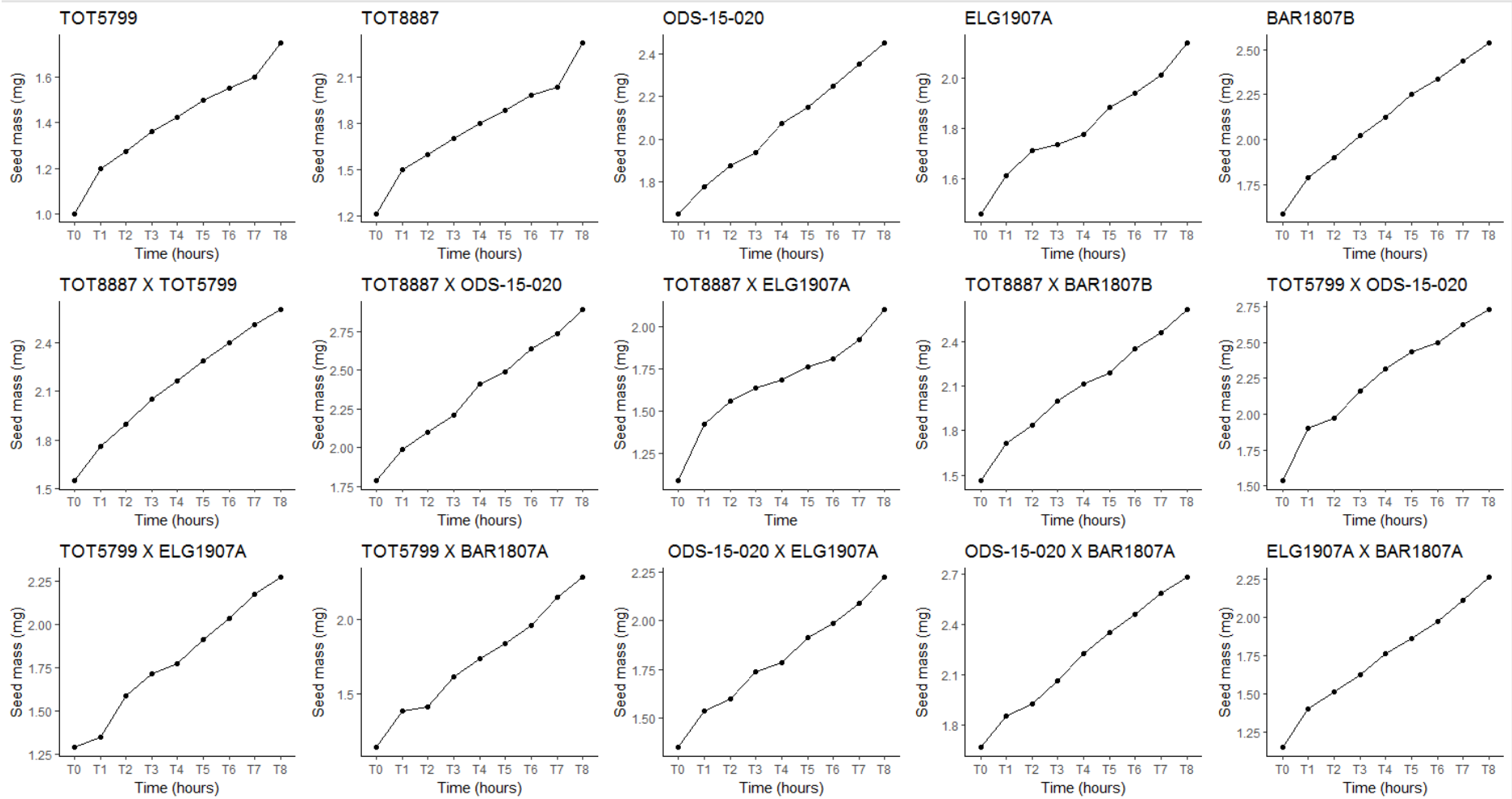

Figure 4.7: Imbibition curve of 15 *Gynandropsis gynandra* genotypes of seeds
T0= 0 hour, T1= 2 hours, T2= 4 hours, T3= 6 hours, T4= 8 hours, T5= 10 hours, T6= 12 hours, T7= 24 hours, T8= 48 h

Table 4.6: Effect of different pre-treatments on the germination pecentage of fresh seeds of of *Gynandropsis gynandra*

Genotype	**Control**	**GA3**	**Heating**	**KNO3**	**F value**
TOT8887	1.00±2b	**22.00±8.33a**	**25.00±6.83a**	0.00a	23.73***
TOT5799SC	**65.00±3.83a**	32.00±8.64b	**68.00±4.61a**	0.00c	147.8***
ODS-15-020	23.00±3.83b	26.00±5.16b	**79.00±14.37a**	2.00±2.31c	67.89***
ELG1907A	0.00c	**68.00±10.33a**	18.00±9.52b	0.00c	83.76***
BAR1807B	3.00±3.82c	13.00±6.83b	**40.00±6.53a**	0.00c	50.97***
TOT8887 X TOT5799SC	0.00b	**20.00±3.26a**	**19.00±3.83a**	0.00b	80.16***
TOT8887 X ODS-15-020	7.00±3.83b	0.00b	**22.00±8.3a**	0.00b	20.49***
TOT8887 X ELG1907A	16.00±3.26b	15.00±6.83b	**29.00±3.83a**	4.00±3.26c	20.26***
TOT8887 X BAR1807B	15.00±8.87b	16.00±3.26b	**48.00±11.77a**	0.00c	28.65***
TOT5799SC X ODS-15-020	3.00±2.00b	12.00±5.66b	**27.00±5.03a**	0.00b	38.35***
TOT5799SC X ELG1907A	4.00±0.00c	14.00±5.16b	**21.00±3.82a**	0.00c	35.19***
ODS-15-020 X ELG1907A	0.00b	**20.00±8.64a**	**17.00±6.83a**	0.00b	15.24***
ELG1907A X BAR1807B	6.00±4.00b	0.00c	**18.00±5.16a**	0.00c	27.00***
TOT5799SC X BAR1807B	13.00±6.00a	**17.00±3.83a**	**22.00±7.66a**	0.00b	12.98***
ODS-15-020 X BAR1807B	11.00±6.00c	27.00±7.57b	**40.00±6.53a**	4.00±4.62c	26.61***

*** p<0.001, Data in bold indicate treatment with highest value for each genotype

4.4 Discussion

This study aimed to (1) investigate the pattern of seed development to identify the critical stage for harvest maturity, (2) to document the variability in seed dormancy and identify the kind of dormancy and (3) evaluated different dormancy breaking methods (heating, gibberellic acid and potassium nitrate) in *G. gynandra*.

The results showed highly significant differences (p<0.001) among *Gynandropsis gynandra* genotypes with respect to pod and seed colour, and germination percentage at maturity stage.

Spider plant seeds attained harvest maturity stage about six weeks after appearance of the flower bud. At this stage, the pods turned yellow or brown depending on the genotype. Seed colour is also an important parameter to take into account when harvesting spider plant seeds. At harvest maturity stage, spider plant seed appeared black or brown in colour depending on the genotype. In some genotypes, such as ELG1907A, pods became yellow while the seed colour was still green-black. This observation emphasizes the need to check for the seed colour before harvesting. These result agree with those of Ekpong (2009), who found that spider plant seeds attained physiological maturity stage when the pod colour turned yellow and the seed colour turned black. In addition, the seed moisture content of freshly harvested mature seeds was on average 18.69%, which indicated that a drying period was necessary to reduce seed moisture content before germination or storage. Spider plant seeds must be dried up to 5% for better seed conservation and germination (Kamotho et al. 2014).

A seed is said to be dormant if no or only a few seeds germinate under suitable conditions however, if the majority (80-100%) of the seeds germinate, they are said to be non-dormant (Baskin and Baskin 2014). In this study the germination percentage results suggest that seeds of *G. gynandra* undergo dormancy. However, the dormancy period and degree are genotype dependent. Based on the germination percentage, mature spider plant seeds can be classified into 3 dormancy classes. Firstly, genotypes with strong dormancy for which the germination percentage was less than 6% (<6%), secondly, genotypes with intermediate dormancy for which the germination percentage was between 6% and 50% (6%-50%) and thirdly genotypes with weak dormancy for which the germination percentage was between 50% and 80% (50%-80%). Similar results were reported in a cross between variety CV (30%) and a landrace from Zimbabwe ZV (3%). K'Opondo et al. (2011); Tibugari et al. (2012); Essou (2017) reported the same variation between four morphotypes in Kenya and three Benin accessions respectively. Zharare (2012) reported the same trend but assumed that the differences reflected habitat-specific selection in genotypes that were collected in different environments. In our case, since all genotypes were grown in a single environment, the differences obtained should emanate from genotypic differences with site-specific and adaptive germination traits (Alonso-Blanco et al. 2003; Veasey et al. 2004). McWilliams et al. (1968); Loha et al. (2006); Mavengahama and Lewu (2012) reported similar results in *Amaranthus* species, large-leaved cordia (*Cordia africana)* and jute mallow *(Corchorus olitorius),* respectively.

The imbibition curves showed that the 15 spider plant genotypes were able to imbibe water 2 hours after imbibition and continue to imbibe after the 48 hours of imbibition period showing that they were continuing to imbibe water. This result showed that spider plant seed are water permeable which indicate that the dormancy in the species might not be attributed to a hard seed coat as a physical barrier to water absorption as reported by Ekpong (2009). These

results are contrary to those of Ochuodho (2005) who observed that the species germinated better after seed punctuation with a razor blade, and suggest that spider plant seeds do not exhibit physical dormancy. In addition, images of spider plant fresh seed embryo showed that the species have a fully developed embryo and help us to identify the type of dormancy in the species. The effect of dormancy breaking methods was also highly dependent on genotype in this study. Most of the genotypes responded well to heating at 41°C for 3 days and some responded well to GA at 0.01%. This explained the contradiction found in the literature by Shilla et al. (2016), where Ekpong (2009) found that heating was the most effective method for breaking dormancy in spider plant whereas Zharare (2012) reported that GA3 was the most effective breaking dormancy method. In contrast, potassium nitrate 0.1% inhibited the germination capacity of the species as reported by Muasya et al. (2009); Zharare (2012). But the results of Essou (2017) on the contrary showed that potassium nitrate was the most effective method for breaking dormancy in the species.

Following the classification of seed dormancy by Nikolaeva (1977) the dormancy in *Gynandropsis gynandra* is written as C_a whereby C stands for physiological dormancy broken by heat stratification (a). Further studies need to be done to distinguish between a non-deep, intermediate and deep physiological dormancy. Seeds with physiological dormancy have physiological inhibiting mechanisms in the embryo that prevents radicle emergence (Baskin and Baskin 2014). In the case of spider plant, it may be possible that at harvesting, the embryo is not fully mature and needs an after ripening period. To confirm this hypothesis, several studies have shown that freshly harvested seed of spider plant had a lower germination percentage than old seeds and after a period of 3 to 5 months the germination percentage could increase to up to 90% without any pre-treatments (Ochuodho and Modi 2007; Ekpong 2009; Zharare 2012)

4.5 Conclusion

Spider plant seed can be harvested when the pods turn yellow or brown and the seeds are black or brown depending of the genotype. The species exhibits a physiological dormancy with the level of dormancy varying among genotypes. Heating at 41°C for three days and gibberellic acid were able to break the dormancy in the species although each genotype responded differently to the two methods. The genotypes with different levels of seed dormancy identified in this study can be used for breeding programmes in development of segregating populations to perform genetic and molecular study for dormancy in *Gynandropsis gynandra* species. Future studies can look into the heritability of the dormancy in the species and also focus on the understanding of phytohormones production in each

genotype and factors that induce changes in phytohormones such as abscisic and gibberellic acids during seed development, seed storage and germination.

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CHAPTER 5

COMPARING THE STORAGE POTENTIAL OF NEWLY HARVESTED (FRESH) AND STORED (OLD) *GYNANDROPSIS GYNANDRA* (L.) BRIQ SEEDS USING THE ACCELERATED AGEING TEST

Abstract

The spider plant (*Gynandropsis gynandra* (L.) Briq) is an annual vegetable with high reproductive ability and high nutrient contents. This study assessed the storage potential of the seeds of four spider plant accessions using accelerated ageing test. Fresh seeds and four-month-old seeds were assessed for moisture content, electrical conductivity, percentage viability, percentage germination, mean germination time, seedling shoot and root length under traditional ageing (40 ml of distilled water), saturated ageing (40 g NaCl/100 ml water) and unaged (zero day of ageing) conditions. The traditional ageing for 48h method was also efficient but exhibited higher moisture content. Accession ELG 1907A performance was significantly superior to that of the other three accessions and its appeared to be the most tolerant to different ageing conditions. A highly significant difference was observed between the fresh and stored seed, with stored seed showed higher seed vigour. Accelerating seed ageing, using the saturated solution for 48 h, can be used to evaluate the physiological quality in spider plant seeds

Keywords: spider plant, germination, storage potential, seed vigour.

5.1 Introduction

Gynandropsis gynandra (L.) Briq (spider plant) is one of the highly consumed and prioritized traditional leafy vegetable in several African countries and is exclusively reproduced by seed (Raju and Rani 2016). The species is sold in rural and urban markets in eastern, southern and some west African countries (Abasse et al. 2006; Sogbohossou et al. 2018) and is still collected in wild in some communities (Segnon and Achigan-Dako 2014). In urban and rural areas, the plant becomes more and more popular with demand sometimes exceeding supply (Shackleton et al. 2009). The poor germination rate and the inappropriate seed supply are the principal constraints to large-scale production of the species, thus the need to investigate factors affecting seed germination and quality in the species. Seeds of major crops have been extensively investigated with regard to their physiological aspect, while neglected species like spider plant have received very little attention.

Previous studies on spider plant seeds have reported better germination after a storage period of at least 3 months (Ekpong 2009; Muasya et al. 2009). However, physiological mechanisms leading to seed deterioration during the storage period remain unclear. Seed deterioration is a major problem in developing countries where seeds are stored under uncontrolled environments without proper control over the humidity and temperature ranges (Makkawi and van Gastel 2007). In addition, previous investigations on the germination ability of spider plant seeds have been limited to standard germination tests (Ochuodho 2005; Muasya et al. 2009; Tibugari et al. 2012). As a result, there is lack of information on mechanisms underlying the germination of the seed of spider plant and genetic diversity among accessions with respect to seed vigour and germination. To supplement the standard germination test, seed vigour and viability are important aspects, which need to be assessed to gain deeper insights on the quality of spider plant seeds at different storage periods. One the most acknowledged and popular tests employed to evaluate the physiological potential and vigour of diverse seed species is the accelerating seed ageing test (Tekrony 1995). Initially developed to estimate the storage potential of seeds, the accelerated ageing test has proved its potential to assess seed vigour and sorted seed lots according to their ability to germinate and establish under field conditions (Madruga De Tune. et al. 2011). This method is based on the principle that exposing seeds to high temperature and relative humidity accelerates their deterioration (Marcos Filho et al. 1999). These conditions result in the production of reactive oxygen species, which damage the cell membranes, resulting in a loss of cell membrane integrity. Seeds with high vigour are able to tolerate ageing conditions and produce higher percentage of normal seedlings (Baalbaki et al. 2009). Seeds that are able to tolerate high temperatures and relative humidity are also considered to have good storage potential. Storage potential as

evidenced by high germination percentage, and rapid and uniform seedling emergence is also indicative of high seed vigour.

The objective of this study was to compare the storage potential of freshly harvested and 4-month stored spider plant seeds using the accelerating seed ageing test. The basic hypothesis of this study is that accessions with high vigour (storage potential) will tolerate ageing conditions better and show higher percentage of germinated seeds than accessions with low vigour.

5.2 Materials and methods

5.2.1 Plant material and experimental site

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. Seeds of four spider plant accessions from different origins (Table 5.1) were used in this study. The accessions were divided into two sets depending on the storage period. The first set comprised of seeds harvested and stored for four months under ambient temperatures and considered as old seeds. The second set comprised of seeds, which were stored for seven days and referred to as fresh seeds. The seeds of the two sets were harvested at the experimental site of the University of KwaZulu-Natal.

Table 5.1: List of accessions and their origins

Accessions	Country of origin	Region	Source
TOT8887	Uganda	East Africa	AVRDC
TOT5799	Thailand	Asia	AVRDC
ODS-15-020	Benin	West Africa	GBioS
ELG 19078	Kenya	East Africa	KENRIK

GBioS: Laboratory of Genetics, Horticulture and Seed Science, AVRDC: World Vegetable Center, KENRIK: Kenya Resource Centre for Indigenous Knowledge

5.2.2 Data collection

Spider plant accessions were tested for ageing, seed moisture content, viability, germination and electrical conductivity.

(i) Ageing treatments

Traditional accelerated ageing: 0.1 g of seed was weighed per accession using an analytical balance. The seed samples were placed in inner chamber plastic boxes containing 40 ml of distilled water in the bottom, preventing the seeds from touching the water. The test was

performed at 40ºC for 48 hours (Baalbaki 2009). Thus the test was performed under an environment with 100% relative humidity as recommended by Delouche and Baskin (2016) followed by germination, viability and electrical conductivity tests at 27ºC in filter paper moistened with an adequate amount of water.

Saturated salt accelerated ageing: This test was performed as mentioned above, but with the water replaced by 40 ml of a saturated sodium chloride solution (40 g NaCl /100 ml distilled water). This test was performed at 40ºC for 48, 72 and 96 hours under an environment with 75% relative humidity, as described by Winston and Bates (1960). After these periods, the germination, viability and electrical conductivity tests were performed. Unaged seeds (zero-day ageing treatment) were used as a control.

(ii) Seed moisture content determination

The moisture content was determined for both fresh seed and old seed. Each treatment consisted of four replications of ten seeds. The oven method (17 h at 103°C in the oven) prescribed by International Seed Testing Association ISTA procedure (ISTA. 2012) was used and the seeds were weighed using the digital balance Ohaus® Pioneer™ Plus analytical balance Model PA114C, AC/DC input 230 V AC, universal plug set. The moisture content was calculated using the following formula.

$$\mathrm{MC} = \frac{\text{Loss of weight}}{\text{initial weight}} \mathrm{x}100 = \frac{(\mathrm{M1} - \mathrm{M2})}{\mathrm{M1}} \mathrm{x}100$$

Where, M1 (g) is the weight of the seed before drying and M2 (g) is the dry weight of the seeds. Seed moisture content determination was repeated after the different accelerating aging tests.

(iii) Viability test

The tetrazolium viability test as described by the International Seed Testing Association ISTA book (ISTA. 2012) was done to ensure that the seeds were viable before conducting a germination tests. Four replicates of 10 seeds were used due to the limited number of available seeds. Seeds were imbibed in distilled water for 24 h at 20°C and cut into two parts before 1.0% of 2,3,5 triphenyl tetrazolium chloride solution (TZ) were added. After 24 h of incubation at 30°C the number of viable and non-viable seeds were identified by the staining of the embryo and counted under a light microscope at the Plant Pathology laboratory of the University of KwaZulu-Natal.

(iv) Germination test

The germination test was carried out using moistened paper towels. Four replicates of 25 seeds each from each ageing treatment were placed on moistened paper towels, which were rolled and then placed in a germination chamber. The germination experiment was set using a completely randomized block design (CRBD). The number of germinated seeds was recorded based on radicle protrusion every 24 hours for fourteen days and the number of normal seedlings per replicate were counted and expressed as the percentage of germination according to ISTA book. The mean germination time (MGT) was calculated using the following formula from Baskin and Baskin (2014).

$$MGT = (\Sigma \mathrm{nidi}) / \Sigma \mathrm{N})$$

Where *ni* = the number of germinated seeds at day *i*, *di* = incubation period in days, and *N* = number of germinated seeds in test. The data for the shoot length (mm) and root length (mm) were measured five days after germination.

(v) Electrical conductivity

For each treatment, 10 seeds were soaked in 30 ml of distilled water for 24 hours in a room set to a constant temperature of 25°C. After the 24 hours of soaking, the conductivity of the soak solution was immediately tested with OHAUS Starter 3100C Conductivity Meter and readings were expressed in $\mu S.cm^{-1}$. Four runs of 10 seeds were tested for each treatment.

5.2.3 Data analysis

Descriptive data analysis was performed on moisture content, electrical conductivity, seedling shoot and root length using the R package “psych” (Revelle 2017). A bar line was generated on percentage germination data as well as percentage viability and mean germination time using the R package “ggplot2” (McMurdie and Holmes 2013) and the plot from the 4 month stored and fresh seeds data were arranged into the same file using the function *ggarrange* of the R package “ggpur” (Kassambara 2017). The general linear model analysis was performed on the results of laboratory test (unaged and aged), to examine whether there was significant difference among accessions, storage, ageing treatments and their interaction. All the data were analysed using the R software version 3.5.1 (Crawley 2012).

5.3 Results

The results of the general linear model analysis on the different viability and vigour tests in *G. gynandra* seeds are presented is Table 5.2. The results showed a highly significant difference ($p<0.001$) between the ageing treatment for seeds’ moisture content, electrical conductivity, percentage viability, percentage germination, seedling shoot length and seedling root length. A highly significant difference ($p<0.001$) between accessions and the interaction between

accession and storage was observed for the seeds percentage germination showing that the percentage of germination of the spider plant accessions during the ageing treatments depends on the storage length. A significant difference ($p<0.01$) was also observed between the accessions for seedling shoot length and root length.

Table 5.2: Comparison of results for accelerated ageing treatment on viability and vigour tests in *Gynandropsis gynandra*

AA levels	MC	EC	%viability	%germination	MGT	SL	RL
0 h	7.1±2.2	19.5±1.8	87±12.2	81.7±21.7	4.0±0.3	28.7±7.7	18.5±7.9
48 h H20	28.8±5.2	8.3±2.0	82.5±12.3	73.5±30.3	3.8±0.2	30.5±6.8	19.5±6.5
48 h NaCl	9.0±3.7	6.7±2.3	83.1±13.0	47±31.78	4.1±0.3	44.3±14.9	19.4±8.5
72h NaCl	10.6±5.3	10±2.3	64.7±23.7	50.75±31.21	3.9±0.2	36.4±10.3	12.6±5.5
96h NaCl	11.0±2.6	8.8±1.6	52.5±22.14	39.5±32.75	4.3±0.4	40.3±13.6	11.9±4.6
STATISTICS							
Mean	13.3	10.67	73.97	58.5	4.06	36.03	16.41
CV (%)	66.17	47.42	19.97	31.04	4.19	18.07	23.03
Aging	***	***	***	***	ns	***	***
Accessions	ns	ns	ns	***	ns	**	**
Storage	ns	ns	ns	*	ns	ns	***
Accession*storage	ns	ns	ns	***	ns	ns	ns

MC= Moisture content, EC= electrical content, % = percentage, MGT= mean germination time, SL= shoot length, RL= root Length, ace*sto= interaction between accession and storage. CV= coefficient of variation, *** $p<0.001$; ** $p<0.01$; ^{ns}= non-significant

5.3.1 *Gynandropsis gynandra* seeds moisture content and electrical conductivity before and after ageing

The results of the seed moisture content, initially and after each accelerating ageing period, in the traditional method (using distilled water) and in the saline method (using 40% saturated saline solution) for both fresh and old seeds of spider plant accessions are summarized in Table 5.3. The initial seed moisture content varied little among the accessions, varying between 5.52% to 6.97%. The same trend was observed among fresh seed where the variation among TOT8887, ODS-15-020 and ELG1907A range from (6.53% to 7.57%) and slightly higher for TOT5799 (11.22%). Any differences between the results above could cause changes in the intensity of deterioration during the ageing period. However, the average initial moisture content of spider plant accessions was similar for both fresh and old seeds 7.98 % and 6.58% respectively (Table 5.2). This was appropriate for the execution of the different ageing tests, as uniform moisture content is essential to ensure the consistency of results (Torres and Marcos-Filho 2005).

The seed moisture content analysis under traditional ageing (48h H_2O) revealed the highest values for both fresh and old seeds in comparison to saturated saline solution ageing method (40% of NaCl). The moisture content of fresh seeds under traditional ageing ranged from 20.2% to 30.36% with an average of 24.80%. The moisture content of old seeds ranged from 21.99% to 35.36% with an average value of 28.38%. The saline solution restricted seeds water absorption with an increase of seed moisture content with the number of days of ageing. On average, the fresh seeds showed 8.15%, 10.26% and 11.99% of moisture content after 48 h, 72 h and 96 h of saturated ageing, respectively. Old seeds showed in average 7.49%, 10.29% and 13.61% of moisture content after 48 h, 72 h and 96 h of saturated ageing respectively

Table 5.3: Moisture content before and after the traditional and saturated accelerated ageing, at 41 ºC, in *Gynandropsis gynandra* fresh and old seeds

Accessions	**Fresh seeds moisture contents %**					**Old seeds moisture contents %**				
	Initially	TRA[1]	SSA[2]			Initially	TRA[1]	SSA[2]		
		48 h	48 h	72 h	96 h		48 h	48 h	72 h	96 h
TOT8887	6.6	24.23	10.9	12.72	13.32	5.88	29.82	6.22	10.05	16.97
TOT5799	11.22	30.36	7.34	9.7	12.99	6.97	35.68	8.98	11.35	11.73
ODS-15-020	6.53	20.92	6.55	8.1	10.6	7.96	26.03	6.12	10.33	11.31
ELG 1907A	7.57	23.7	7.81	10.51	11.06	5.52	21.99	8.64	9.45	14.44
Mean	7.98	24.80	8.15	10.26	11.99	6.58	28.38	7.49	10.29	13.61
CV (%)	27.71	16.04	23.38	18.75	11.36	16.80	20.52	20.44	7.70	19.34

[1]Accelerated aging test following the traditional methodology with water;
[2]Accelerated aging test using saturated solution (40% NaCl)

The results of the seed electrical conductivity of spider plants accessions are presented in Table 5.4. The seeds electrical conductivity before applying the ageing methods varied from 16.58 $\mu S.cm^{-1}$ to 19.49 $\mu S.cm^{-1}$, with an average of 17.41 $\mu S.cm^{-1}$ for spider plant fresh seeds. In fresh seed, the initial electrical conductivity ranged from 17.14 $\mu S.cm^{-1}$ to 21.42 $\mu S.cm^{-1}$ with an average value of 19.48 $\mu S.cm^{-1}$. Accession ODS-15-020 showed the highest value (19.49 $\mu S.cm^{-1}$) of initial electrical conductivity in fresh seed while accessions TOT5799 showed the highest value (21.42 µS.cm) in old seeds. In both fresh seed and old seeds, accession ELG1907A showed the lowest values, namely 16.58 $\mu S.cm^{-1}$ and 17.14 $\mu S.cm^{-1}$, respectively. In general, the results of the electrical conductivity test in spider plant seeds was higher in traditional ageing than in saturated ageing methods showing, that seeds under saturated ageing were more vigorous than seeds under traditional ageing. The fresh seed electrical conductivity for seeds aged for 48h ranged from 5.47 $\mu S.cm^{-1}$ to 6.37 $\mu S.cm^{-1}$ with

an average of 6.54 µS.cm^{-1}. In addition, the spider plants old seeds electrical conductivity under 48 h of ageing in H_2O solution ranged from 5.89 µS.cm^{-1} to 9.69 µS.cm^{-1} with an average value of 8.30 µS.cm^{-1}

Seeds' electrical conductivity under saturated seed ageing was lower at 48 h, followed by 96 h and 72 h for both fresh seeds and old seeds. The spider plants' fresh seeds average electrical conductivity under saline solution ageing were 6.80 µS.cm^{-1}, 6.90 µS.cm^{-1} and 6.31 µS.cm^{-1} after 48h, 72h and 96h respectively. In addition, old seed showed on average 6.74 µS.cm^{-1}, 10.00 µS.cm^{-1} and 8.84 µS.cm^{-1} electrical conductivity after 48h, 72h and 96 h, respectively.

Table 5.4: Electrical conductivity (µS.cm^{-1}) before and after the traditional and saturated accelerated ageing, at 40 ºC, in *Gynandropsis gynandra* fresh and old seeds

Accessions	**Fresh seeds electrical conductivity**					**Old seeds electrical conductivity**				
	Initially	TRA[1]	SSA[2]			Initially	TRA[1]	SSA[2]		
		48 h	48 h	72 h	96 h		48 h	48 h	72 h	96 h
TOT8888	16.69	6.37	4.28	6.23	4.58	19.65	9.69	5.38	7.43	7.01
TOT5799	16.86	6.47	8.94	6.02	5.78	21.42	9.035	9.84	13.27	10.66
ODS-15-020	19.49	7.84	8.89	9.69	7.86	19.70	8.59	6.17	9.156	9.56
ELG 1907A	16.58	5.47	5.07	5.66	7.03	17.14	5.89	5.56	10.15	8.12
Mean	17.41	6.54	6.80	6.90	6.31	19.48	8.30	6.74	10.00	8.84
CV (%)	8.01	14.95	36.34	27.17	22.76	9.05	20.11	31.11	24.51	18.12

[1]Accelerated aging test following the traditional methodology with water;
[2]Accelerated aging test using saturated solution ((40% NaCl)

5.3.2 *Gynandropsis gynandra* seeds viability percentage, germination percentage and mean germination time before and after ageing

The tetrazolium test (TZ) is a rapid test to estimate seed viability and vigour based on colour alterations of seed living tissues in contact with a solution of 2,3,5 triphenyl tetrazolium chloride, thus reflecting the degree of activity of the dehydrogenase enzyme system closely related to seed respiration and viability (Marcos Filho 2015). Viable seed embryos stain red after soaking in tetrazolium solution as was observed in the spider plant seeds (Figure 5.1)

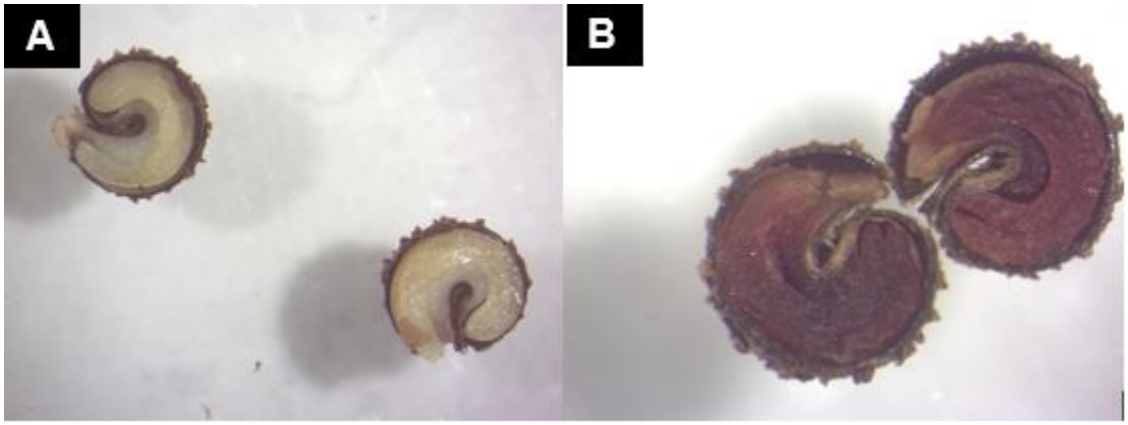
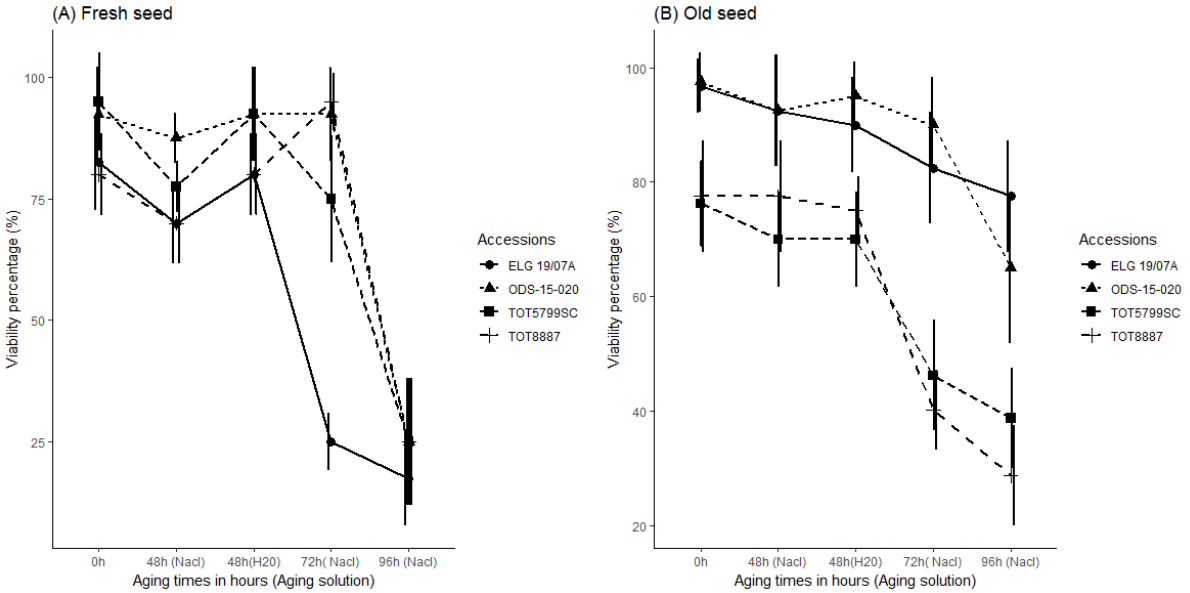


Figure 5.1: Light microscopy images of spider plant seed cross section showing how its stain with a tetrazolium solution; **A**: before tetrazolium test **B**: Viable seed after tetrazolium test (scale 200 µm)

The effect of accelerated seed ageing on the four accessions for seed viability using tetrazolium solution were different (Figure 5.2). The percentage viability values in the unaged controls were high for both fresh seeds and old seeds, namely: ELG1907A (96.75%), ODS-15-020 (97.5%), TOT5799SC (76.25%), TOT8887 (77.5%) fresh seeds and ELG 1907A (82.5%), ODS-15-020 (92.5%), TOT5799SC (95%), TOT8887 (80%) old seeds (Figure 5.3). In comparison with the control treatment, there was a slight decrease in fresh seeds viability after 48 h of H_20 ageing in ELG 1907A, ODS-15020, TOT 5799, TOT8887 (90%, 95%, 70% and 75%, respectively). In old seeds, there was a slight reduction of viability in ELG 1907A (80%) and TOT 5799 (92.5 %); in ODS-15020 and TOT8887 no change was observed in seed viability after 48 h H_2O of seed ageing.

The effect of saturated seed ageing in spider plant seeds viability was also different for the four accessions. After 48 h of saturated ageing a slight decrease or no change in spider plant seeds viability was observed for fresh seeds of ELG1907A (92.5%), ODS-15-020 (92.5%), TOT5799SC (70 %), TOT8887 (77.5 %), and old seeds of ELG1907A (70 %), ODS-15-020 (87.5%), TOT5799SC (77.5%), TOT8887 (70%). An increase in saturated ageing (NaCl) time led to decrease the percentage of viable seeds in both fresh and old seeds. A slight reduction was observed after 72 h of saturated ageing and a greater reduction was observed after 96 h. The TZ test proved to be an effective test to judge the viability of seed of spider plant at different ageing periods.



The vertical bar indicates standard deviation

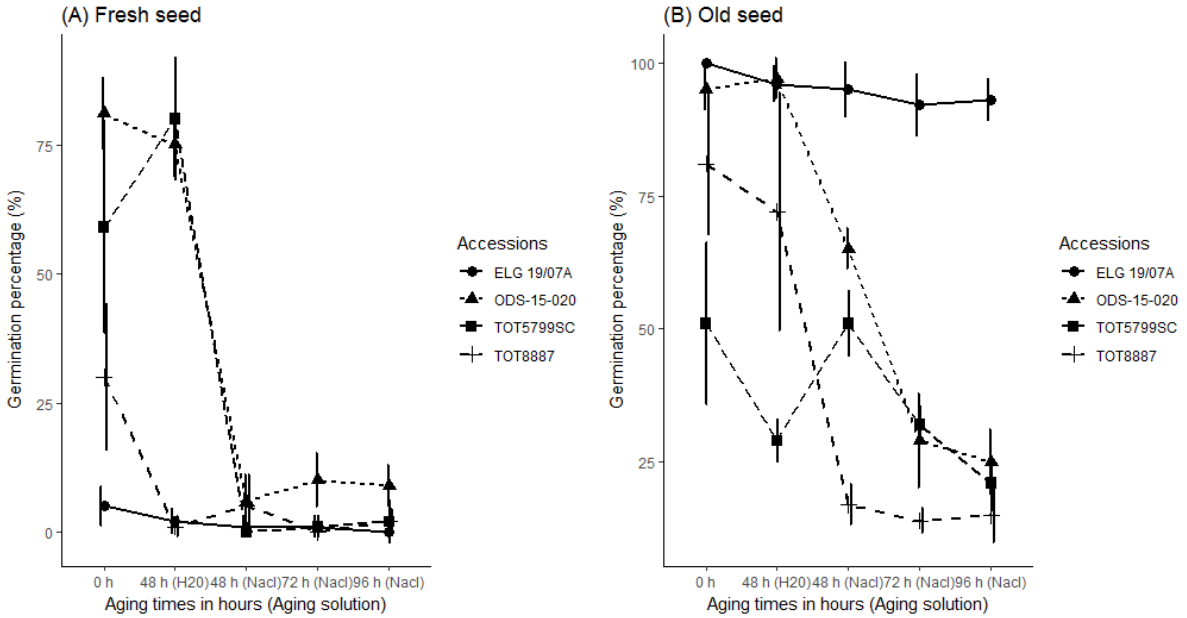
Figure 5.2: Effect of accelerating ageing treatments on percentage of viability using tetrazolium solution of (A) fresh seeds and (B) old seeds of *Gynandropsis gynandra*.

With regard to the effect of ageing treatments on germination percentage, similar trends were observed to those of viability percentage. However, although fresh seeds showed high viability percentage their percentage of germination was low compared to the old seed, which suggests that they were still in the dormant state. The initial percentage of germination of spider plant fresh seeds was low compared to old seeds: Fresh seeds of the accession ODS-15-020 (81%) showed the highest initial percentage of germination in fresh seed, while accession ELG1907A (5%) showed the lowest value. In old seed (stored for 4 months), accessions ELG1907A showed the highest percentage of germination (100%), while TOT5799 showed the lowest value (51%) (Figure 5.3.).

The response of spider plants accessions to the ageing treatments differed with respect to percentage off germination. The germination percentage of fresh spider plant seeds for the accessions subjected to 48 hours of H_2O ageing, as follows: ELG 11907A (2%), TOT 8887 (1%) and ODS-15-020 (75%). In contrast, the germination capacity of TOT5799 increased up to 80%. Ageing at 40°C with H_2O broke dormancy in accession TOT5799.

Under the same ageing condition, a similar trend was observed for old seeds with a slight decrease in the germination percentage of accessions TOT579SC (29%), ELG1907A (96%), and TOT8887 (72%) and an increase in the germination of accession ODS-15-020 (97%).

The germination capacity of spider plant accessions decreased with the saturated ageing period. Saturated ageing for 48h caused a rapid decline in the germination percentage of fresh seeds of accessions ODS-15-020 (25%), TOT8887 (17%) and TOT 5799SC (0%). The same trend was observed for old seeds of accessions ODS-15-020 (65%) and TOT8887 (17%). In contrast, no change was observed in the germination percentage of the old seeds of accession TOT5799 (51%). For both fresh seed and old seeds of accession ELG1907A only a slightly decrease was observed after saturated ageing.



The vertical bar indicates standard deviation

Figure 5.3: Effect of accelerating ageing treatments on percentage of germination of **(A)** Fresh seeds and **(B)** old seeds of *Gynandropsis gynandra*.

Figure 5.4 presents the effect of ageing treatments on the mean germination time of spider plant accessions. All spider plant accessions in this study germinated within 5 days, after this period no germination was observed for the control. The initial mean germination time of spider plant fresh seed accessions ranged from 4.66 to 4.92 days with the highest value obtained by accessions TOT8887. Old seeds' initial mean germination time ranged from 3.69 to 4.44 days with an average of 4.04 days (Figure 5.4.). After two days of H_2O ageing, there was a slight decrease in the mean germination of fresh seed of accessions TOT8887 (4 days) and no significant change was observed in the mean germination time of accessions ELG1907A, ODS-15-020 and TOT5799SC. Traditional ageing for 48 h reduced the mean germination time of old seed of all accessions presented in this work except for the fresh seed of accession TOT5799SC. Like for percentage germination and percentage viability, under saturated ageing, the mean germination time decreased with the number of days of ageing.

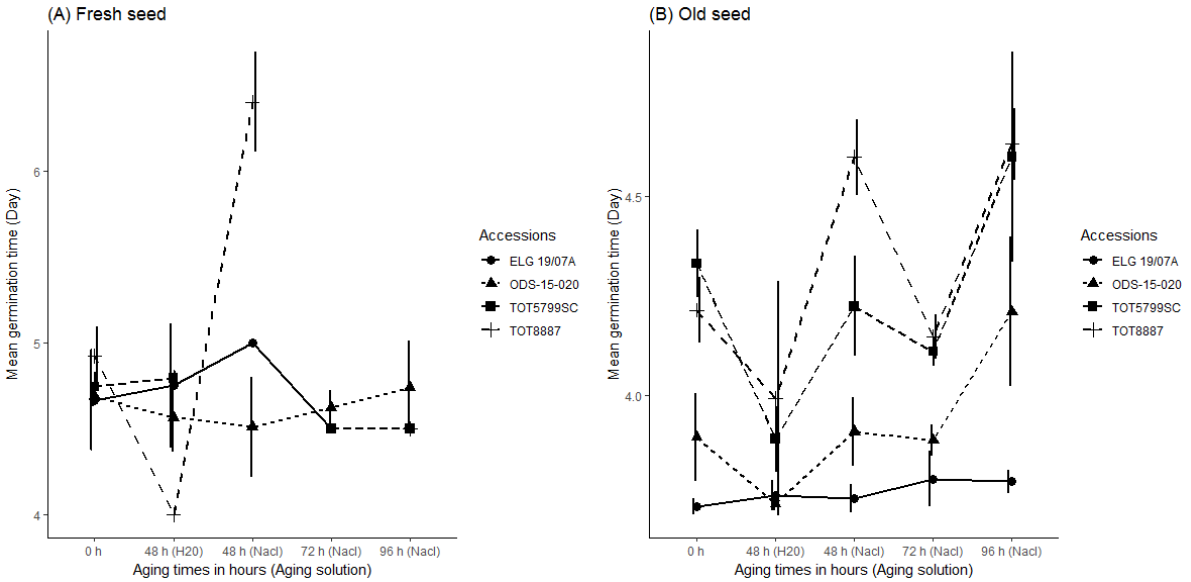


Figure 5.4: Effect of accelerating aging treatments on mean germination time of **(A)** Fresh seeds and **(B)** old seeds of *Gynandropsis gynandra*.

5.3.3 *Gynandropsis gynandra* seedling root and shoot length before and after ageing

The results of spider plant shoot length before and after ageing are presented in Table 5.5. The initial shoot length of spider plant fresh seed accession ranged from 24.16 mm to 37.7 mm with an average of 26.59 mm. Without any ageing treatment, fresh seed of accession ODS-15-020 was the most vigorous with the tallest shoot length (37.7 mm) and accession TOT 8887 was the less vigorous with the shortest shoot length (18.7 mm).

Old seed showed a higher vigour than fresh seed. The initial shoot length for old seeds of spider plant accessions ranged from 22.40 mm to 37.22 mm with an average of 28.75 mm. However, seeds aged for 48 h using traditional method (48 h) showed a higher shoot length except for the fresh seed of accession ODS-15-020 (29.4 mm) and old seed of accession TOT 5799SC (28.60 mm). Under saturated seed ageing (NaCl solution), the average seedling shoot length of *G. gynandra* accessions after 48 h of ageing was higher than the control: 31.61 mm and 44.25 mm, respectively for fresh seeds and old seeds. In addition, the spider plant average seedling length decreased with the number of days of saturated seed ageing.

Table 5.5: Seedling shoots length before and after the traditional and saturated accelerated ageing, at 40 ºC, in *Gynandropsis gynandra*

Accessions	Fresh seeds shoot length (mm)					Old seeds shoot length (mm)				
	Initially	TRA[1]	SSA[2]			Initially	TRA[1]	SSA[2]		
		48 h	48 h	72 h	96h		48 h	48 h	72 h	96h
TOT8887	18.7	20.8	12.33	13.8	-	22.4	33.1	28.6	30	31
TOT5799SC	25.8	26.7	-	20	-	32.8	28.6	50.5	30	51.8
ODS-15-020	37.7	29.4	57.5	44.66	38.5	27.8	32.6	61.4	48	50.8
ELG 19/07A	24.16	26.16	25	35	30	32	37.22	36.5	37.7	27.5
Mean	26.59	25.77	31.61	28.37	34.25	28.75	32.88	44.25	36.43	40.28
CV (%)	30.10	13.98	73.71	49.51	17.55	16.58	10.72	32.96	23.41	31.82

[1]Accelerated aging test following the traditional methodology with water;
[2]Accelerated aging test using saturated solution ((40% NaCl)

Regarding the seedling root length, a similar trend was observed with seedling shoot length. Data of spider plant accessions' root length before and after seed ageing are summarized in Table 5.6. The fresh seed, initial root length ranged from 11.50 mm to 15.83 mm with an average of 13.41 mm. Accession ELG1907A had the longest seedling root length and accession TOT5799SC had the shortest. The old seeds' initial root seedling length ranged from 10 mm to 23.6 mm with an average of 18.55 mm. The average spider plant seedling root length was longer under traditional ageing conditions than under saturated solution conditions. In addition, the seedlings' root length decreased with the number of saturated ageing days, showing that seed vigour in spider plant decreases with the number of days of saturated ageing.

Table 5.6: Seedling shoots length before and after the of traditional and saturated accelerated ageing, at 40 ºC, in *G. gynandra*

Accessions	Fresh seedling root length (mm)					Old seedling root length (mm)				
	Initial	TRA[1]	SSA[2]			Initial	TRA[1]	SSA[2]		
		48 h	48 h	72 h	96 h		48 h	48 h	72 h	96 h
TOT8887	14.5	15.9	16.33	17	-	23.6	23.5	29	14.3	11.5
TOT5799SC	11.5	14.1	-	10	10	18	20.4	18.5	8.2	15
ODS-15-020	11.8	14	6.75	8.1	5	10	12.3	11.9	9.2	7.9
ELG 19/07A	15.83	17.33	7	20	-	22.6	22	18.4	18.3	13.5
Mean	13.41	15.33	10.03	13.77	7.5	18.55	19.55	19.45	12.5	11.97
CV (%)	15.69	10.38	54.45	65.44	47.14	33.42	25.55	36.38	37.59	25.65

[1]Accelerated aging test following the traditional methodology with water;
[2]Accelerated aging test using saturated solution ((40% NaCl)

5.4 Discussion

Accelerating seed ageing in *Gynandropsis gynandra* was shown to be highly affected by high temperature (40°C) and high relative humidity (100% RH for traditional ageing and 75% RH for saturated ageing). These conditions caused a decline in seed germination and viability with an increase in seed vigour. Similar results were observed in lentil (*Lens culinaris* Medikus) by Makkawi and van Gastel (2007) and in soybean (*Vigna radiata* (L.) Wilczek**)** by (Murthy et al. (2003). Under traditional and saturated ageing, spider plant seeds of genotypes used in this study showed a reduction in electrical conductivity and an increase in the seedling shoot and root length which are an indication of better vigour of seeds under accelerated ageing (Table 5.6). In contrast, seeds exhibited reductions in percentage viability and percentage germination under both traditional and saturated ageing. The results showed that increased saturated ageing time implies low germination percentages in *G. gynandra* accessions. Different results were observed in Amaranth (*Amaranthus cruentus* L.), where the percentage germination after accelerating ageing test increased as a result of increasing the exposure time of the seeds to high humidity and temperature (Rosa et al. 2018). The germination capacity of accession TOT5799 increased up to 80% showing that ageing at 40°C with H_2O broke dormancy. The germination of a seed is related to a combination of factors including temperature, water availability, light and others (Guillemin et al. 2013). In some species, temperature may interfere in overcoming seed dormancy (Taab and Andersson 2009), and seeds subjected to conditions of high or low temperatures can undergo metabolic changes, which affect the enzymatic expression responsible for the germination process.

The results showed highly significant differences ($p<0.001$) among accessions in their ability to tolerate different seed ageing treatments as reported by da Silva Almeida et al. (2014) in tomato (*Lycopersicum esculentum* L.). In germination tests, the four accessions of *Gynandropsis gynandra* responded differently to the accelerating ageing treatments. Accession ELG1907A, exhibited a low decrease in germination percentage after 2 days of saturated ageing, showing a lower sensitivity to saturated ageing in comparison to accessions TOT8887, ODS-15-020 and TOT5799SC. Seedling shoot and root length were highly affected by the ageing time. The capacity of the seed to germinate and seed vigour are thought to be related to the genetic constitution and the capacity to support ageing conditions (Madhava Rao and Kalpana 1994).

Among the vigour tests, seed moisture content and electrical conductivity showed a wide range of variation among ageing treatments. The results indicated that a duration of two days of ageing was sufficient to differentiate among ageing periods. On the contrary, germination parameters including percentage of germination shoot and root length exhibited a wide range of variation among accessions. Significant differences ($p<0.01$) were observed among storage period for percentage of germination and seedling root length. Seed stored for four months germinated better than freshly harvested seed. This could possibly explain the highly significant differences ($p<0.001$) observed between seed storage and accessions. Similar findings have been reported by Muasya et al. (2009), Kamotho et al. (2014) who found that spider plant seeds stored for three and six months respectively germinated better than freshly harvested seeds.

5.5 Conclusion

Spider plant accessions responded differently to ageing treatments and seeds of accession ELG1907A were less susceptible to deterioration. The use of saturated solutions decreased the water absorption and the results suggested that NaCl treatment for 48 h was an appropriate condition for accelerating ageing in spider plant. It is thus concluded that low vigour seeds rapidly lose their viability during ageing compared to high vigour, seed indicating their good storage potential. Seeds with good storage potential can be preserved for longer periods and hence the accelerated ageing test could be used in breeding programmes to screen accessions with higher storage potential for greater storability.

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CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Findings and implications

Traditional leafy vegetables are currently considered of less importance compared to exotic crops in terms of production area, consumption trends and research attention. Consequently, indigenous vegetable yields in sub-Saharan Africa are declining. Nevertheless, there are studies in the literature that have reported on the importance of including these vegetables in gardens and field production systems. However, major production constraints include poor quality seed and lack of technical packages for seed (Abukutsa-Onyango 2005; Adebooye et al. 2005). Farmers are not able to store the seeds under optimal conditions of temperature and relative humidity, and this leads to the loss of seed viability. Poor and delayed germination are usually observed for many traditional leafy vegetable species, and lower number of viable seeds harvested by farmers often prevent an all year-round production of traditional leafy vegetables (Shilla et al. 2016). Spider plant (*Gynandropsis gynandra*) is one of the most consumed leafy vegetable in sub-Saharan Africa. This study aimed to gain a deeper understanding of factors influencing the relationship between dormancy, low and non-uniform germination in *Gynandropsis gynandra* and environmental conditions during seed development, germination and storage.

One crucial step to the study was the need to conduct a review (chapter 2) of available knowledge on seed germination and dormancy in *G. gynandra*. The review found that dormancy in spider plant seeds could be broken using certain dormancy breaking methods including heating, gibberellic acid, soaking and stratification. The review revealed that seed stored for a minimum period of three months germinated better than freshly harvested seed. It was also found that exposing spider plant seeds to dark conditions promoted germination. Despite this, the review found some contradictory reports regarding the use of pre-treatments by different authors and the identification of the critical moment when the seed of the species enters in a dormancy state. The review concluded that further studies on seed dormancy and germination with more diverse and large number of accession are needed to explain the narrow scientific information available with regard to low germination in spider plant.

To this end, a phenotypic characterization of twenty-nine (29) spider plant accessions from diverse origin including Asia, and West, South and East Africa was done (chapter 3). The study showed that spider plant seeds are considerably diverse with respect to shape, size, mineral composition, germination percentage and mean germination time. The Asian

accessions recorded the highest values with respect to percentage germination and phosphorus content while Western Africa accessions showed highest values with respect to seed size. We concluded that materials from different geographical origins could be used as prospect for the genetic improvement to increase the germination capacity and the yield of the species. The study also provided new insights on the external and internal seed structure of *G. gynandra*. This information will be useful for further studies on the species.

The study confirmed that *Gynandropsis gynandra* seed are mature when the pod colour turns brown or yellow and the seeds are black or brown depending genotype/ accession. Freshly harvested spider plant seeds exhibit physiological dormancy with the degree of dormancy varying from one genotype to another. The effect of pre-treatments was also highly genotype dependent, with dormancy of one (01) genotype (ELG1907A) broken by GA3, dormancy of nine (09) genotypes (i.e. ODS-15-020) broken by heating and dormancy of three genotypes broken by both GA3 and heating. Potassium nitrate was not able to break spider plant dormancy, in contrast it inhibited the germination capacity of the species. This result explained the narrow differences observed in the literature review regarding the effect of breaking dormancy methods on *G. gynandra* germination. These results also confirmed our hypotheses that *G. gynandra* seed dormancy, composition and germination are strongly genotype dependent and that different genotypes are adapted to different ecological zones will respond differently to breaking dormancy methods. As an implication for breaking dormancy in *Gynandropsis gynandra*, the researcher must test both heating and GA3, and identify the best method for his lot of seed before undertaking a germination test (Chapter 4).

Another challenge was to assess the storage potential of spider plant seed. There are suggestions in the literature that stored seed germinates better than freshly harvested seed. The storage potential and physiological quality of the seeds of four spider plant accessions assessed using accelerating ageing showed that accelerating seed ageing, using the saturated solution for 48 h, can be used as a technique to evaluate the physiological quality in spider plant seeds during storage (chapter 5). Accession ELG1907A's performance was significantly superior to that of the other three accessions and it appeared to be the most tolerant to different ageing conditions. Since viability and vigour are controlled genetically, the ageing test could be used in a breeding program to screen for accessions with higher vigour and greater storability.

Although not consistent, in general we observed that Asian genotypes performed better with respect to seed germination, dormancy, and seed vigour, compared to the African accessions. Fifteen (15) months stored seed used for the phenotypic characterization and four-month seeds used for the accelerating seed ageing germinated better than freshly harvested seeds.

However, to farmers it is not common practice to store *C. gynandra* seed, as well as other traditional African vegetables for such a long storage period; normally seeds are stored for not more than three months (Muasya et al. 2009). As one of the strategies to avoid the dormancy period, in most cases farmers could collect seeds and keep them for the next season's planting.

6.2 Recommendations

Further experiments are suggested to develop genotypes with little dormancy to improve germination capacity in the species. The genotypes with varying levels of seed dormancy identified in this study can be used for breeding programmes in the development of segregating populations to perform genetic and molecular studies for dormancy in *Gynandropsis gynandra* species. Future studies can be focused on the understanding of phytohormone production in each genotype and factors that induce changes in phytohormones such as abscisic and gibberellin acids during seed development, storage and germination. It is further recommended that other studies should focus on the comparison of proteins between genotypes, and gene identification for instance, the delay of dormancy genes homologous in *Gynandropsis gynandra*, and other genes that are implicated directly or indirectly in seed dormancy control. A deeper study to understand the type of physiological dormancy in the species is needed for better crop improvement. This will be an opportunity for seed companies to offer their customers quality seed with good germination. These genotypes can be recommended to farmers if they fulfil the other desired traits.

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