ENVIRONMENT, PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS ON SEED GERMINATION CHARACTERISTICS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.)

BY

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DECLARATION

DECLARATION BY THE STUDENT

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DEDICATION

This thesis is dedicated to my late father Mr. Michael Heneson Botey, may his soul continue to rest in peace. It is also dedicated to my mother, Joyce Arthur for her prayers and support throughout this journey.

ABSTRACT

The Gilo group of the African eggplant (*Solanum aethiopicum* L.) is one of the most important vegetable crops cultivated by smallholder farmers in Ghana and other West African countries. In the absence of a formal quality seed production and supply system for the African eggplant, over 90% of farmers rely on farmer-saved and other informal sources for seed. Seeds from these sources show no or low (0 – 25 %) germination, which affects seedling emergence and field establishment. The seeds are also known to possess short longevity. The quality of seed and its viability is affected by environmental, physiological and biochemical factors independently or interactively. The objectives of this study were to determine the effect of temperature and light on germination of two cultivars of African eggplant, evaluate the influence of fruit harvesting maturity stage on the physical and physiological quality of African eggplant, determine the effect of seed maturity stage on biochemical characteristics of African eggplant, evaluate the effect of harvest time and after-ripening on seed physiological characteristics of African eggplant and lastly to evaluate the effect of fermentation and drying methods on seed physiological characteristics of two cultivars of African eggplant. Field experiments were conducted under tropical monsoon (Bungoma, Kenya) and temperate oceanic climates (Chepkoliel, Eldoret, Kenya) between April, 2019 and January, 2021. Laboratory experiments were conducted at Seed physiology laboratory of University of Eldoret and International Livestock Research Institute (ILRI, Kenya). Field experiments were laid out in a randomized complete block design with factorial arrangements depending on the specific objective. Results showed that the environment, temperature, light, harvesting maturity stage, after-ripening, fermination and drying methods independently and interactively ($p \leq 0.01$) affected seed physical, physiological and biochemical characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.). Temperature and light interactively improved seed germination of African eggplant. The maximum seed germination (76 and 95%) was obtained when seeds were germinated at alternating temperatures of 30/20 °C under alternating 8/16 hours light/dark periods. This condition also gave the shortest time (4-5 days) required to complete the germination process. The results established that seeds harvested precociously (20 and 34 days after anthesis), did not germinate or recorded very low percentage germination (≤ 15%). The suitable maturity stage to harvest fruits for maximum seed quality was 76 days after anthesis. Fruit characteristics such as weight, size and colour have shown to be suitable indicators of seed physiological quality with a strong positive correlation. Seed sugars and crude protein were not strongly associated with seed quality characteristics of African eggplant. The results showed that tannin content positively influenced physiological quality in terms of germination and vigour of African eggplant seeds. Further, after-ripening of fruits for 10 to 15 days improved seed vigour and germination quality when fruits were harvested 40 or 50 days after anthesis. It was however, confirmed that after-ripening is inconsequential and not necessary when seeds are harvested at physiological maturity, which coincides with maximum germination at 70-76 days after anthesis for cv. *Kpando*. Lastly, results from the study suggest that African eggplant seeds do not require fermentation prior to extraction for enhanced seed germination percentage, although, fermentation up to 12 hours can improve and maintain seed vigour. All drying methods used in this study were able to reduce seed moisture content to an ideal level (< 10 %) for safe storage and maintained seed physiological quality. For the purpose of seed production of African egpplant, it is recommended that seeds should be extracted from fruits harvested between 60 and 76 DAA for maximum seed quality. Oven drying at 30 °C for 24 hours or 48 hours of shade drying can maintain seed quality.

TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
ABSTRACT iv
TABLE OF CONTENTS v
LIST OF TABLES ix
LIST OF FIGURES xi
LIST OF PLATES xiv
ABBREVIATIONS AND ACRONYMS xv
ACKNOWLEDGEMENT xvi
CHAPTER ONE 1
INTRODUCTION 1
1.1Background 1
CHAPTER TWO 13
GENERAL LITERATURE REVIEW 13
1.2 Taxonomy, Botany and Distribution of the African eggplant 13
1.3 Economic Importance of the African eggplant 15
1.4 Factors that influence Seed Germination and dormancy 16
1.5 Effect of harvesting maturity stage of fruits on the physical and physiological quality 19
1.6 Biochemical changes in seeds during seed development and maturation 23
1.7 Postharvest Handling Practices and their influence on Seed Quality Characteristics 29
CHAPTER THREE 34
DETERMINATION OF THE EFFECT OF TEMPERATURE AND LIGHT ON SEED GERMINATION OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.) 34
3.1 Material and Methods 37
3.1.1 Plant Materials used for the study 38
3.1.2 Determination of Initial Seed physical and biochemical characteristics 39
3.2 Data collected 41

3.2.1 Percent Seed Germination 41
3.3 Data analysis 41
3.4 Experimental layout and Design 42
3.5 Data collected 42
3.5.1 Determination of seed germination characteristics 42
3.6 Data analysis 43
3.7 Results and Discussion 43
3.7.1 Initial Seed Lot physical and germination characteristics 43
3.8 Conclusion 53
3.9 Recommendation 54
3.10 Suggestions for further study 54
CHAPTER FOUR 55
EVALUATION OF THE INFLUENCE OF FRUIT HARVESTING MATURITY STAGE ON THE PHYSICAL AND PHYSIOLOGICAL QUALITY OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.). 55
4.1 Objectives of the study 59
4.2. Research questions 59
4.3 Materials and Methods 60
4.4 Results and Discussion 67
4.5 Conclusion 101
4.6 Recommendations 101
4.7 Suggestions for further research 101
CHAPTER FIVE 102
DETERMINATION OF THE EFFECT OF SEED MATURITY STAGE ON THE BIOCHEMICAL CHARACTERISTICS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.) AND ITS RELATIONSHIP WITH SEED PHYSIOLOGICAL QUALITY 102
5.1 Objectives of Study 105
5.2 Research Questions 105
5.3 Materials and Methods 106
5.4 Results and Discussion 114

5.5 Conclusion..... 131
5.6 Recommendation..... 131
5.7 Suggestions for further research..... 131
CHAPTER SIX 132
EVALUATION OF THE EFFECTS OF HARVEST TIME AND AFTER-RIPENING ON SEED PHYSIOLOGICAL CHARACTERISTICS OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.) 132
6.1 Objective of Study..... 135
6.2 Research question..... 135
6.3 Materials and Methods..... 135
6.4 Results and Discussion..... 137
6.5 Conclusion..... 153
6.6 Recommendation..... 153
6.7 Suggestion for further research 153
CHAPTER SEVEN..... 154
EVALUATION OF THE EFFECTS OF FERMENTATION AND DRYING METHODS ON SEED PHYSIOLOGICAL CHARACTERISTICS OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.) 154
7.1 Objective of the Study..... 156
7.2 Research questions 156
7.3 Materials and Methods..... 157
7.4 Results and Discussion..... 160
7.5 Conclusion..... 171
7.6 Recommendation..... 171
7.7 Suggestions for further research..... 171
CHAPTER EIGHT..... 172
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 172
8.1 General Discussion..... 172
8.2 Conclusions 178
8.3 Recommendations 179
8.4 Suggestions for further Research 180

REFERENCES **181**
APPENDICES **214**

LIST OF TABLES

Table 1: Treatment combination of cultivar (2) x temperature (8) 40

Table 2: Seed moisture content, 1000 seed weight and proximate composition (mean ± standard deviation) between two African eggplant cultivars used for the study. 44

Table 3: Mean germination time and time to reach 50% germination (mean ± standard deviation) as influenced by temperature and light durations on two cultivars of African eggplant. 50

Table 4: Correlation dynamics among seed quantitative measurements of African eggplant as influenced by the interactive effect of temperature and light. 52

Table 5: Parameters characterising germination rate of seeds lots as influenced by harvest maturity stage and produced under tropical climate. 88

Table 6: Parameters characterising germination rate of seeds lots as influenced by harvest maturity stage and produced under temperate oceanic climate. 89

Table 7: Parameters characterizing germination synchrony of seeds lots as influenced by harvest maturity stage. 93

Table 8: Correlation matrix for fruit morphometric and seed germination metrics of cv. *Oforiwa* produced under tropical climate. 94

Table 9: Relationship of Fruit Colour Changes during development and maturation of African eggplant, cv. *Oforiwa* and percentage seed germination produced under a tropical (A) and temperate oceanic (B) climates). 96

Table 10: Relationship of Fruit Colour during development and maturation of African eggplant, cv. *Kpando* and percentage seed germination produced under a tropical (A) and temperate oceanic (B) climates. 97

Table 11: Correlation matrix of biochemical components and physiological variables of African eggplant cv. *Oforiwa* seeds. 127

Table 12: The effect of after-ripening for 15 days on dry seed weight and 1000 seed weight of cv. *Oforiwa* extracted from fruits harvested at 30 -70 days after anthesis. 139

Table 13: The effect of after-ripening for 15 days on dry seed weight and 1000 seed weight of cv. *Kpando* extracted from fruits harvested at 30 -70 days after anthesis. 140

Table 14: The effect of after-ripening for 15 days on first germination count (%) and Germination (%) of cv. *Oforiwa* seeds extracted from fruits harvested at 30 -70 days after anthesis. 143

Table 15: The effect of after-ripening for 15 days on first germination count (%) and Germination (%) of cv. *Kpando* seeds extracted from fruits harvested at 30 -70 days after anthesis. 144

Table 17: Influence of Harvest maturity and After-ripening on Germination Index (GI) and Mean daily germination (MDG) of cv. *Oforiwa* and cv. *Kpando* seeds. 150

Table 18: Correlation of qualitative and quantitative dynamics among seed germination variables of cv. *Oforiwa* in relation to harvest time (50, 60 and 70 DAA) and after-ripening for up to 15 days under ambient condition. 152

Table 19: Effect of fermentation on first germination count, percentage germination and accelerated ageing (mean ± standard deviation) for cv. *Ofoirwa* (CV1) and cv. *Kpando* (CV2) African eggplant seeds subjected to varied durations of natural fermentation.....163

LIST OF FIGURES

Figure 1: Cumulative Percent Germination of cv. *Oforiwa* seeds under various constant (A) and alternating temperatures (B) and cv. *Kpando* seeds under constant (C) and alternating temperatures (D) germinated under 8 hours/16 hours light and dark photoperiods. 44

Figure 2: Effect of Temperature and its interaction with light exposure on seed germination (%) ± SEM of two cultivars of African eggplant. (CV1: cv. *Oforiwa*, CV2: cv. *Kpando*. 48

Figure 3: A Sketch Map of the Field Experimental locations (Author, 2019) 60

Figure 4: Field Layout for field experiments conducted at both Mabanga (Tropical monsoon) and Chepkoliel (Temperate Oceanic) Climates. 62

Figure 5: Seed moisture content and dry seed weight of African eggplant seeds (cv. *Oforiwa* (CV1) and cv. *Kpando* (CV2) harvested at different maturity stages (DAA) produced under Tropical climate (A) and Temperate Oceanic climate (B)..... 68

Figure 6: Mean Seed length (SL) and thousand seed weight (TSW) during seed development of African eggplant cv. *Oforiwa* (CV1) and cv. *Kpando* (CV2) under both tropical (TCC) and temperate oceanic climates (TOC). 72

Figure 7: Relationship of seed moisture content and Field emergence of cv. *Oforiwa*) and cv. *Kpando*) at different maturity stage 74

Figure 8: 'Changes in percentage seed germination of cv. *Oforiwa* and cv. *Kpando* extracted from fruits harvested at different maturity stages and produced under tropical' climate (A) and temperate oceanic climate (B). 76

Figure 9: Effects of time of harvest on emergence percentage of African eggplant seeds for cv. *Oforiwa* and cv. *Kpando* grown at a tropical climate (TCC, A) and temperate oceanic climate (TOC, B) 78

Figure 10: Relationship of harvest maturity on time to reach 50% (T_{50}) seed germination and emergence for seeds produced under both tropical (A) and temperate oceanic (B) climates for cv. *Oforiwa* (CV1) and cv. *Kpando* (CV2). 82

Figure 11: Effects of harvest maturity stage on fruit weight and diameter of cv. *Oforiwa* and cv. *Kpando* produced under tropical and temperate oceanic climates. CV1 (A): cv.

Oforiwa produced under tropical climates; CV2 (B): cv. *Kpando* produced under temperate oceanic climates. 84
Figure 12: Number of seeds per fruit differed between cv. *Oforiwa* and cv. *Kpando* and under both tropical and temperate oceanic climates.. 86
Figure 13: Effect of harvest maturity stage on Coefficient of variation of germination time (CVt) as a measure of uniformity of seed germination of seed lots produced under tropical (CVt, A) and temperate oceanic (CVt, B) climates. 92
Figure 14: Means for seed germination (A), germination index (B), mean daily germination (C) and mean germination time (D) of African eggplant cv. *Oforiwa* harvested at six maturity stages. 114
Figure 15: Content of crude protein and crude fat during seed development in African eggplant cv. *Oforiwa*.....117
Figure 16: Means of Antioxidant activity (AOA) and Tannin content during seed development in African eggplant cv. *Oforiwa*.....120
Figure 17: Changes in soluble sugar contents (sucrose, glucose and fructose) of African eggplant cv. *Oforiwa* seeds during development. 123
Figure 18: Principal component analysis (PCA) for biochemical components and physiological quality of African eggplant cv. *Oforiwa* seeds. %G: germination; GI: germination index; MDG: mean daily germination; MGT: mean germination time; AOA: Antioxidant activity. 128
Figure 19: Partial least squares regression analysis (PLS-R) for association of biochemical components with physiological quality of African eggplant seeds. 130
Figure 20: Seed moisture content for African eggplant cultivars harvested at 30, 40, 50, 60 and 70 DAA and after-ripened for 0, 5, 10 and 15 days.. 137
Figure 21: Mean values of the interactive effect of maturity stage and after-ripening on time to reach 50% germination (T_{50}) and mean germination time (MGT). 148
Figure 23: Seed moisture contents (SMC) and Seed dry weight (SDW) of cv. *Oforiwa* and cv. *Kpando* African eggplant seeds as influenced by various fermentation duration .161
Figure 24: Seed Moisture content of cv. *Oforiwa* and *Kpando* African eggplant seeds as affected by different drying methods.. 166

Figure 25: First germination count (%) and final germination (%) of cv. *Oforiwa* (A) and cv. *Kpando* (B) African eggplant seeds subjected to different methods of drying.....168

LIST OF PLATES

Plate 1: Ripened African eggplant fruits of cv. *Kpando* (A) and cv. *Oforiwa* (B) (photo by, Botey, HM. 2020) 38

Plate 2: African eggplant with fruits in the field (photo by Botey, HM, 2020).....39

Plate 3: African eggplant with fruits in the field (photo by Botey, HM, 2020).....61

ABBREVIATIONS AND ACRONYMS

ABA	-	Abscissic acid
AOAC	-	Association of Official Agricultural Chemists
CRI	-	Crops Research Institute, Ghana
CSIR	-	Council for Scientific and Industrial Research, Ghana
CV1	-	Cultivar Oforiwa
CV2	-	Cultivar Kpando
DAA	-	Days after anthesis
FAO	-	Food and Agriculture Organization of United Nations
GA	-	Gibberellic acid
HCl	-	Hydrochloric acid
ILRI	-	International Livestock Research Institute, Kenya
ISTA	-	International Seed Testing Association
MoFA	-	Ministry of Food and Agriculture, Ghana
MT	-	Metric tonnes
PM	-	Physiological maturity
PPMED		Policy, Planning, Monitoring and Evaluation Directorate
RH	-	Relative Humidity
RHS	-	Royal Horticultural Soceity Colour Chart
TCC	-	Tropical monsoon climate (Bungoma, Kenya)
TOC	-	Temperate Oceanic climate (Eldoret, Kenya

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CHAPTER ONE

INTRODUCTION

1.1 Background

The Gilo group of the African eggplant (*Solanum aethiopicum* L.) is one of the most important vegetable crops cultivated by many resource constrained smallholder farmers in Ghana and other West and Central Africa countries (Owusu-Ansah *et al.,* 2001; Weinberger and Msuya, 2004; Grubben and Denton, 2004). It is reported to be the third most frequently grown non-leafy vegetables in Ghana (Horna *et al.,* 2007) and Tanzania (Weinberger and Msuya, 2004) and plays a major role as a source of income among rural and urban resource poor communities (Chadha, 2000).

The fruits and leaves are excellent sources of vitamins A and B, calcium, phosphorus and iron and known to possess carminative and sedative properties and reported to be used in the treatment of blood pressure (Grubben and Denton, 2004).

While production data on African eggplant (*Solanum aethiopicum* L.) is scanty, estimated production figures in some countries in West Africa ranges from 8,000 MT to 60,000 MT (Mshida, 2014). In Ghana, national production is estimated to be around 30,000 MT but exact figures are not available for the whole country. Statistics available for a region in Ghana however, indicates a decreasing trend in total output and yield (Horna *et al*., 2007). From 1993 to 2004, production has declined drastically from 10,100 MT to 570 MT and corresponding yields per ha has also reduced from 3.26 t/ha to 1.97t/ha (PPMED-MoFA, 2005; Hornal *et al.,* 2007).

The continuous low and inconsistent yields of this important crop has necessitated the need to initiate more research to gain a better understanding of the factors influencing the yield trends.

Farmers who cultivate African eggplant are confronted with myriads of production and postharvest handling constraints which affect their yields and incomes. Paramount among is inadequate access to high quality seeds which is known to be one of the most important agricultural inputs for increase productivity (Adentumbi & Daniel, 2004; Asare-Bediako *et al.,* 2007; Bortey, *et al*., 2011; Chauhan *et al.,* 2013; Elias, 2018).

Generally, the public sector in Ghana has invested very little resources in African eggplant (‘garden egg’) research. There are currently no officially released variety of this crop for commercial cultivation, hence no formal seed system exist for the crop. However, a number of cultivars are used by farmers to meet the local demand (Owusu-Ansah *et al.* 2001). More than 90% of seeds are sourced through recycled seeds or bought from market women who sell fresh fruits of the crop (Horna *et al*., 2007), which are not certified and often with low, unpredicatable germination and loose viability shortly after storage.

The African eggplant is known for its low germination even when placed under conditions regarded to be favourable. There are also reports that have suggested cultivated varieties of *Solanum melongena* (eggplant), possess seed dormancy (Gao and Yamata, 1991; Krishnasamy and Palaniappan, 1990; Yogeesha *et al*., 2006), which could explain such unpredictable germination behavior of *Solanum aethiopicum* L. However, no such information is available for the African eggplant. While the Food and Agriculture Organization of the United Nations (FAO) proposes a minimum germination percentage

of 70 % for eggplant (FAO, 2010), this is hardly achieved. The absence of a certification system at the national level and procedures in Ghana for this crop makes it imperative to determine and establish the appropriate internal and external factors required to achieve maximum seed germination of this crop.

Further, since the crop is usually grown under rain-fed open field conditions, the environmental factors particularly the temperature during the development stage can greatly affect the fruit quality and seed quality therein resulting in poor emergence and crop establishment (Dranski *et al.*, 2010; Kortse and Oladiran, 2013; Singkaew *et al*., 2017). Farmers who cultivate this crop experience poor germination, poor seedling emergence during nursery stage and rapid loss of viability during storage (Personal communication with African eggplant farmers). It is therefore important to investigate how different seed production environments influence the seed quality of the seeds lots of this crop under open field production.

Although the seed physiological and biochemical quality are genetically determined (Finch-Savage and Leubner-Metzger, 2006; Linkies and Leubner-Metzger, 2012; Yan *et al*., 2014), it is often influenced by external factors including the fruit maturity stage at harvest (Demir and Ellis 1992a; 1992b; Passam *et al*., 2010a; Vidigal *et al*., 2011; Bortey and Dzomeku, 2016), harvesting and postharvest handling methods (Malik, 2013) such as time and method of seed extraction and drying (Demir and Samit, 2001b; Bortey *et al.*, 2011; Takac *et al*., 2015, Bortey and Dzomeku, 2016). Any alteration of these factors could influence the seed quality aspects leading to poor germination, field emergence and ultimately lower yields.

For species whose seeds are borne in fleshy fruits, there are quite a number of literature on the influence of environmental conditions during seed development and maturation on seed quality. Other studies on the effect of harvest maturity on seed quality have been demonstrated for various crop species (Demir and Ellis, 1992b; Demir & Okcu, 2005; Incalcaterra and Caruso, 1994; Demir & Samit 2001a; Vidigal *et al*., 2011; Dias *et al.*, 2006a; Amini and Izadkhah, 2013; Bortey and Dzomeku, 2016). However, most of these studies have yielded inconsistent results between and within crop species. Further, these studies have been restricted to other families of the Solanaceae with the closest relative of African eggplant being *Solanum melongena* (Ozden and Demir, 2018; Passam *et al*., 2010a).

As considered an underutilized and researched crop, very little is known on how the seed production environment, harvesting practices and postharvest handling practices employed by farmers ultimately affect the physical, physiological and biochemical quality of the African eggplant seeds. The present study therefore sought to determine the seed germination requirement with focus on temperature and light for germinating seeds of African eggplant under controlled conditions for certification purposes. It further investigated how fruit harvesting practices during development and maturation affect the seed physiological and biochemical quality under contrasting seed production environments. Finally, we investigated the effects of postharvest handling practices with focus on after-ripening techniques, fermentation and drying methods on seed physiological quality of the African eggplant.

The results generated from this study should lead to an improvement of African eggplant seed production practices in a formal seed system and ultimately ensure the production

and supply of quality seeds to farmers/growers to increased fruit yields. It is further hoped that the information contained in this work will spur the interest in public research on other important but underutilized indigenous crops in Ghana and Africa.

1.1.1 Statement of Problem

The absence of formal quality seed production and distribution system in Ghana and most part of Africa where the African eggplant (*Solanum aethiopicum,* L.) is cultivated has led to the over-reliance of farmer-saved seeds. Consequently, farmers of this crop experience poor and unpredictable seed germination, seedling emergence and loss of seed viability in a short time during storage.

Over 90 percent of farmers source their own seeds extracted from fruits that have been left on the mother plant to ripen in the field after the marketable fruits have been harvested for the fresh market. This implies that often, fruits are harvested for the fresh market for consumption well before the seeds therein matures. The other source of seeds is from traders who extract seeds from fruits which are unsold in the market. These seeds are sent to the markets unripe but get ripened (after-ripened) in the cause of selling in the markets. These fruits thus come from unknown production sources and processes which results in poor germination.

Due to the lack of formal seed multiplication system for underutilized crops in Ghana, there currently exist no protocol regarding the temperature and light requirements for germinating these crops under controlled conditions. Hence, no national standards have been developed for the African eggplant on methods and procedures for conducting standard germination tests for the purposes of seed certification. A preliminary study of

the African eggplant seeds obtained from farmers and breeders sources recorded a wide variation in percentage germination under ambient conditions (25±2 °C).

The germination percentage ranged from 0 % to 25 % while freshly ungerminated seeds ranged between 53% and 87% (Botey, 2019, unpublished data). This percentages fall far short of the FAO recommended 70 % germination for *Solanum melongena*. This observation could be attributed to several factors that influences germination including a possible inherent dormancy as previously reported in cultivated varieties of eggplant (*Solanum melongena* L.), (Gao and Yamata, 1991; Yogeesha *et al.,* 2006). This type of dormancy if exist could be either due to seed immaturity at harvest or external stimuli such as temperature and light. How these external stimuli affects germination of this crop is however unknown.

1.1.2 Justification of Study

Generally, seed is regarded as one of the most critical input for increasing the productivity of agricultural and horticultural crops, thereby ensuring food security. While other agricultural inputs and practices directly or indirectly influence crop yields, the use of high quality seed alone increases productivity by 15-20 per cent (Chauhan *et al*., 2013, Elias, 2018). Therefore, lack of knowledge on the effects of production environment and the associated harvesting and postharvest handling techniques will ultimately render the seeds produced of poor quality (Demir and Ellis 1992a; Demir & Samit 2001a, 2001b; Silva *et al.,* 2012; Miklic *et al.,* 2006; Takac *et al.,* 2015; Elias, 2018).

The use of poor quality seeds by resource poor farmers cultivating the African eggplant has significant implication on their fruit quality, yields and their incomes (Horna *et al*., 2007; Bortey and Dzomeku, 2016). The cost of producing this crop also increases due to

high usage of agro-chemicals and seeds to compensate for poor seedling emergence, crop stand and disease incidences (Bam *et al.,* 2008; Bortey *et al.,* 2011). Thus, the most viable option for farmers is access to high quality seeds.

Like many African crops, the African eggplant (*Solanum aethiopicum* L.) falls into the category of underutilized species (Jaenicke and Hoeschle-Zeledon, 2006). Although, they are locally cultivated by resource poor smallholder farmers in West and Central Africa, the African eggplant has received very little scientific research, particularly in relation to quality seed production relative to its economic prospects (Gruère *et al*., 2006; Horna *et al*., 2006). With current efforts by the national agricultural research institutes in Ghana to develop and release improved varieties of the crop, it is imperative that information on quality seed production techniques are simultaneously developed to foster a readily available production and supply system for quality seed. This is the surest way farmers and growers can derive the maximum genetic potential of the improved varieties.

Seed development and maturation is one of the main factors that determine the quality of seed. It is a requirement for successful germination and seedling emergence of vegetable seeds (Dias *et al*., 2006; Tetteh *et al.,* 2018). However, several studies have demonstrated the existence of variations among plant species regarding the time and point in the development process where seed quality is maximum and how it is related to seed and fruit maturation characteristics (Ellis and Filho 1992a; Demir and Ellis 1993; Welbaum 1999; Vidigal *et al*. 2011; Tetteh *et al.,* 2018).

In addition, after- ripening of fruits has been reported as as a technique that has the ability to improve or negatively impact on the quality of seeds (Demir & Ermis, 2005; Passam, *et al*., 2010a). Studies on Aubergine (*Solanum melongena*) a close relation of the African

eggplant (*Solanum aethiopicum*) revealed that after-ripening (post-ripening) significantly increased total germination (Ozden & Demir, 2018).

The produce good quality seeds of this crop requires an understanding of how the various factors aforementioned influence independently or interactively the seed physiological and biochemical quality. While various studies have assessed seed quality of selected species of the *Solanaceae* family in relation to some aspects of seed quality, little is known for the African eggplant, particularly the Gilo group, which is cultivated by many poor resource farmers in West and Central Africa. Further, there has not been a comprehensive studies on fruit and seed development, production environment effects, harvesting and postharvest handling practices and how they influence the quality of seeds of the African eggplant, hence the need for this study.

The expectation of this study was to provide a good scientific bases for setting national standards for seed certification purposes for this crop, provide private seed producers, farmers or growers knowledge and techniques for producing and obtaining high quality seeds of this crop. Finally, the information generated will offer farmers higher yields and incomes and reduce the cost of production associated with use of poor quality seeds.

1.1.3 Broad objective

To establish environmental, physiological and biochemical effects on seed quality of African eggplant to improve the sustainable production and supply of quality seeds for farmers.

1.1.4 Specific objectives

- To determine the effect of temperature and light on germination of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- To evaluate the influence of fruit harvesting maturity stage on the physical and physiological quality of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- To determine the effect of seed maturity stage on biochemical characterictics of African eggplant (*Solanum aethiopicum* L.) and its relationship with seed physiological quality.
- To evaluate the effect of harvest time and after-ripening on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- To evaluate the effect of fermentation and drying methods on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.).

1.1.5 Hypotheses

- H_0: Temperature and light has no significant effect on germination of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- H_1: Temperature and light has a significant effect on germination of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- H_0: Fruit harvesting maturity stage has no significant influence on the physical and physiological quality of two cultivars of African eggplant (*Solanum aethiopicum* L.)

- $H_{1:}$ Fruit harvesting maturity stage has a significant effect on the physical and physiological quality of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- H_0: Seed maturity stage has no significant effect on biochemical characterictics of African eggplant (*Solanum aethiopicum* L.)
- $H_{1:}$ Seed maturity stage has a significant effect on biochemical characterictics of African eggplant (*Solanum aethiopicum* L.)
- H_0: Fruit harvest time and after-ripening treatment has no significant effect on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- H_1: Fruit harvest time and after-ripening treatment has a significant effect on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- H_0: Fermentation and drying methods has no significant effect on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.)
- H_1: Fermentation and drying methods" has a significant effect on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.)

1.1.6 Research questions

i. What is the seed germination response of two cultivars of African eggplant to different constant and alternating temperatures?

ii. To what extent does light independently or interactively with temperature influence seed qualitative and quantitative characteristics of African eggplant (*Solanum aethiopicum* L.)?

iii. What is the physiological maturity stage and the suitable time to harvest two cultivars of African eggplant for high seed germination and field emergence under contrasting seed production environments?

iv. What is the relationship of fruit morphological traits such as fruit weight and size (diameter and length) at different maturity stages with seed physiological quality and to what extent does fruit epicarp colour changes during maturation indicative of seed physiological quality in African eggplants?

v. What is the accumulation pattern of biochemical components such as total protein, fats, soluble sugars, antioxidant activity and tannin during seed evelopment of African eggplant (*Solanum aethiopicum* L.) and how does it affects seed physiological quality?

vi. Which of the biochemical component (s) identified in (v) have the greatest contribution to the maintenance of physiological quality of African eggplant seeds?.

vii. Does seeds within harvested fruits of African eggplant after-ripen (fill and mature) under ambient storage conditions prior to extraction and to what extent does it influence the physiological quality and germination behaviour of African eggplant seeds?

viii. To what extent does fermentation techniques prior to seed extraction affects seed quality?

ix. What drying methods are suitable for African eggplant seeds and to what extent does it maintain seed viability?

1.1.7 Scope and limitations of study

This study was conducted in Mabanga in Bungoma County of Kenya and Chepkoliel in Eldoret, located in Uasin Gishu County of Kenya with a tropical monsoon and temperate oceanic climate classifications respectively. Therefore, the results presented herein reflects these climates and may be applied in same or similar climatic conditions.

The two cultivars selected and used for this study are popular African eggplant currently been improved by CSIR-Crops Research Institute to enable official release as varieties into the vegetable seed system in Ghana. The observed differences of the cultivars to the various treatments thus suggests that the results herein presented apply only to these two cultivars.

The present study focused on the various factors that affect the seed germination behaviour of the African eggplant seed at the physiological and biochemical levels. It addressed the conditions that are required to germinate the seeds under laboratory conditions with focus on temperature and light conditions only. It further focused on how contrasting seed production environments influence the appropriate time to harvest fruits for maximum seed physiological and biochemical quality. Final aspects of the study investigated how post-harvest handling techniques practiced by farmers also influence the seed quality, with focus on after-ripening, fermentation and drying. Hence, any interpretation of the results should be within this context.

CHAPTER TWO

GENERAL LITERATURE REVIEW

1.2 Taxonomy, Botany and Distribution of the African eggplant

The African eggplant (garden eggs), *Solanum aethiopicum* L. is an important crop of the Solanaceae family. Other close relatives are *Solanum macrocarpon* (Gboma eggplant) and *Solanum melongena* L. (Brinjal eggplant) (Sękara *et al.,* 2007). *Solanum aethiopicum* cultivars could be divided into four groups namely; Gilo Group, Shum, Kumba and Aculeatum Group (Lester, 1986; Caruso, 2001; Lester & Daunay, 2003). The first three are the most important in Africa. The common name 'eggplant' covers three closely related cultivated species, which belongs to the genus *Solanum* L., subgenus *Leptostemonum* (Levin *et al.* 2005; Levin *et al.,* 2006; Sekara *et al.,* 2007). The subgenus *Leptostemonum* comprises more than 30 % of the species of the genus *Solanum*. The first section comprises *Solanum melongena* L. (brinjal eggplant, aubergine), synonymous to *Solanum cumingii* Dunal, *Solanum pressum* Dunal, *Solanum undatum* Poiret sensu Ochse; and *Solanum macrocarpon* (Gboma eggplant) which is also synonymous to *Solanum integrifolium*; Poiret var. *macrocarpum,* and *Solanum melongena* L. var. *depressum* Bail.

The second section is the Oliganthes (*Solanum aethiopicum* L. (scarlet eggplant), synonymous to *Solanum integrifolium* Poiret, *Solanum integrifolium* Poiret var. *microcarpum*, *Solanum zuccagnianum* Dunal (Sekara *et al.,* (2007).

The African eggplant (*S. aethiopicum* L.) is phenotypically diverse species. It is a fairly woody deciduous annual, or perennial, which grows up to 100-150 cm tall. It has bisexual partially self-pollinated flowers and or cross-pollinated and produces single or

group of fruits (trusses) depending on the subspecies and varieties (Seek, 1997; Macha, 2005). The fruits vary from being bitter to sweet taste depending on the content of saponin. When fruits reach full maturity, the colour of fruits turn red or reddish-orange attributed to high carotene content (Macha, 2005).

Depending on the sub-groupings, *Solanum aethiopicum* cultivars could have hairy, inedible leaves, and fruits differentiated in shape (round, elongated, egg-shaped or spindly). Others have glabrous leaves eaten as a green vegetables; very small with inedible elongated fruits (Macha, 2005).

The Aculeatum group, which is described as ornamental eggplant, possesses hairy leaves and large fruits, often used for disease resistance breeding under the synonym *Solanum integrifolium* (Lester 1986, Caruso 2001, Lester and Daunay 2003). According to Anaso (1991), the Gilo cultivar group might have evolved from the Shum cultivar group through hybridization and selection. *Solanum aethiopicum* is reported to be more nutritious especially the leaves compared to tomato and *S. melongena* (Abdoulaye, 2009). The seeds scattered through the fruit also contain vitamin C and carotene and other nutrients (NRC, 2006).

Solanum *aethiopicum* and *Solanum macrocarpon*, are the most popular native, traditional vegetables in West and Central Africa (Agnieszka *et al*., 2007). It is reported to be present in virtually all of sub-Saharan Africa, but is less well known in South Africa and Madagascar (Edmond and Chweya, 1997). The centre of these eggplants diversity is Western Africa (Sękara *et al.,* 2007).

1.3 Economic Importance of the African eggplant

The African eggplant (garden eggs), *Solanum aethiopicum* (Gilo) is very popular and plays an important part in many diets. It is one of the most important vegetable crops in Ghana and West Africa (Owusu-Ansah *et al*., 2001; Grubben and Denton, 2004; Osei *et al.,* 2010). Not only is this crop consumed on an almost daily basis by rural and urban families but it also represents the main source of income for many rural households, millions of farmers, most of them women in the forest zone of the country and other parts of Africa (Asenso-Okyere *et al*., 2000; Owusu-Ansah *et al.,* 2001; Weinberger and Msuya, 2004). In Tanzania and Ghana, it is the third most frequently grown non-leafy vegetables (Weinberger and Msuya, 2004, Horna *et al.,* 2007). The most common group in Ghana is the Gilo group, which is thought to be more genetically heterogeneous due to higher cross-pollination than Kumba although some cultivars of the Kumba Group are available in the Ghanaian market (Horna *et al.,* 2007).

It is reported that domesticated and wild relatives of the African eggplant (garden eggs) have important breeding traits that remain to be explored (Alba *et al.,* 2005; Rizza *et al.,* 2002). Field observation of the crop has shown its ability to withstand drought and tolerate heat compared to tomato or conventional eggplant. It has also shown lower susceptibility to pests and diseases than that of the exotic eggplant (Grubben & Denton 2004).

Despite these immense nutritional, medicinal, economic and breeding research potential of the African eggplant, it has received very little research attention. There is little known on its fruit and seed development and the various changes that occur during such processes. Information on pre and postharvest handling to produce high quality seed of

the crop is also scanty. Thus, any attempt to explore these aspects of the crop is imperative to ensure that farmers and growers benefit from the full potential this crop has to offer.

1.4 Factors that influence Seed Germination and dormancy

2.3.1 Influence of Temperature on seed germination and dormancy

Seed germination refers to the protrusion of the primary root or radicle from the seed coat and marks as the starting point of plant growth. The ability of a viable seed to germinate and at what point this process begins is determined by a series of factors, including the inherent causes of germination and external environmental conditions (Bewly *et al.,* 2014). Temperature and light are two ecological factors of importance in terms of regulating the seed germination process (Bewly *et al.,* 2014).

Temperature has been shown to be one of the primary variable that affects both the percentage and speed of germination (Bewly and Black, 1994). Temperature affects germination through seed imbibition of moisture and the biochemical reactions that regulate the metabolism involved in the germination process such as hormone production and enzyme activity (Finch-Savage and Leubner-Metzger, 2006).

For seeds to germinate, it must imbibe water and a warmer environment will lead to evaporation hence decreasing the available moisture required for this process.

Further, Finch-Savage and Leubner-Metzger (2006) reported that two major hormones that regulate seed germination are abscisic acid and gibberellins. Abscisic acid promotes dormancy and inhibits germination while gibberellins advance germination and the genes

that control the production of these hormones are influenced by external stimuli including prevailing temperature (Finch-Savage and Leubner-Metzger 2006).

Temperature also play a major role in metabolic activities leading to seed germination such as the degradation of the endosperm tissue and seed coat rupture to allow radicle protrusion (Finch-Savage and Leubner-Metzger 2006). This implies that the chemical signaling the production of the activity of enzymes is in turn regulated by temperature (Finch-Savage and Leubner-Metzger 2006). Thus, if the appropriate temperature is not provided at the right time, these enzymes may become inactive (Peterson *et al*. 2007) and delay the germination process.

The germination percentage usually increases linearly with temperature up to an optimal temperature, after which the germination percentage decreases (Tolyat *et al.,* 2014; Laghmouchi *et al.,* 2017). Thus increased temperatures not only affect the germination process but also directly affect seedling growth after germination.

If dormancy should not just be associated with the absence of germination but rather a characteristic of the seed that determines the conditions required for germination as proposed by Vleeshouwers *et al.*, (1995); and Fenner & Thompson, (2005), then the germination behaviour of the African eggplant seeds either possess an inherent dormancy (physiological) or may just be lacking the suitable conditions required for optimum germination. Information on the suitable conditions in relation to temperature required for maximum seed germination in African eggplant is however not known, hence this study.

2.3.2 Influence of Light on seed germination and dormancy.

While temperature is broadly believed to regulate both dormancy and germination, light on the other hand is known to regulate germination (Bewley & Black, 1994; Baskin & Baskin, 2004; Fenner & Thompson, 2005).

Light independently has been considered to stimulate germination (Vleeshouwers *et al*., 1995) and to terminate dormancy (Benech-Arnold *et al*., 2000; Batlla & Benech-Arnold, 2005) or interact with temperature to regulate germination (De Villiers *et al.*, 2002). Like temperature, light is considered another important external stimuli that acts directly on germination (Thompson *et al.*, 2003; Fenner and Thompson, 2005).

The sensitivity of seeds to light, however varies according to species. Some seeds germinate equally well in light and darkness, while others germinate better under only light or darkness (Bewley and Black, 1994; Chanyenga *et al.*, 2012). According to Aud and Ferraz (2012), light helps to alleviate the adverse effects of germination when the incubating temperature is higher than is favorable.

Another role light plays is breaking of secondary dormancy in seeds that require light for germination (Hilhorst, 1990). This is achieved through the biosynthesis of GA and increase sensitivity of seeds to GA, which is known to advance germination (Finch-Savage and Leubner-Metzger, 2006).

While light effects on seed germination independently or its interaction with temperature has been studied for various species (Benvenuti *et al.*, 2001; Ochuodho and Modi, 2005; Motsa *et al.*, 2015), information on temperature and its interaction with light for optimum seed germination in African eggplant is scanty.

1.5 Effect of harvesting maturity stage of fruits on the physical and physiological quality.

One of the important factors that affect quality seeds during seed production is the ability to determine the suitable harvest maturity stage. Generally, seed crop should be harvested when seed quality in relation to germination and vigour is maximum (Welbaum, 1999). The end results of the process of seed forming and filling, which starts right after pollination, is to enable the seed to germinate after a rest period (Miklic *et al.*, 2006). For decades, it was held that most crops usually reach maximum germination and vigour at the end of the seed-filling period, when physiological seed maturity is attained and thereafter declines (Harington, 1972).

This was held to be true for seeds that are borne in fleshy-fruited species as well (Welbaum, 1999). According to Rajanna and Andrews (1980), the term physiological maturity (PM) occurs when the seed exhibits maximum dry weight accumulation and substantial loss of water. It is thus suggested that when the maximum germination capacity and vigour are attained at physiological maturity, it is recommended that harvesting be carried out (Patrick and Offler, 2001).

Nevertheless, reports on the stage when maximum seed quality is attained in the cause of its development varies substantially from crop to crop. Several studies have demonstrated that there are variations among plant species in occurrence of maximum seed quality during development (Demir & Ellis, 1992b; Vidigal *et al.*, 2011; Takac *et al.*, 2015; Bortey & Dzomeku, 2016; Tetteh *et al.*, 2018) and this is associated with seed and fruit maturation characteristics (Ellis & Filho, 1992; Demir & Ellis 1992a).

Seed maturation is one of the main factors that determine the quality of vegetable seed (Dias *et al*., 2006; Tetteh *et al.,* 2018). However, some studies have reported that seeds borne in fleshy-fruited species generally attain maximum germination and seed vigour after the seed-filling phase, which is reported to coincide with physiological maturity (Berry & Bewly, 1991; Welbaum, 1999; Demir & Samit, 2001a; Vidgal *et al.,* 2011). Others, however, suggest that maximum dry matter accumulation, associated with the seed-filling period does not conincide with maximum seed quality. This includes crops such as pepper (*Capsicum annuum* L.) Demir and Ellis (1992b; Oliveira *et al.,* 1999), melon (*Cucumis melo* L.) Welbaum and Bradford (1988) and tomato (*Solanum lycopersicon* Mill.) (Berry and Bewly 1991; Demir and Ellis, 1992; Demir and Samit, 2001b; Dias *et al.,* 2006).

Welbaum (1999) reported the optimum harvest maturity stage for cucurbit seed crops varies with the environment and cultivar. For muskmelon seeds, maximum viability and vigour in the field were obtained only for a relatively short period (45-60 DAA). Maximum standard germination, 5-day germination count and seedling emergence percentages occurred at 30 days after anthesis and coincided with maximum seed dry weight of Okra (Demir and Ermis, 2005). Bortey and Dzomeku (2016), however observed maximum germination and vigour at 40 DAA for the cultivar *Asontem*, which also coincided with maximum seed dry weight. For sweet pepper seeds (*Capsicuum annuum),* mass maturity of the seeds occured 75 days after anthesis, when seed moisture content was 47.3 % and the fruit colour had turned reddish. It also had highest seed high germination and vigour (Vidigal *et al*., 2011).

Using the fruit colour as an indicator of physiological maturity, Silva *et al.,* (2012) reported that *Jatropha curcas* L., seeds gave higher germination from seeds extracted from yellow and yellow-brown fruits compared to seeds extracted from green fruits, which had lower physiological quality and dry matter content. Recently, Tetteh *et al.*, (2018) using fruit ripening stage as an indicator of maturity in tomato cultivars concluded that high vigour and germination can be obtained from fruits harvested at half ripe, fully ripe red tomatoes.

On the contrary, others studies have suggested that maximum seed germination and vigour does not coincide with maximum dry weight. In an earlier study by Kwon and Bradford (1987), it was reported that tomato seeds obtained maximum germination and vigour 15 days after physiological maturity. Subsequent studies according to Demir and Ellis (1992a), confirmed this stage to be 70 days after anthesis. In their studies, maximum seed dry matter occurred 50 days after anthesis while maximum germination was attained later at 70 days after anthesis. Similar results have been reported for pepper seeds, where maximum seed dry matter and germination was attained 50 and 60 days after anthesis respectively (Oliveira *et al.*, 1999). In studying *Cucumis melo*, Oluoch and Welbaum, (1996) had earlier indicated that optimum seed quality occurred when the cultivar '*Top Mark'* fruit were harvested 50 to 55 DAA which is 10 days after edible maturity and 20 days past seed mass maturity.

In other words, the development of physiological maturation increases seed vigour and continues after seed mass maturity when seeds are already fully viable. These results contradict the hypothesis that maximum seed quality is attained at the end of the seed-

filling period and that seed viability and vigour begin to decline immediately thereafter (Demir & Ellis, 1992a).

For eggplant (*Solanum melongena*), which is a close relative of *Solanum aethiopicum,* it was reported that seed inside the fruit matures at 50-55 days after fertilization (Chen *et al.,* 1995, Passam *et al.,* 2010a). According to Demir *et al.* (2002), seed-filling with nutrients occurs 40-42 days after flowering and maximum seed quality is achieved 10-20 days later for eggplant. Yogeesha *et al.,* (2006) indicated that germination was possible at 41 days after flowering with maximum germination occurring at 57 days after flowering.

Most researchers on eggplant have concluded that to produce high quality seeds of eggplant, seeds should be harvested 55-60 days after flowering (Demir *et al.,* 2002; Rashid & Singh 2000; Chen, 2001; Yogeesha 2008; Passaam *et al.,* 2010a; Takac *et al.,* 2015).

Accordingly, the occurrence of maximum seed quality during development and its relation with seed and fruit characteristics are key factors used to define the suitable stage to harvest fruits. Since these features and characteristics are noted to be varied between crop species and even within same species, and further influenced by environmental stresses and postharvest handling practices, studies in determining how these factor solely and interactively effect various seed quality aspects becomes important. Such information currently is not available for the African eggplant, hence this study was to determine the ideal maturity stage to harvest fruits of this crop for maximum seed physiological quality and establish whether the fruit colour changes during maturation can serve as indicator for seed quality.

1.6 Biochemical changes in seeds during seed development and maturation.

Several changes in the physiology and biochemistry of seeds occur during the development and maturation process. Most of the reported physiological changes include seed dry weight, loss in moisture content, germination percentage and emergence rate, seed vigour, fruit and seed colour and their relationship with seed quality aspects have been studied (Yang *et al.,* 2004; Samarah and Abu-Yahya, 2008; Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009; Vidigal *et al.,* 2011; Eskandari, 2012). However, other biochemical techniques have also been demonstrated as effective tools for assessing seed quality. Ramya *et al*. (2012) observed the enzymatic activities that are involved in cell respiration during seed development and maturation of onion (*Allium cepa* L.) while Oliveira *et al.,* (2013) and Silva *et al.,* (2015) studied amylase enzyme expression in maize seeds and reserve mobilization and metabolites accumulation during development of *Capsicum baccatum* respectively. These numerous cellular and biochemical events that occur during seed development are reported to be associated with the acquisition of desiccation tolerance in seeds especially orthodox (Leprince *et al.,* 1993; Bewley *et al.,* 2013). These changes include activation of anti-oxidation defenses (Leprince *et al.,* 1993), accumulation of storage reserves and metabolites such as structural proteins, carbohydrates, lipids among other. These are reported to be indirectly related to the integrity of cell membranes and by implication confers seed vigour and storability (Weber *et al.,* 2005; Carvalho *et al.,* 2009).

2.5.1 Changes in protein during seed development and their effect on seed physiological quality

Silveira *et al.,* (2004), observed that buffer-soluble protein contents and dry matter increased progressively during development, reaching their maximum values at the matured stage *Pinus taeda*, a conifer. Similar observations was made by Chandra and Keshavkant (2016), when protein content in Madhuca (*Madhuca latifolia* Roxb.) seeds increased about 13 folds during the developmental stages. In sunflower seeds (*Helianthus annus* L.) however, the protein content decreased during seed maturation, which further sharply decreased preceding the oil biosynthesis indicating an inverse relationship of these two reserves (Renganayaki and Krishnasamy, 2001).

In studying the seed storage proteins accumulation in *Cleome gynandra* L. and *Brassica kaber* L., Ochuodho *et al.,* (2006) observed that the content of seed proteins increased as the seeds of Cleome matured. The authors further reported that green seeds (immature) extracted from green pods showed the least number of protein bands using SDS-PAGE profile. On the contrary, brown seeds extracted from the green pods accumulated more seed proteins compared to the green seeds, but less than the black seeds, which were considered fully matured. Silva *et al.,* (2015) also reported that **t**he soluble protein (SP) content in *Capsicum bacatum* seeds increased 66% from 10 to 40 days after anthesis (DAA) and remained almost unchanged until the last harvest. The identified polypeptide bands in their study showed molecular weights which well-matched the chains of different seed storage proteins previously reported in *Capsicum annuum* cultivars (Vladova *et al.,* 2000).

In the latter study, it was concluded that the intensity of all of the identified polypeptide bands increased progressively over the course of pepper seed development and was maximum at 50 days after anthesis. Protein accumulated in seeds during development and maturation is known to have a protective role. This is achieved through the synthesis of genetic material and the enzymatic reactions which are essential to cellular breakdown (De Souza *et al.,* 2018). Macgregor and Matsuo, (2016) also reported protein may act as an alternative substrate for respiration.

2.5.2 Changes in fats/lipids during seed development and their effect on seed physiological quality

Lipid reserve in seeds play a major role in cell membrane composition. It has a direct relation with seed quality due to its function as source of energy reserves during germination (De Souza *et al*., 2018). This was demonstrated when Sett *et al.,* (2016) in studying the *Albizia procera* during seed germination observed a steady decline in the level of total lipid concentration from 0.110 to 0.004 (mg/g) from the 1^{st} to 15^{th} day of germination. The relationship of seed proteins to the lipid bodies preventing lies in their ability to amalgamate through the action of hydrolytic enzymes (Pavithra *et al.* 2014). Silva *et al.,* (2015) reported that neutral lipids content increased 14-fold between 10 and 30 days after anthesis, reduced drastically 78% from 30 to 60 days after anthesis and doubled from 60 to 80 DAA in *Capsicum baccatum*. The pattern of lipids accumulation in rubber tree *Hevea brasiliensis* L. seeds increased steadily with from 30 DAA and peaked at 180 DAA, and was similar to that of protein content accumulation (De Souza *et al.,* 2018).

2.5.3 Changes in sugars during seed development and their effect on seed physiological quality

The role of starch in carbon storage, embryo formation and reserve biosynthesis in the early stage of seed development has been previously described in *Arabidopsis thaliana* (Baud *et al.,* 2002), *Medicago truncatula* (Djamel *et al.,* 2005) and soybean (Saldivar *et al.,* 2011). It is considered as the primary source of carbon during germination process and seedling establishment (Shaik *et al.,* 2016). During seed development of *Capsicum baccatum*, Silva *et al.,* (2015) found out in their study that the content of starch content decreased 67 percent at the first harvest (14 DAA) until 30 days after anthesis and thereafter increased 23 percent between 30 and 60 days after anthesis and remained steadily until about 85 DAA. Silveira *et al.,* (2004) also reported earlier that starch contents and dry matter increased progressively during development, reaching their maximum values at the mature stage of *Pinus taeda* L. During seed development of Madhuca (*Madhuca latifolia* Roxb.), total sugar content declined (3-folds), from 10 DAA (59.06 to 19. 14 mg/g FM) suggesting their supportive role in germination and early seedling growth (Chandra and Keshavkant, 2016).

Gill and Singh (1985) reported that seeds that are subjected to varied environmental stresses could influence germination, respiration and other related metabolic activities. Bernal-Lugo and Leopold (1992) observed decreased levels of soluble sugar and starch in Maize (*Zea mays*) as seed deteriorates. This they alluded to the low levels of substrate being available for respiration, thereby reducing seed germination and vigour. Accumulation of non-reducing sugars has often been shown to be associated with acquisition of dehydration tolerance of orthodox seeds (Corbineau *et al.,* 2000; Bailly *et*

al., 2001). Steadman *et al.* (1996) and Obendorf (1997) have suggested that seed sugar content could be used as an indication of seed vigour and storability.

During the development of *Capsicum baccatum* seed, Silver *et al.,* (2015) reported a sharp decrease in total soluble sugars (TSS) and total free amino acids (TFAA). The total soluble solids and total free amino acid contents decreased by 80% and 60 % respectively when seeds were first harvested 10 days after anthesis until 30 days after anthesis (Silver *et al.,* 2015). This decrease corresponded with the accumulation of neutral lipids and soluble protein, suggesting that these metabolites serve as precursors of carbon and nitrogen reserves (Silva *et al.,* 2015). The authors further reported differences in the non-reducing sugar (NRS) content during *Capsicum baccatum* seed development. The NRS content increased 5-times between 10 and 60 days after anthesis and then reduced 40% until the last harvest. The maximum non-reducing sugar content was at 60 DAA. It was established that NRS, accumulation during maturation drying plays a crucial role in desiccation tolerance (Silva *et al.,* 2015).

2.5.4 Antioxidant and enzyme activities during seed development and maturation and their role in seed quality maintenance

The activities of enzymes involved in cell respiration during seed development up to maturation have been studied for crop species such as onion (*Allium cepa* L.) by Ramya *et al.* (2012), Maize by Oliveira *et al.,* (2013) and *Capsicum bacatum* (Silva *et al.,* 2015). During development, as a by-product of metabolic reactions, reactive oxygen species (ROS) are generated, which have been shown to play dual functions as cytotoxic or

playing a role in development, dormancy breakage and defense against biotic and abiotic stresses (Berjak and Pammenter, 2008).

These ROS are popularly known to react with all Superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX), which constitute the major enzymatic systems by which cells catabolize free radicals, thus minimizes the severity of oxidative damage (Chandra *et al.,* 2015). SOD plays key role in conversion of superoxide into oxygen and hydrogen peroxide. While CAT, POX and APX catalyzes the conversion of hydrogen peroxide into water and molecular oxygen and therefore prevent further generation of free radicals (Chandra *et al.,* 2015).

Matamoros *et al.* (2010) observed that superoxide dismutase activity increased while APX activity was stable towards the end of maturation in fruits and pea seeds. According to the authors, relatively stable behaviour of APX may be attributed to the stabilization of ractive oxygen species production and decline in seed moisture content. De Souza *et al.,* (2018) observed higher activity of sodium dismutase, APX and catalase towards the end rubber tree seed development. The auther attributed such increase in enzyme activity to the high moisture content. At higher seed moisture content coupled with high temperatures, there is accelerated metabolism with associated enzymatic activities.

Studies to generate in-depth knowledge regarding pattern and mechanisms of reserves accumulation in seed is important for both quality seed production and handling and also serve as good indicators for plant-breeding activities (Kok *et al*., 2013). The metabolic changes taking place during seed development have been studied for several species (Baud *et al.,* 2002, Silveira *et al.* 2004, Saldivar *et al.* 2011, Ramya *et al.,* 2012; Oliveira *et al.,* 2013; Pavithra *et al.,* 2014; Silva *et al.,* 2015; De Souza *et al.,* 2018), however

there is an absolute lack of knowledge for the African eggplant (*Solanum aethiopicum*, L.).

1.7 Postharvest Handling Practices and their influence on Seed Quality Characteristics

2.6.1 Effect of after-ripening on seed physiological quality

Although, the eggplant fruit (*Solanum melongena*) a close relative of the African eggplant (*Solanum aethiopicum*) is non-climacteric and therefore not considered to ripen further after harvest (Passam and Karapanos, 2008). However, most crop species whose seeds are borne in fleshy berries are known to continue to mature after it has been detached from the mother plant, meaning the seeds within the harvested fruit may continue to develop and mature (Passam and Karapanos, 2008). Some of the seeds harvested may be less matured and result in dormancy due to the insufficiently developed embryo (Demir *et al.,* 2002). However, after-ripening is a technique that is reported to enhance seed quality and release partial dormancy in such fruits (Yogeesha *et al.,* 2006; Iglesias-Fernandes *et al.,* 2010; Passam *et al.,* 2010a) and promote germination in pepper, another member from the *Solanaceae* family (Edwards and Sundstrom, 1987, Passam *et al.,* 2010a; 2010b).

For instance, Passam *et al.,* (2010b) harvested eggplant fruits (*Solanum melongena* L.) 25 days after anthesis (DAA) and stored under ambient conditions of 25 °C for 20 days before seed extraction. The results was that, 1000 seed weight was significantly higher than seeds extracted from fruit harvested on the same day but without after-ripening.

After-ripening was also observed to improve the rate of seed germination in from fruit harvested 45–65 days after anthesis. They concluded that storage of prematurely

harvested fruit prior to seed extraction allows the seeds of these fruits to after-ripen *in situ* "and thereby increases seed size and germination (Passam *et al.* 2010a). Earlier, Dias *et al.* (2006b) found that allowing a short period of post-harvest fruit storage of tomato improves physiological seed quality. Kortse and Oladiran (2013) observed that after-ripening durations significantly influence 100 seed weight and germination percentage of melon (*Citrullus lanatus* (Thumb) seeds.

Shaheb *et al*. (2015) also found the highest germination of French bean seeds from the latest harvest than those harvested and extracted earlier. Fresh and dry seed weights, 100-seed weights and germination percentage responded positively from five to ten days after-ripening of eggplant (*Solanum melongena*) (Kortse, *et al.*, 2017). Ozden and Demir (2018) in studying Aubergine (*Solanum melongena*) a close relation of the African eggplant (*Solanum aethiopicum*) also revealed that after-ripening (post-ripening) significantly increase total germination and concluded that aubergine (*Solanum melongena*) seed germination can be increased through after-ripening treatment. Unfortunately, little information regarding this phenomenon is known on other species of *Solanum* such as the *Solanum aethiopicum*, particularly the Gilo Group popularly grown and consumed in West and Central Africa.

2.6.2 Effects of seed extraction and drying methods on seed germination capacity

Seeds that are borne in fleshy fruits are subjected to several postharvest handling practices which do affect the quality of seeds positively or negatively. Two of such important practices are seed extraction and drying methods. Several seed extraction methods such as wet, dry, natural fermentation, chemical fermentation, and mechanical extraction have been used to extract seed from fruit vegetables such as tomato (Demir

and Samit, 2001b), egusi melon (Ogbonna and Odo, 2011), cucumber (Chethan *et al.*, 2013) and eggplant (Franca *et al.,* 2013; Rahman *et al.,* 2015).

These various methods resulted in varied effects on seed quality among different species. Silva *et al.,* (1982) observed that natural fermentation gave a lower germination than acid treatment fermentation in tomato which concurred in a later study by Demir and Samit (2001b). However, in the latter, seeds extracted from fruits of 70 days after anthesis showed much resistance to the effects of acid extraction and gave a relatively higher germination comparable to the natural fermentation treatment. In evaluating some tomato varieties and how they respond to different extraction methods, Vishwanath *et al*., (2006) reported that 2.5% HCl for 30 minutes and fermentation for 24 hours or 48 hours showed above 90 percent germination and gave better seedling vigour index. They however, observed a low mycoflora load in acid extraction method compared to natural fermentation. For eggplant, Franca *et al.,* (2013) observed no significance difference among seed extraction methods used on first germination count.

Most farmers of the African eggplant usually extract seeds manually from ripened fruits or allow them in water for some time to ferment prior to extraction. A practice which may be termed as natural fermentation. Natural fermentation and manual seed extraction methods are commonly used methods for other crops especially tomato and eggplant (Demir and Samit, 2001; Nemati *et al.,* 2010). Empirical evidence is however scanty on the potential effect of these extraction methods and time of fermentation (soaking fruits in water before extraction) on seed vigour and germination quality.

After seed extraction from fleshy fruits, the next important step in maintaining the quality of seed is drying. The rationale for drying seeds is to reduce their moisture content to a level safe enough to prolong longevity during storage. This is so because seed moisture content in addition to storage temperature are the most important factors influencing seed longevity in several vegetables (Dickie *et al.,* 1990; Wang *et al.,* 2001). Several drying methods have been used for drying seeds. Due to the sensitivity of most vegetable crops to desiccation tolerance, drying procedures to reduce seed moisture content should not cause damage to the seed tissue.

Seeds are generally harvested at high moisture content especially seeds borne in fleshy fruits (Demir and Ellis, 1992a) and need to be dried before storage with a careful attention to the rate and extent of post-harvest drying (Babiker *et al.,* 2010).

Manish *et al.,* (2015) applied several drying methods to *Sorghum bicolor* (L.) Moench) seeds and observed that seed quality was preserved in conventional drying method (drying chamber at 15°C and 15% RH), and comparable to seeds dried using lithium chloride. Drying rate was however slow in the latter method. Silica gel according to the authors resulted in faster rate of drying to maintain moisture content and seed quality. Achigan *et al.,* (2004) also subjected Zea mays, *Vigna unguiculata* and *Vigna subterranea* to various low-cost drying methods and concluded that sun drying as a low-cost alternative allowed low moisture contents to be reached for maize (3.9-7.8%), (3.2-5.1%) for cowpea and (8.4-9.6%) for Bambara groundnut. These moisture levels are safe enough for long term storage. All drying methods including shade, sun and silica gel also resulted in good germination at shorter mean germination time (3-5 days) for crops studied (Achigan *et al.,* 2004).

While several studies have demonstrated the potential of various drying methods in reducing the seed moisture content to an acceptable levels for long term storage, information on African eggplant in this regard is scanty.

CHAPTER THREE

DETERMINATION OF THE EFFECT OF TEMPERATURE AND LIGHT ON SEED GERMINATION OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.)

Abstract

A preliminary study of the African eggplant seeds obtained from farmers and breeder sources recorded a wide variation in percentage germination under ambient conditions (25± 2 °C). The germination percentage (normal seedlings) ranged from 0% to 25%, while fresh seeds ranged between 53% and 87%. As temperature and light are important factors for seed germination, this study investigated the effect of constant and alternating temperatures and light on the seed germination of two cultivars of African eggplant (*Solanum aethiopicum* L.). Two independent experiment were conducted in this study. Exepriment involved 8 levels of temperature and two cultivars. The design was laid out in completely randomized design with 16 treatment combinations replicated four times (8 x 2). The second experiment was laid out in a 5 x 3 x 2 CRD in factorial arrangement. The factors were temperature (5), light (3) and cultivars (2). Seeds of two cultivars of African eggplant were subjected to constant and alternating temperatures and under three light exposure regimes for 14 days in a Germinator. Seed quality was accessed by first germination count (seed vigour), percent germination, mean germination time, time to reach 50% germination, germination index and mean daily germination. Cummulative seed germination of the two cultivars of African eggplant was significantly affected by both constant and alternating temperatures. The highest percentage germination under constant temperatures was recorded at 25 °C (76%). The maximum seed germination quality (76 and 95%) for the two cultivars were obtained when seeds were germinated at 30/20 °C alternating temperatures under alternating 8/16 hours light and dark periods. This condition also gave the shortest time (4-5 days) required to complete the germination process. Seeds of African eggplant exhibited neutrally photoblastic to percentage germination. The interactive effect of temperature and light was significant and improved germination, mean germination time and time to reach 50% germination. It is recommended that the two cultivars of African eggplant seeds should be germinated under 30/20 °C alternating temperatures and light/dark (8/16 hours).

Introduction

The African eggplant (*Solanum aethiopicum* L.) is one of the most commonly consumed fruit vegetable in Ghana and other West African countries. Both in quantity and value, the crop is the third, after tomato and onion and before okra (Osei *et al.,* 2010). Unfortunately, the long neglect of this crop by formal crop improvement programmes has resulted in absence or lack of formal seed system for the production and supply of quality seeds. Farmers therefore, rely largely on saved and recycled seeds for planting with observed low germination, field emergence and poor establishment.

A preliminary study of the African eggplant seeds obtained from farmers and breeder sources recorded a wide variation in percentage germination under ambient conditions (25 ±2 °C). The germination percentage ranged from 0% to 25% while fresh ungerminated seeds ranged between 53% and 87% (Botey, 2019, unpublished data). This observation could be attributed to several factors that influences germination. Previously, there are reports on seed dormancy in cultivated varieties of eggplant (*Solanum melongena*), which is a close relative of *Solanum aethiopicum* (Gao and Yamata, 1991; Krishnasamy and Palaniappan, 1990). This type of dormancy could be either due to seed immaturity at harvest or external stimuli such as temperature and light.

Among the various germination factors, temperature is the most prominent environmental factor regulating growth and development of plants (Koger *et al.,* 2004; Ghaderi *et al.,* 2008). The temperature at which the maximum germination and emergence occurs tends to differ among crops and within species. Motsa *et al.,* (2015) reported in their study of some African leafy vegetable seeds that optimum percentage germination temperatures ranged between 29 °C and 32 °C while minimum temperatures ranged between 8 °C to 15 °C.

Abdel *et al.*, (2016) also reported that germination were inhibited for warm season fruit vegetables such as pepper and tomato crops at 5 °C but responded to germination when temperatures were raised to 15 °C and above. Both pepper and tomato seeds however exhibited thermo dormancy, with no seed germination at 40 °C. Wilcox and Pfeiffer (1990), had earlier also demonstrated the effect of temperature on time to germinate (days). They reported that eggplant (*Solanum melongena* L.) and pepper (*Capsicum* spp.) took between 8 to 50 days when germination temperatures were reduced from 24 °C to 12.3 °C respectively.

The requirement of light for the germination of seeds of certain plant species prevents germination in places and times not favourable for seedling establishment (Fenner and Thompson, 2005). The light requirement of such seed acts as a mechanism that determines where and when germination takes place, and it is important for survival of the plant species concerned, as it prevents stored seed reserves from being depleted. Some seeds germinate equally well in light and darkness, whilst others germinate better under only light or darkness (Bewley and Black, 1994; Chanyenga *et al.*, 2012). Ochuodho and Modi (2005) reported that seed germination of *Cleome gynandra* was influenced by both light and temperature and this differed between the seed lots of same species. Seeds that were subjected to $\geq$ 12 h day^{1} at 20 °C continuously significantly ($p < 0.001$) reduced germination compared to those exposed to $\leq$ 8 hour day^{1}. Thus, it the requirement of these two important factors for germination of especially neglected crops becomes imperative.

For a rapid and uniform germination to occur, the seed must be placed in an optimum environmental condition such as temperature, moisture and in some cases light (Bewley

and Black, 1994; Fenner and Thompson, 2005). There is currently little known established temperature and light requirement for the African eggplant seeds and how they interact to influence their seed germination pattern under controlled conditions. Therefore, the objective of this study was to investigate the effect of temperature on the germination pattern and the influence of light in interaction with temperature on seed germination of African eggplant (*Solanum aethiopicum* L.) under controlled conditions.

Objective of the study

- To determine the effect of temperature and light on germination of two cultivars of African eggplant (*Solanum aethiopicum* L.)

Research Questions

i. What is the seed germination response of two cultivars of African eggplant to different constant and alternating temperatures?
ii. To what extent does light independently or interactively with temperature influence seed qualitative and quantitative characteristics of African eggplant (*Solanum aethiopicum* L.)?

3.1 Material and Methods

This study was conducted in two separate independent experiments (Experiment 1 & 2). An initial study on the proximate composition of the seed samples including, moisture, protein, fats, ash, fibre and carbohydrates was carried out at the Biochemical laboratory of CSIR-Crops Research Institute, Kumasi, Ghana. All other physical and physiological studies were conducted at the Seed physiology laboratory of the department of Seed, Crop and Horticultural Sciences, University of Eldoret, Kenya.

3.1.1 Plant Materials used for the study

Seeds of two cultivars of African eggplant (*Solanum aethiopicum* L.) cv. *Oforiwa* (Round-shaped fruit) and cv. *Kpando* (Elongated to Blocky shaped fruit) were obtained from CSIR-Crops Research Institute, Kumasi, Ghana for this study. These are most popular cultivars grown in Ghana and are currently been improved for released into the seed system. The initial seed lots used for objective 1 were produced in 2018 and about 4 weeks old in cold storage when they were collected for the experiment. Plate 1 and 2 shows the ripened fruits of the two cultivars and dried seeds respectively.

Plate 1: Ripened African eggplant fruits of cv. Kpando (A) and cv. Oforiwa (B) (photo by, Botey, HM. 2020)

Plate 2: African eggplant with fruits in the field (photo by Botey, HM, 2020)

3.1.2 Determination of Initial Seed physical and biochemical characteristics

i. Determination of Seed Weight and Seed moisture content

The thousand seed weight (TSW) was determined from a sample of five replicates of 1000 seeds using an analytical balance with 2 decimal digits and mean weight expressed in grams. The seed moisture content (SMC, %) was determined using the low constant temperature oven method at 105± 3°C for 24 hours with two replicates of 2 g of seeds (Brazil, 2009). Seed moisture content was expressed as a percentage on fresh weight basis (fwb).

ii. Determination of initial proximate composition of cultivars used for the study

The proximate composition of the dried seeds was determined by the official method of the Association of Official Analytical Chemists, AOAC (2005) as follows: Protein

(section 978.04C), crude Fat (section 930.09), ash (section 930.05) and crude fiber and Carbohydrate was calculated by difference (section 962.09).

Experiment 1: The effects of constant and alternating temperatures on seed germination behaviour of African eggplant.

Experimental layout and Design

The experiment was a two factor experiment comprising temperature and cultivar. Factor one was cultivar at two levels (cv. *Oforiwa* and *Kpando*) corresponding to CV1 and CV2 respectively while factor two comprised temperature regimes at 8 levels (15, 20, 25, 30, 35, 20/30, 30/25 and 35/20 °C) corresponding to T1, T2, T3, T4, T5, T6, T7 and T8. This was laid in completely randomized design (CRD) with 16 treatments combinations replicated four times as shown in Table 1.

Table 1: Treatment combination of cultivar (2) x temperature (8)

Cultivar	Temperature	Treatment combination
CV1	‘T1	CV1T1
	T2	CV1T2
	T3	CV1T3
	T4	CV1T4
	T5	CV1T5
	T6	CV1T6
	T7	CV1T7
	T8	CV1T8
CV2	T1	CV2T1
	T2	CV2T2
	T3	CV2T3
	T4	CV2T4
	T5	CV2T5
	T6	CV2T6
	T7	CV2T7
	T8’	CV2T8

3.2 Data collected

3.2.1 Percent Seed Germination

Four replicates of 25 seeds were placed in petri dishes on two layers of Prat Dumas (A009108-100 units, 90 mm) filter papers, moistened with 5 ml of distilled water. Additional water was added to filter paper as and when necessary to keep the filter paper moist. The seeds were then incubated in growth chamber (WTB binder BD 400) at the various constant and alternating temperatures regimes at 8/16 hours of light/dark periods. Seeds were observed and counted daily for germination for 14 days. Seeds were considered germinated when the radicle had protruded at least 2 mm from the testa.The cumulative percentage seed germination was calculated by the number of seeds germinated at the end of the test period against the total number of seeds sown and expressed as a percentage.

3.3 Data analysis

The cumulative percentage germination over time were calculated and presented in graphs. Germination percentages was arcsine transformed before subjected to statistical analysis. Data was checked for normality using Shapiro-Wilk test, followed by analysis of variance (ANOVA). Mean separation was carried out using Tukey HSD test at a significance level of 5% ($\alpha = 0.05$). The Statistical Tool for Agricultural Research (STAR) was used for analysing the data. Data on initial physical and proximate composition was subjected to ANOVA using and means separated by Fischers' LSD at 5% significance level.

Experiment 2: The effect of light and its interation with temperature on seed germination characteristics of African eggplant.

3.4 Experimental layout and Design

This experiment was a three factor experiment comprising temperature, light and cultivars (5 x 3 x 2) laid in a completely randomized design (CRD) with four replications. The temperature regimes applied were 15, 20, 25, 30/20 and 35/20 °C (five levels). The light regimes had three levels comprising 24 hours dark (L1), 24 hours full light (L2) and 8/16 hours of light/dark alternating (L3). The third factor was cultivar at two levels (CV1 and CV2). Sub-samples of seeds of the same cultivars used in the previous studies were used for this study. The light source was 6 tubes (100 cm) of TL-D 36W (Philips EcoBright®).

3.5 Data collected

3.5.1 Determination of seed germination characteristics

The germination procedure was same as previously described in 3.3.4. Germination capacity (G %) was determined as the proportion of germinated seeds in relation to the total number of seeds sown in the petri dishes. Other seed quantitative characteristics measured were Mean Germination Time (MGT), Time to 50% germination (T_{50}), Germination index (GI), Coefficient of velocity of germination (CVG), Mean daily germination (MGD) and were calculated using the Advanced Germination Measurement Tool (Khalid, 2018).

3.6 Data analysis

Data was checked for normality using Shapiro-Wilk test, followed by analysis of variance (ANOVA). Variables expressed in percentages were arcsine transformed before subjected to statistical analysis. Mean separation was carried out using Tukey's HSD test at a 5% probability. Statistical Tool for Agricultural Research (STAR) was used for data analysis. The relationship dynamics among these seed quantitative traits were measured by Pearson correlation using Statistical Tool for Agricultural Research (STAR).

3.7 Results and Discussion

3.7.1 Initial Seed Lot physical and germination characteristics

The thousand seed weight (g) and seed moisture content (%) of cultivars used for this study were significant ($p \leq 0.01$). Seeds of cv. *Kpando* were heavier (3.13g) than cv. *Oforiwa* (2.36g). Seed moisture content of cv. *Oforiwa* was slightly higher (11.5%) compared to 8.0% in cv. *Kpando* prior to germination (Table 1). The proximate analysis also showed a significantly higher total crude protein content in cv. *Oforiwa* (28.5 %) than cv. *Kpando*. Inversely, Total percentage carbohydrates was significantly higher in cv. *Kpando* (25%) than cv. *Oforiwa* (7.2%). Both had approximately same level of percentage fat (Table 2).

Table 2: Seed moisture content, 1000 seed weight and proximate composition (mean ± standard deviation) between two African eggplant cultivars used for the study

Cultivar	TSW (g)	% SMC	% Protein	% Fat	% Carbohydrate
cv. *Oforiwa*	2.36	11.5	28.5 ± 4.39	21.96 ± 1.79	7.2 ± 3.25
cv. *Kpando*	3.13	7.9	13.2 ± 1.13	22.55 ± 1.63	25.2± 3.27
LSD (5 %)	0.008	0.217	7.26	4.11	6.58
P (5%)	<0.001	<0.001	0.004	0.71	0.002
Mean	2.74	9.74	20.9	22.26	16.2

TSW: Thousand Seed Weight; SMC: Seed Moisture Content.

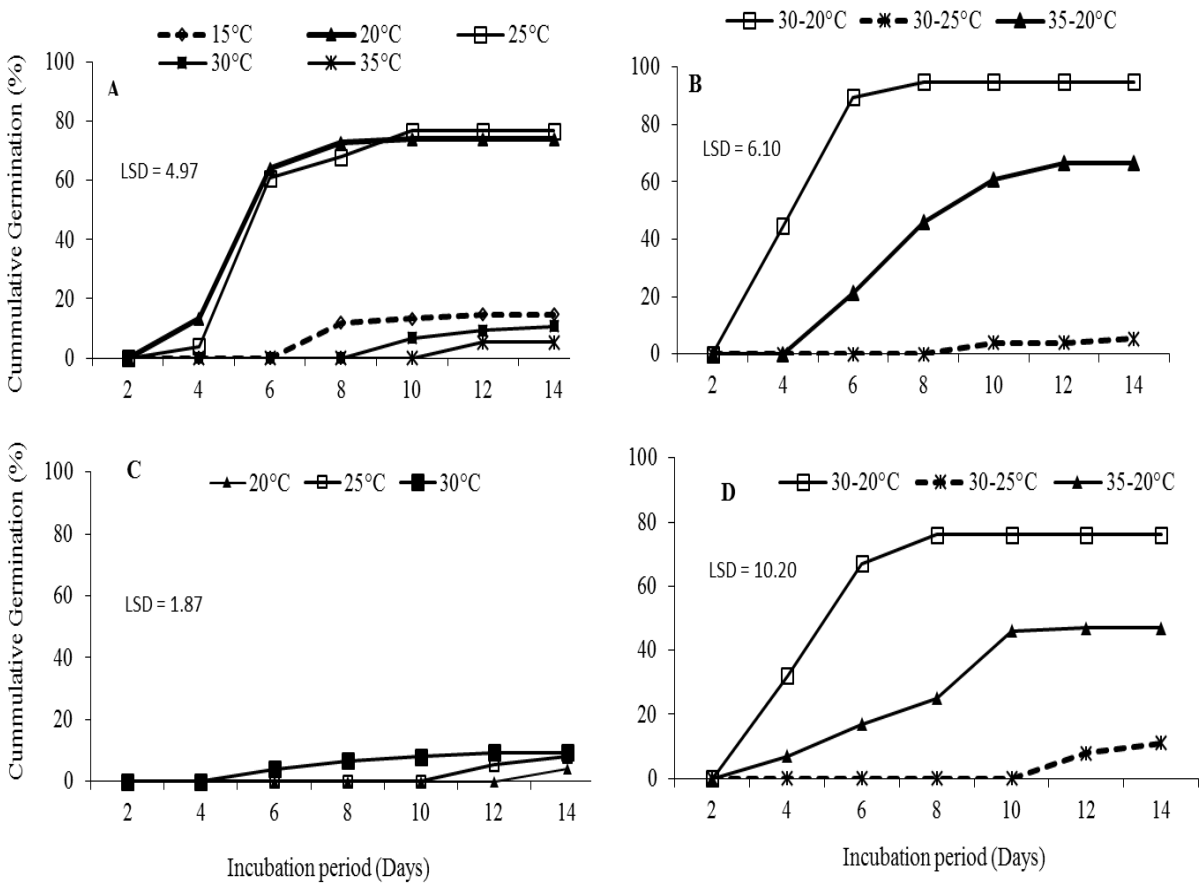


Figure 1: Cumulative Percent Germination of cv. *Oforiwa* seeds under various constant (A) and alternating temperatures (B) and cv. *Kpando* seeds under constant (C) and alternating temperatures (D) germinated under 8 hours/16 hours light and dark photoperiods. Note: LSD $_{0.05}$.

3.7.2 Effect of constant and alternating temperatures on cumulative percentage seed germination (G %)

The interactive effect of temperature and cultivar on cumulative seed germination percentage was highly significant ($p \leq 0.01$). The total germination of *cv. Oforiwa* was significantly higher than *cv. Kpando* regardless of constant and alternating temperature conditions during germination (Figure 1). However, under constant temperatures (Fig. 1A), germination was maximum (74%) at 20 °C and 25 °C (76%) while lower temperatures of 15 °C and higher temperature of 30 °C and 35 °C gave the least percent seed germination ≤ 15 % for *cv. Oforiwa*. No germination was recorded at lower (15 °C) and higher (35 °C) constant temperatures for *cv. Kpando* (Fig. 1C).

The results is consistent with other solanum species. Kamgar (2009) observed maximum seed germination for *Solanum nigrum* at constant temperatures of 26 and 30 °C. Similarly, Finch-Savage and Leubner-Metzger (2006) recorded optimum germination for five solanum species at 28 -33 °C constant temperatures. The maximum germination (74%) recorded for cv. *Oforiw*a (Fig. 1A) at 20 °C is consistent with that of *Solanum ptychchanthum* (Zhou *et al.,* 2005) and *Solanum betaceum* (Mavi and Uzunoğlu, 2020). At lower temperatures, no germination occurred in both cultivars. This is consistent with other reports for *Solanum lycopersicum and Solanum nigrum*, where seeds failed to germinate at low temperatures (5-10 °C) (Abdel *et al.,* 2016; Dong *et al*. 2019). This observation confirms that the African eggplant (*Solanum aethiopicum* L.) as related to the commonly cultivated relative (*Solanum melongena* L.) is a warm tropical crop and requires relatively warmer environment for germination, characterized by slow germination rates (Chen and Li, 1996).

At constant higher temperatures, seeds exhibited thermo inhibition, especially in *cv. Kpando*. This could be attributed to the inhibitory effects of protein denaturing at higher temperatures or embryo immaturity as reported for eggplant (Yogeesha *et al.,* 2006). Saeidnejad *et al*., (2012) also asserted that such observed differences could be due to genetic variability among the seeds. The inability of seeds to germinate at higher than optimal temperatures is attributed to a condition called thermos-inhibition (Hills and van Staden, 2003). The current study corroborates studies of other solanaceous crops. Abebe (1993) observed that seeds of *Solanum lycopersicum* seeds did not germinate at high temperatures of 35 °C but germinated when temperatures reduced to 25 °C or 30 °C. Abdel *et al.,* (2016) later concluded that the optimal temperature for tomato is 25°C for maximum germination. Similarly, *Solanum nigrum* failed to germinate at higher temperatures of 35 and 40 °C (Dong *et al*., 2019).

The current study further showed that seed germination increased significantly ($p \leq 0.01$), when subjected to alternating temperatures (Fig. 1B and D) for both cultivars. The highest seed germination were 95 % and 76 % for *cv. Oforiwa* and *cv. Kpando* respectively at alternating temperatures of 30/20°C. This suggests that African eggplant germinates well under alternating temperatures of 30/20°C, which simulates the tropical temperatures. This result concurs with reports of Chen and Li (1996) and Ullio (2003) that seed germination in *Solanum melongena* ranges from 20-32°C with the highest germination at 27-30°C. Similarly, Cutti and Kulckyzynski (2016) reported maximum germination (86 -95%) for S. *torvum* at 20/30 under 16/8 hours light and dark periods. Torres-Gonzalez (2019) and Dong *et al.,* (2019) concluded that alternating temperatures of 25/15 °C and 30/20 °C, gives maximum germination for *Solanum betaceum* and

Solanum nigrum respectively. In a non-dormant seed, temperature alternations may accelerate germination by regulating the balance of growth inhibitors and promoter hormones (Copeland and McDonald, 2001; Ferreira and Borghetti, 2004). This suggests that alternating temperature is effective in increasing the germination percentages of most seeds than constant temperatures (Govindaraj *et al*., 2017).

3.7.2 Effect of light and its interaction with temperature on seed germination characteristics of two African eggplant seeds

Figure 2 illustrates the effect of three light exposure regimes (A, B and C) and its interaction with temperature on seed germination characteristics. Light independently did not have any significant effect on percentage seed germination ($p > 0.05$). It did improve significantly ($p \leq 0.01$) mean germination time and T_{50}. There was also a significant interactive effect ($p \leq 0.01$) of light x temperature and cultivar on percentage germination, mean germination time and time to reach 50 percent germination.

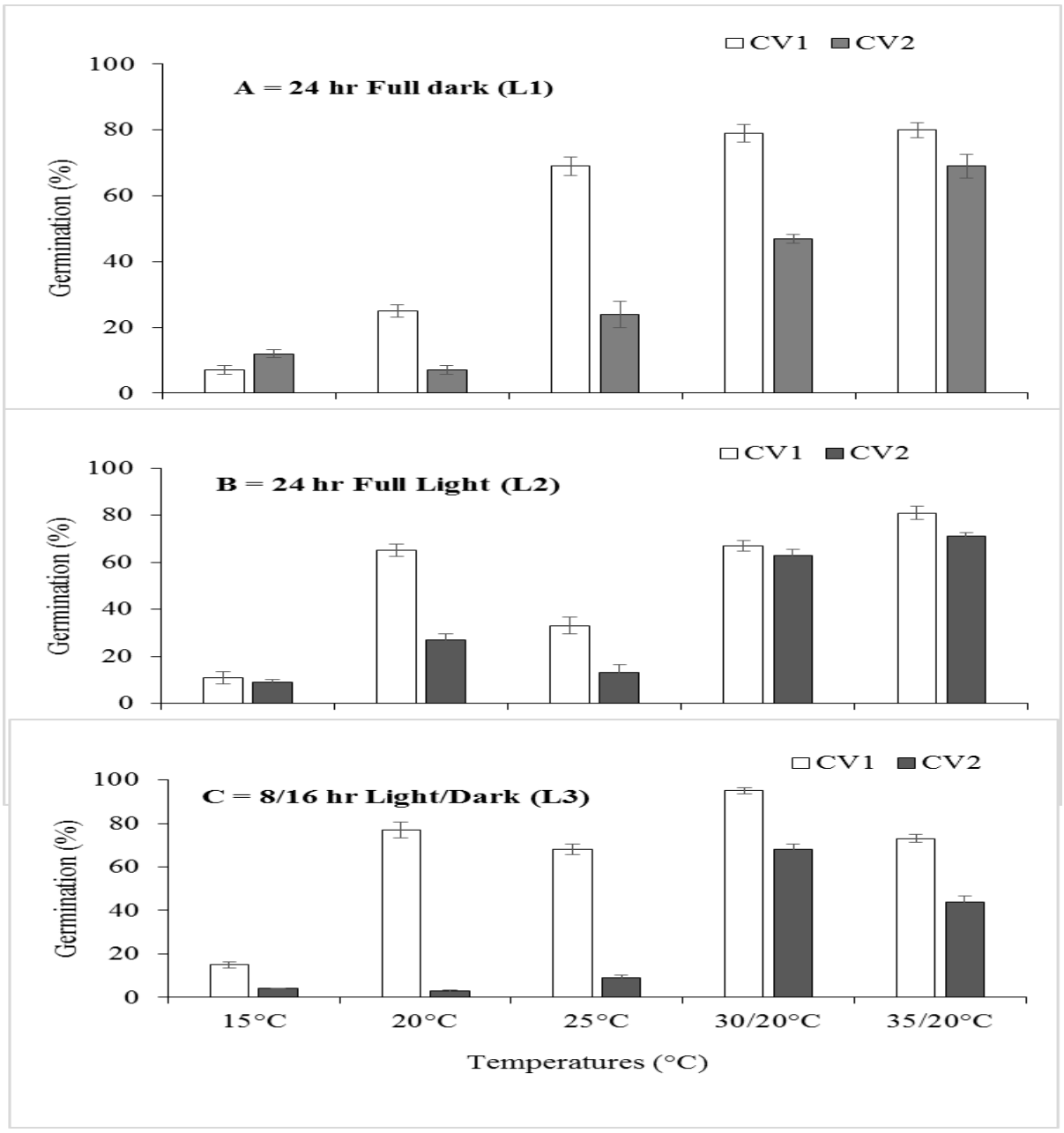


Figure 2: Effect of Temperature and its interaction with light exposure on seed germination (%) ± SEM of two cultivars of African eggplant. (CV1: cv. *Oforiwa*, CV2: cv. *Kpando*, SEM: Standard error of means).

The seed germination of *cv. Oforiwa* was essentially indifferent to light although there was some minor negative effect when germinated at 20 °C under full darkness (Figure 2A). This concurs with the report that many cultivated species are indifferent to light to germinate (Miranda *et al*., 2017). This result further concurs with the germination behaviour of *Solanum nigrum*, where light had no effect on seed germination at certain constant and alternating temperatures (Dong *et al.* 2019).

Seed germination of *cv. Kpando* however, was significantly ($p \leq 0.01$) affected when placed under full darkness (Fig. 2A) or limited light (Fig. 2C) at constant temperatures (20 °C and 25 °C). Germination improved at alternating temperatures (30/20°C or 35/20 °C) and full light (Fig. 2B) or limited light (Fig. 2C). This germination behaviour of *cv. Kpando* could be a temperature or light imposed dormancy (Baskin and Baskin, 1998). It further suggests that seed germination in African eggplant requires an interaction of light and temperature. At alternating temperatures (15/5 °C and 20/10 °C), germination was greater in darkness (75.5 and 93%) than in light/dark for *Solanum nigrum* (Dong *et al*., (2019). Similarly, Ochuodho and Modi (2005) observed that *Cleome gynandra* seeds lots failed to germinate or were lower <10% under 20 °C with 24h light but improved significantly under alternating temperatures of 30/20 °C. Zhou *et al*., (2005) also observed that seed germination of S. *physalifolium* was not sensitive to photoperiod and germinated well under 14 hour photoperiod or continuous darkness at 30 °C. This suggests that light effects on seed germination depends on the surrounding temperatures. Thus, seed germination in most solanum species will occur at favourable temperature regimes irrespective of the presence or absence of intermittent exposure to light as observed in the present study.

3.7.3 Effect of Light and Temperature on mean germination time and time to reach 50% germination of two cultivars of African eggplant seeds

Table 3: Mean germination time and time to reach 50% germination (mean ± standard deviation) as influenced by temperature and light durations on two cultivars of African eggplant.

Cultivar	Temp.	Mean Germination Time (MGT, days)			Time to reach 50% germination (T_{50}, days)		
		L1	L2	L3	L1	L2	L3
cv. *Oforiwa*	15 °C	5.3 ± 0.58 a	8.3 ± 1.89 a	8.5 ± 1.75 a	4.8 ± 0.58 c	8.2 ± 2.02 a	7.6 ± 1.51 a
	20 °C	7.4 ± 1.83 bc	6.3 ± 0.31 bc	5.6 ± 0.22 b	6.7 ± 2.14 bc	5.2 ± 0.21 bc	5.1 ± 0.21 b
	25 °C	9.7 ± 0.41 a	7.7 ± 2.00 ab	6.0 ± 0.65 b	9.5 ± 0.28 a	7.0 ± 2.08 ab	5.1 ± 0.39 b
	30/20 °C	8.3 ± 0.40 ab	4.9 ± 0.33 c	4.5 ± 0.25 b	7.6 ± 0.22 ab	3.7 ± 0.15 c	3.9 ± 0.37 b
	35/20 °C	5.9 ± 1.03 c	5.6 ± 0.36 bc	8.2 ±0.94 a	4.9 ± 0.99 c	5.0 ± 0.33 bc	7.7 ± 1.07 a
cv. *Kpando*	15 °C	9.2 ± 3.70 b	8.3 ± 2.89 ab	*	8.5 ± 3.50 b	7.7 ± 2.84 b	*
	20 °C	12.0 ± 1.00 a	10.2 ± 0.21 a	*	11.5 ± 1.00 a	10.5 ± 0.63 a	*
	25 °C	11.0 ± 2.00 ab	9.3 ± 1.28 a	6.9 ± 1.83 ab	10.8 ± 1.67 a	9.0 ± 1.32 ab	6.3 ± 1.89 a
	30/20 °C	10.6 ± 0.32 ab	6.0 ± 0.59 c	4.9 ± 0.23 b	9.8 ± 0.54 ab	5.5 ± 0.88 c	4.1 ± 0.25 b
	35/20 °C	7.0 ± 0.39 c	6.3 ± 0.43 bc	7.9 ± 0.43 a	6.1 ± 0.39 c	5.5 ± 0.37 c	7.0 ± 0.56 a

Means followed by the same letter (s) within a column are not significantly different at 0.05 probability level according to Tukey's HSD test. Temp.: Temperature; L1: 24 hours Full Dark; L2: 24 hours Full Light; L3: Alternating 8/16 hours of light/dark periods; *: No germination occurred.

The results of this study indicated a highly significant ($p \leq 0.01$) interactive effect of light and temperature on mean germination time (MGT) and time to reach 50 % germination (T_{50}) (Table 3). The interactive effect of cultivar x temperature x light regimes was also highly significant ($p \leq 0.001$) for MGT and T_{50}. The results showed that, under constant lower temperatures (15 °C) at full light or alternating light/dark periods, seeds of both cultivars took more days to reach 50 % germination (T_{50}) and complete germination (MGT) Table 3. Seeds took 8.3 to 8.5 days to complete germination (MGT) under 15 °C but gradually reduced to (5-7 days) when temperature was increased to 20 °C or 25 °C.

This results concurs with an earlier observations by Wilcox and Pfeiffer (1990) that eggplant and pepper seeds took between 18 to 44 days to complete germination when temperature was reduced from 16.7 to 14.5 °C respectively. At higher temperatures of 21.1 to 24 °C however, germination was completed in 7-8 days (MGT). Simon *et al.*, (1976) has earlier reported similar trends for cucumber seeds where the time required for 50 % of seeds to germinate increased to 14 weeks at about 14 °C or below. The rate of seed germination (the reciprocal of MGT) is reported to usually increase as the temperature rises (Iannucci *et al.*, 2000; Al-Ahmadi and Kafi, 2007) and this is attributed to the reactivation processes occurring within the imbibing seeds (Hills and van Staden, 2003).

Thus, the temperature effect observed in this study suggests that at lower temperatures, the rate of metabolic activities was retarded or enzymatic activities were inhibited (Kamala and Maguire, 1992; Thygerson *et al.*, 2002). This low temperature condition slows down the diffusion process which causes a disruption of imbibition and escape of solutes from seeds, which are critical in the protrusion of the radicle, hence delaying

germination (Thygerson *et al*., 2002). Consequently, lower temperatures are not ideal for germinating African eggplant seeds.

3.7.4 The correlation dynamics of seed quantitative traits as a function of temperature and light interactive effect

Table 4 shows the relationship among the seed quantitative variables measured and how they relate to the percentage seed germination.

Table 4: Correlation dynamics among seed quantitative measurements of African eggplant as influenced by the interactive effect of temperature and light.

	G (%)	MGT	MGR	MDG	CVG	GI	T_{50}
G (%)	1		.				
MGT	-.481**	1					
MGR	.453**	-.966**	1				
MDG	1.00**	-.481**	.453**	1			
CVG	.453**	-.966**	1.000**	.453**	1		
GI	.934**	-.675**	0.675**	.934**	.675**	1	
T_{50}	-.491**	.987**	-.954**	-.491**	-.954**	-.665**	1

*G (%): Germination percentage; MGT: Mean germination time (days); MGR: Mean germination rate (day^{-1}); MDG: Mean daily germination (seed/day); CVG: Coefficient of velocity of germination (%); GI: Germination Index (seed/day); T_{50}: time to 50% germination (days); **: Correlation is significant at the 0.01 level; *: Correlation is significant at the 0.05 level.*

All the quantitative parameters measured significantly ($p \leq 0.01$) relate to seed germination (Table 4). While mean germination time (MGT), correlates significantly with percent seed germination, giving an indication of the time taken for a seed lot to

germinate, it was not strong (-0.481**) because, this measure fails to account for the time spread and uniformity of germination (Kader, 2005).

Germination index (GI) and Mean daily germination (MDG) however, strongly correlated to seed germination 0.934** and 1.00** respectively (Table 4). Mean daily germination indicates the percentage of filled-seed germinating at the end of the test period divided by the number of days of the test (Diavanshir and Pourbeik, 1976). GI in the other hand from this study appears to be the most comprehensive measured parameter as it combines both the germination percentage and its speed in terms of spread and duration (Kader, 2005).

It is well known that seed germination percentage represents the number of seeds germinated within a specified period under favourable conditions such as suitable temperature, adequate moisture and in some seeds light as observed for African eggplant in this study. However, the important parameters measured gives further understanding of the seed germination behaviour and represent significant impact from agronomic, planning and physiological perspectives (Jones and Sanders, 1987; Kader, 2005). These parameters are also significant in giving an indication of the seed vigour and stress resistance of the African eggplant seeds studied (Kader and Jutzi, 2002).

3.8 Conclusion

The interactive effect of temperature and light exposure improved seed germination of two cultivars of African eggplant (*Solanum aethiopicum* L.) particularly under alternating temperatures of 30/20 °C with limited light periods (8/16 hours).

3.9 Recommendation

Seeds of African eggplant (*Solanum aethiopicum* L.) should be germinated at alternating temperatures of 30/20 °C under alternating light/dark of 8/16 hours for maximum seed germination percentage and mean germination time.

3.10 Suggestions for further study

It is important to investigate further the temperature gradient for other cultivars to verify if the relative differences observed in their responses to temperature and light exposures is solely due to genetic.

CHAPTER FOUR

EVALUATION OF THE INFLUENCE OF FRUIT HARVESTING MATURITY STAGE ON THE PHYSICAL AND PHYSIOLOGICAL QUALITY OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.).

Abstract

Studies regarding seed maturation contribute significantly in determining the suitable fruit maturity stage for harvesting to obtain maximum seed quality. The suitable harvest time to harvest the African eggplant for maximum seed germination is currently unknown. This work aimed at determining the influence of fruit harvesting maturity stage on the physical and physiological quality of two cultivars of African eggplant under a tropical and a temperate oceanic climatic conditions. The relationship of fruit morphological metrics such as fruit weight, diameter and fruit colour as an indicator of seed germination quality was also evaluated. The experiment was laid out in randomized complete block design with factorial arrangement. Factor one was harvest maturity stage at six levels while factor two was cultivars at two levels (6 x 2) with four replications. Fruits were harvested at different maturation stages from 20 to 82 days after anthesis before seed extraction. Seed quality was assessed according to moisture content, dry seed weight, seed length, thousand seed weight, percentage germination and emergence. The results indicated, seeds harvested precociously (20 and 34 days after anthesis), did not germinate or recorded very low percentage germination (0-20%). The physiological maturity for *cv. Oforiwa* occurred 48 days after anthesis while *cv. Kpando* took 14 to 28 days more to reach physiological maturity and coincided with maximum germination at 76 days after anthesis. The study showed that the harvest maturity stage significantly influenced the seed physical and physiological quality for the two cultivars. Fruit colour changes is also a suitable indicator for seed physiological quality determination. This study has established that when the African eggplant is produced under either tropical or temperate oceanic climates, maximum seed quality is obtained from fruits harvested 76 days after anthesis. For the purposes of seed production, it is recommended to harvest fruits 62 to 76 DAA.

Introduction

The Gilo group of the African eggplant (*Solanum aethiopicum* L.) is one of the most important vegetable crops cultivated by many resource constrained smallholder farmers in West and Central Africa including Ghana (Owusu-Ansah *et al.,* 2001; Weinberger and Msuya, 2004; Grubben and Denton, 2004). The absence of a reliable seed system for this crop has resulted in reliance on informal seed exchange among farmers and fresh fruit traders. Seeds obtained from these informal, farmer-saved or 'trader-saved' sources have low germination and field emergence (Bortey, 2019, unpublished data).

This phenomenon could be attributed to physiological seed dormancy as observed in *Solanum melongena* (Abdoulaye, 1992; Yogeesha *et al.,* 2006) and *Solanum tovum* (Cutti & Kulckzynski, 2016), which are close relatives of S. *aethiopicum*. It could also be due to harvesting and extracting seeds that are not matured. Although seed physiological quality is genetically determined (Linkies and Leubner-Metzger, 2012; Yan *et al.,* 2014), it is often influenced by factors such as fruit maturity stage at harvest and environment (Vidigal *et al*., 2011; Bortey & Dzomeku, 2016; Singkaew *et al.,* 2017).

Studies regarding seed maturation contribute significantly in determining the suitable fruit maturity stage for harvesting to obtain maximum seed quality. Particularly, for crops that experience continuous flowering and fruiting such as the African eggplant due to their indeterminate growth habit, fruits of different physiological maturity can be found on the same plant, making it difficult to establish the most suitable fruit harvesting time.

Seed maturation is one of the important components of seed quality and a prerequisite for successful germination and emergence (Perry, 1982). However, the stage of seed development and maturation when maximum seed quality is attained and its relationsip

with seed and fruit characteristics is subject of controversy'. Several studies have demonstrated that there are variations among plant species and growing conditions in occurrence of maximum seed quality during development (Takac *et al*., 2015; Bortey & Dzomeku, 2016; Ribalta *et al*., 2019; Tetteh *et al*., 2021). Additionally, the quality of seed and its association with fruit maturation characteristics have been reported with varied conclusions (Kortse *et al*., 2017; Tetteh *et al.*, 2018).

During seed development, the maximum seed germination may coincide with the maximum dry matter accumulation that characterizes physiological maturity or mass maturity (TeKrony & Egli, 1997) as observed for okra (Demir & Ermis, 2005; Bortey & Dzomeku, 2016), selected *Solanum aethiopicum* spp. (Tetteh *et al*., 2021) and *Allophylus edulis* fruits (Kaiser *et al.,* 2016). Other studies have also reported that maximum seed germination may not coincide with maximum dry matter accumulation as reported for tomato (Borges *et al.*, 2019), pepper (Ruiz and Parera, 2017), safflower (Ramos *et al*., 2021) and eggplant (Demir *et al*., 2002). In the latter reports, seed germination decreases after attaining maximum germination (Ellis, 2019).

Using fruit characteristics such as colour as an indicator of physiological maturity (maximum germination and vigour) has also been studied. Silva *et al.,* (2012) observed that seeds obtained from yellow-brown physic nut (*Jatropha curcas* L.) seeds had maximum vigour and germination. Similarly, Dranski *et al.,* (2010) in their study of the same physic nut concluded that for maximum seed germination quality in *Jatropha curcas,* fruits should be harvested when fruit skin colour gives a colour reading equal or smaller than 82, 70 or 65 nm of red, green or blue scales using a digital colour analyzer.

Tetteh *et al.*, (2018) in their study of two tomato accessions from Ghana (GH 9207 than in GH 9305) concluded that higher seed vigour and germination percentage can be obtained from fruits harvested at half ripe (pink), fully ripe (red) fruit colour stages irrespective of the accession. Thus, the fruit colour changes during maturation could be a suitable indicator for the occurrence of maximum seed quality.

In determining the seed germination characteristics as influenced by the above mentioned factors, not just the final germination percentage attained is necessary but also the speed and distribution of this germination are significant. This gives a broader knowledge of the seed lot under consideration and often used to judge the agronomic relevance of seed lots. Generally, germination is considered a qualitative developmental response of an individual seed that occurs at a point in time, but individuals within a treatment responds at different times (Scott *et al.*, 1984; Kader, 1998). Thus, the final germination alone is unsatisfactory for reporting results and the need to determine other quantitative variables was necessary.

There is little known regarding the African eggplant (*Solanum aethiopicum* L.) in relation to the seed and fruit quality changes that occur during development and maturation and how this influence seed physiological quality. This study was conducted to determine the ideal harvest time for maximum seed germinability and field emergence in two cultivars produced under a tropical and temperate oceanic climatic conditions. We also investigated the association of fruit morphological characteristics, such as fruit weight, diameter, length (size) and colour with physiological quality of African eggplant seeds.

Finally, the quantitative seed germination traits as a function of seed maturity were evaluated to understand the seed germination behaviour.

4.1 Objectives of the study

To evaluate the influence of fruit harvesting maturity stage on the physical and physiological quality of two cultivars of African eggplant (*Solanum aethiopicum* L.)

4.2. Research questions

i. What is the physiological maturity stage and the suitable time to harvest two cultivars of African eggplant for high seed germination and field emergence under contrasting seed production environments?

ii. What is the relationship of fruit morphological traits such as fruit weight and size (diameter and length) and fruit epicarp colour changes at different maturity stages on seed physiological quality of African eggplants?

4.3 Materials and Methods

4.3.1 Experimental Location and Climate

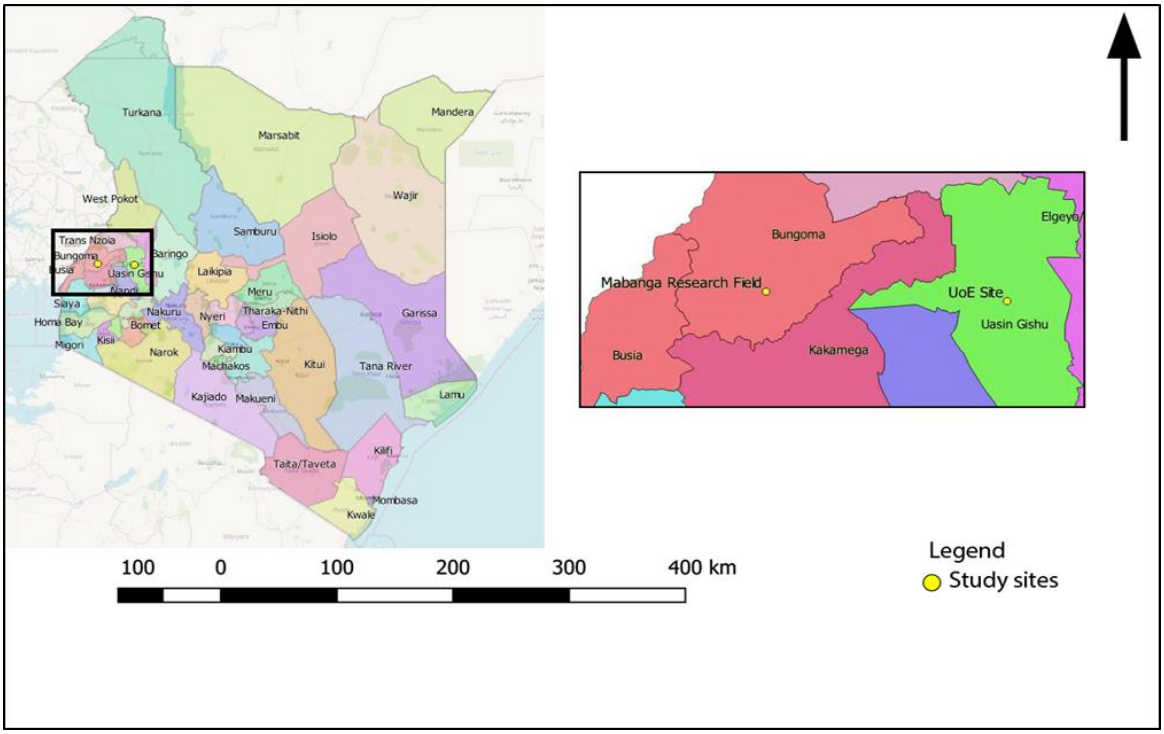


Figure 3: A Sketch Map of the Field Experimental locations (Author, 2019)

The experiments were conducted in two locations: University of Eldoret Agriculture Research Field, Chepkoliel in Uasin Gishu County (N00° 34.468´ E 035° 18.044´) and Mabanga Agriculture Training Centre (ATC) in Bungoma County (Kenya) (N00° 36.222´E034° 37.392´). Chepkoliel (Eldoret) falls under a Temperate Oceanic Climate (TOC), (Cfb) while Mabanga in Bungoma County falls under a Tropical Monsoon Climate (TCC) (Am) according to Koppen Climate classification (Köppen, 1884). Chepkoliel (Eldoret) is a highland plateau with altitude ranging between 1500 to 2100 metres above sea level while Bungoma has an altitude of 1,421 metres.

4.3.2 Planting Materials

Seeds of two cultivars of African eggplant (*Solanum aethiopicum* L.) cv. *Oforiwa* (Round-shaped fruit) and cv. *Kpando* as described in the previous chapter were used for this study.

Plate 3: African eggplant with fruits in the field (photo by Botey, HM, 2020)

4.3.3 Raising Seedlings for field establishment

African eggplants are generally planted using seedlings. Seeds were sown in a seed germination tray (54cm x 28cm x 4.3cm) containing a mixture of top soil mixed with cocopeat at a ratio of 1:1 in the nursery. One seed was sown per cell at a depth of approximately 3mm. Seedlings were transplanted after 6 weeks old (with a minimum of three true leaves) to an open field.

4.3.4 Field Experimentation and Design

In the open field at both locations, six (6) weeks old seedlings of African eggplant were transplanted at a spacing of 75 cm intra-rows and 60 cm inter-rows rows. The experiment was laid out in a randomized complete block design (RCBD) with 6 x 2 factorial arrangement with four replications. Factor one was harvest maturity stages at 6 levels (20, 34, 48, 62, 76 and 82 days after anthesis) while factor two was two cultivars of African eggplant (CV1, CV2), making 12 treatment combinations. Plot size was 6.5 m x 4 m with a total planting area of 19 m x 15 m. There were 8 plants per row with 6 rows per plot constituting a total of 48 plants per plot. The six harvesting stages were superimposed on each treatment plot.

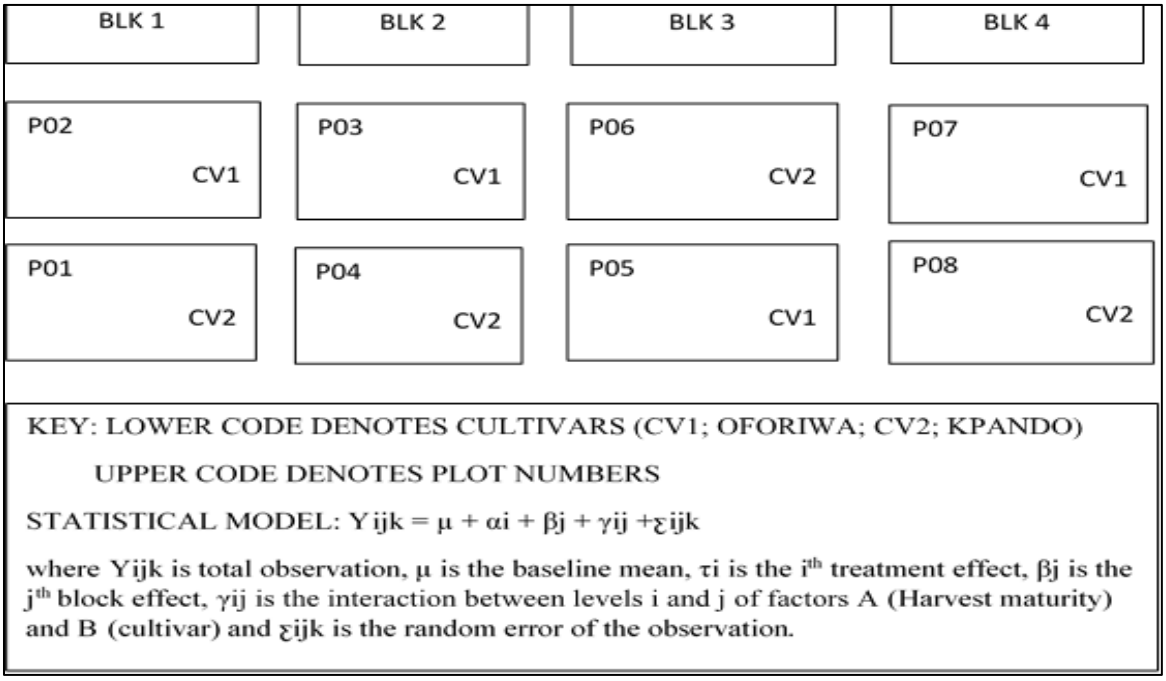


Figure 4: Field Layout for field experiments conducted at both Mabanga (Tropical monsoon) and Chepkoliel (Temperate Oceanic) Climates.

4.3.5 Determining the Harvesting Maturity Stages

Plants started flowering from 65-70 days depending on the cultivar. At anthesis (when flowers have fully opened), flowers were randomly tagged. For each plot/cultivar, a minimum of 60 flowers were tagged for each harvesting stage, making a total of about 240 flowers per plot per harvesting stage. Fruits were harvested at two weeks intervals except the last harvest, which was 7 days interval. In all, six harvesting maturity stages were studied: 20, 34, 48, 62, 76 and 82 days after anthesis (DAA).

4.3.6 Data collected

4.3.7 Fruit morphological data collected

Following each harvest at each maturity stage, 20 fruits were randomly selected to determine the fruit morphometric measurements as follows:

Fruit weight (g): Fruit weights of 20 fruits were measured using a weighing balance (0.01g) and the mean weight recorded and expressed in grams (g).

Fruit length (mm): The fruit length was measured from the shoulder of the fruit to the blossom end, excluding the peduncle using a digital veneer calliper. The mean fruit length was recorded and expressed in millimetres (mm).

Fruit width (mm): The fruit width was measured at the widest middle part of the fruit using a digital veneer-calliper and the mean expressed in millimetres (mm).

Seed number per fruit: Seeds were extracted not later than 24 hours after harvest by cutting fruits opened and removing seeds under water. Seeds were thoroughly washed in running tap water, followed by rinsing in deionised water. The number of seeds per fruit was determined by counting seeds extracted from 5 randomly selected fruits at each harvest stage in five replicates and the mean recorded.

Fresh seed weight (g): To determine the fresh seed weight (g), 100 seeds were drawn from the seed bulk extracted and fresh weight recorded.

4.3.8 Seed physical and physiological data collected

Dry Seed weight (g) and Seed Moisture Content (%): The dry seed weight (g) and seed moisture content were determined by drying seeds at a low constant temperature of 105 ± 3 °C for 24 h (Brasil, 2009). 100 seeds of four replicates were used to determine the dry seed weight. For seed moisture content, 2 grams of two replicates of fresh seeds were dried at the low constant temperature described above and calculated (fresh weight basis).

Thousand seed weight (g) and Seed length (mm): The 1000 seed weight (g) were obtained after seeds were air-dried for 3 days and eight replicates of 100 seeds were counted and weighed using an analytical balance (0.00g). Due to the size of the seed, the seed length (mm) was determined by placing 10 seeds along a measuring rule and the readings were recorded. The seed length per each seed was obtained by dividing the recorded reading by 10.

Percentage Seed Germination

This was conducted as previously described in general methods in chapter three.

Percentage Seedling Emergence determination

The emergence test was conducted for 21 days from sowing to final recordings. Four replicates of 30 seeds at each harvesting maturity stage were sown in top soil mixed with cocopeat at a ratio of 1:1 as used during the nursery stage in plastic trays (54cm x 28cm x 4.3cm) under a screen house condition. Maximum and minimum temperatures during the experiment were 34.4 °C and 12.8 °C respectively.

Seedlings that emerged were counted daily for 21 days and final percentage emergence was calculated at the end of the test using the formula as follows: Percentage Emergence (%) = total seedlings emerged/total number of seeds sown x 100. A seed was considered emerged when the first two true leaves protruded about 5-7 mm above the soil medium.

Germination time measurements: The mean germination time (MGT) and time to reach 50% germination (T_{50}) according to the formulae by Ranal (2009) respectively were used to measure the germination time. These were calculated using the Advanced Germination Measurement Tool Khalid (2018).

Germination rate measurements: The seed germination rate of the seed lots were measured by coefficient of velocity of germination, Germination index, Mean germination rate, Peak value and germination value according to the equations as follows:

i. **Coefficient of Velocity of Germination, CVG** = N1 + N2 + · · · + Nx/100 × N1T1 + · · · + NxTx. Where N = No. of seeds germinated each day, T = No. of days from seeding corresponding to N. (Jones and Sanders, 1987).

ii. **Germination Index, GI** = (10×n1) + (9×n2) + · · · + (1×n10) n1, n2 . . . n10 = No. of germinated seeds on the first, second and subsequent days until the 10th day; 10, 9 . . . and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively (Bench-Arnold *et al.*, 1991).

iii. **Mean germination rate, MGR,** according to Maguire, (1962)

$$MGR = \frac{\text{number of germinated seeds}}{\text{days to first count}} + \ldots + \frac{\text{number of germinated seeds}}{\text{days to final count}}$$

iv. **Mean daily germination (MGD):** It was calculated by the formula by Czabator (1962) as MDG = Total number of germinated seeds/total number of days.

v. **Peak value (PV):** This was calculated by the following formula given by Czabator (1962) as PV = Highest seed germinated/Number of days

vi. **Germination value (GV):** Germination value was calculated by the formula by Czabator (1962) as GV = PV x MDG.

Uniformity of germination measurements: The uniformity of seed germination of the seed lots was measured by the coefficient of variation of germination time using the formula according to Ranal *et al.,* (2009) below:

Coefficient of Variation of the Germination time *(CVt)*:

$CVt = \left(\frac{St}{tm}\right) x\ 100$; where, CVt = coefficient of variation of the germination time, %; s_t: standard deviation of germination time, days; and t_m: mean germination time, days.

Seed germination synchrony measurements: The germination synchrony of the seed lots was measured by the synchronization index (U) and degree of germination overlaps (Z) according to the formula by Ranal & Santana, (2006).

4.3.9 Determining the fruit colour changes during maturation and its relationship with seed germinability

To relate the fruit colour changes during maturation with seed germination traits as an indication of physiologically matured seeds, ten (10) fruit harvested at each maturity stage were visually classified according to the predominant colour using the Royal

Horticultural Society Colour Chart, 6th Edition (2017). The corresponding colour and code were recorded.

4.3.10 Data analysis

In each environment, the effect of African eggplant cultivar and harvesting stages were tested on the parameter measured in a two-way ANOVA. African eggplant cultivar and harvesting time were fixed factors whiles block and replicate were random effects. All values stated in percentages were arcsine transformed. Data were checked for normality of residual distribution and variance homogeneity (Shapiro-Wilk test) before subjecting to analysis of variance at 5% significant level using GenStat 14th Edition. Treatment means were compared by Tukey HSD test at 5% probability level.

4.3.11 Correlation dynamics of fruit traits and seed quality variables

The correlation dynamics between fruit morphological traits and seed quantitative traits were analyzed by Pearson correlation using Statistical Tool for Agricultural Research (STAR).

4.4 Results and Discussion

4.4.1 Seed physical and physiological changes during development of the African eggplant.

The relationship of the seed moisture content and dry content accumulation pattern during seed development and maturation is illustrated in Figure 8.

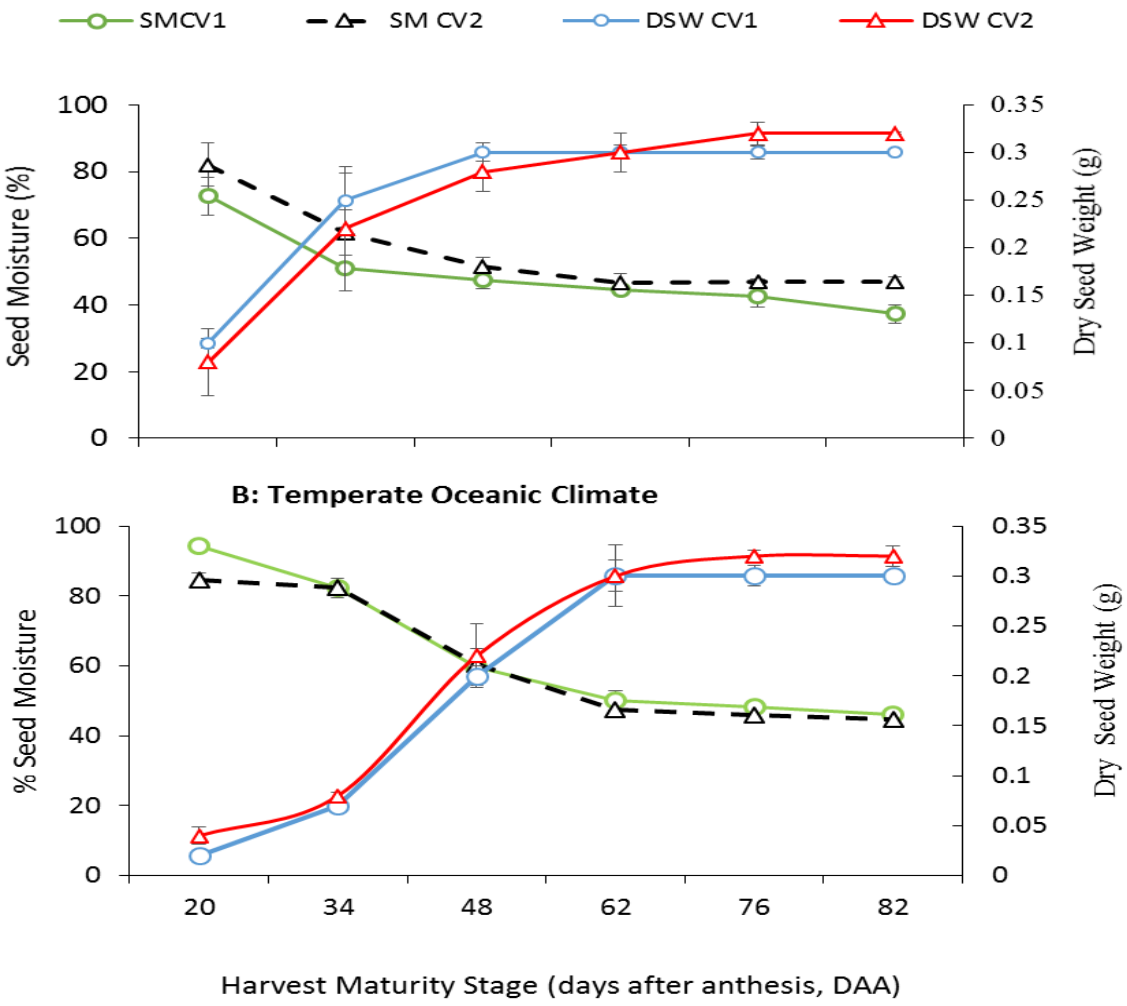


Figure 5: Seed moisture content and dry seed weight of African eggplant seeds (cv. *Oforiwa* (CV1) and cv. *Kpando* (CV2)) harvested at different maturity stages (DAA) produced under Tropical climate (A) and Temperate Oceanic climate (B) (vertical bars are ± standard deviation of means). SMCVI: Seed moisture content of cv. *Oforiwa*; SMCV2: Seed moisture content of cv. *Kpando*; DSWCV1: dry seed weight of cv. *Oforiwa*; DSWCV2: dry seed weight of cv. *Kpando.*

Seed moisture content and dry seed weight were indepently and interactively significantly different ($p \leq 0.001$) for both cultivars and production environments (Fig. 5).

‘Plants began flowering 65 to 70 days after seedling emergence. Seed moisture content at first harvest (20 days after anthesis) was approximately 73% and 82% for cv. *Oforiwa* and cv. *Kpando* respectively and declined rapidly and linearly during fruit development and maturation (Fig. 5). Maximum seed dry weight was observed at 48 DAA in cv. *Oforiwa* while it took approximately a month later for cv. *Kpando* (76 DAA) to attain maximum seed dry weight, when seeds were produced under a tropical climate (TCC, A) (Figure 8A). At this point of physiological maturity, the seed moisture contents for cv. *Oforiwa* and cv. *Kpando*, were 59% and 46% respectively. Similarly, seeds produced under a temperate oceanic climate (TOC, 5B) conditions recorded higher seed moisture at early harvest and declined almost linearly as harvest delayed (Fig. 5B). It was however, observed that physiological maturity (maximum dry seed weight) for cv. *Oforiwa* occurred at a much later stage (62 DAA) (Fig. 5A) while cv. *Kpando* was at the same maturity stage at 76 DAA (Figure 8B). This suggests that, the maximum dry seed weight (PM) for cv. *Oforiwa* was influenced by the seed production environments. Cultivar *Kpando* PM was in the other hand occurred consistently 76 days after anthesis regardless of the seed production environment.

One important feature of seed development in fleshy fruited vegetables is that seed moisture content in these crops remains at values of about 26 - 45% (Borges *et al*., 2019; Ramos *et al.* 2021) and as high as 56 % even at end of maturity (Marcos-Filho, 2016). In this study, seed moisture contents were 57% and 42.5% at physiological maturity of cv. *Oforiwa* and cv. *Kpando* respectively and declined linearly, following the distinctive characteristics of orthodox seeds (Bewley *et al.*, 2013). The moisture content of seeds that develop in fleshy fruits generally fluctuates and remain high during the entire

maturation period and even after accumulation of maximum dry matter (Vidigal *et al.*, 2009). High seed moisture content is expected soon after the fertilization process is completed and thereafter declines gradually during development until seed attains physiological maturation. Subsequently at this moisture level, it is suitable for harvest suggesting that physiological maturity of seeds has been attained or seed-filling phase is completed as observed in the present study. This observations is consistent with results for similar fleshy fruited vegetables such as tomato (Demir and Ellis, 1992a; Borges *et al.*, 2019), okra (Santo *et al.*, 2019), pepper (Demir & Ellis, 1992b; Vidigal *et al.*, 2009; Ruiz & Parera, 2017), aubergine (Demir *et al.*, 2002), and '*Capsicum baccatuum* L. (Silva *et al.*, 2015).

In fleshy fruited vegetables, seed moisture contents declines steadily while dry matter accumulates (seed dry weight increase) until it reaches maximum dry weight (physiological maturity or mass maturity) and remains at high values thereafter. The present study showed similar trend (Fig. 5 A and B). Maximum seed dry weight was attained at 48 or 62 DAA for cv. *Oforiwa* under both tropical and temperate conditions respectively while cv. *Kpando* maximum seed dry weight occurred at 76 DAA regardless of production environment and remained unchanged until the last harvest. This observation means *cv. Oforiwa* attains maximum dry weight 14-28 days earlier than cv. *Kpando*. The seed moisture content progressively decreased but remained high (between 37.4 to 47%) at last harvest (82 DAA).

Series of transformations occur during fruit development and maturation, including fruit tissue degradation, accumulation of sugars and organic acids (Carrari *et al.*, 2006), leading to reduced water potential (Knoche e*t al.*, 2014). Towards the end of the

maturation process however, seeds inside the fruit lose water slowly until the osmotic equilibrium is reached, which explains the lower moisture content of seeds extracted fruits harvested from 62 DAA to 82 DAA. The gradual dehydration observed in seed moisture as maturation advanced is attributed to a decrease in the metabolism of the seed from a limit state of moisture in its tissues, an event reported as part of a natural mechanism in seeds of orthodox species (Leprince & Buitinik, 2010). Our results on the decline in seed moisture content as the fruit ripened corroborates similar observation by Demir and Ellis (1993) for marrow and Valdes and Gray (1998) for tomato. Similarly, seed moisture content of eggplant decreased steadily from 53% when dry matter was maximum, to about 45% at 50 DAA and then remained unchanged (Demir *et al.,* 2002). In pepper, Ruiz and Parera (2017) observed a decline of seed moisture to 41% at physiological maturity, PM.

It has been proposed that at physiological maturity, seeds attain maxium dry seed weight (Rajana & Andrews, 1970) with considerable loss of moisture content (Bewley *et al.,* 2013). The loss in water content is due to the accumulation of sugars and organic acids, and plant/fruit-to-seed nutrient transfer (Alvarenga *et al.,* 1991; Barbedo *et al.,* 1994a), which tend to increase the seed dry weight and thousand seed weight maturation phase (Santos *et al.,* 2020). Similar observations have been made for tomato (Demir and Ellis, 1992a; Borges *et al.,* 2019), pepper (Vidigal *et al.,* 2009), Okra (Santos *et al.,* 2020), aubergine (Demir *et al.*, 2002) and safflower (Ramos *et al*., 2021).

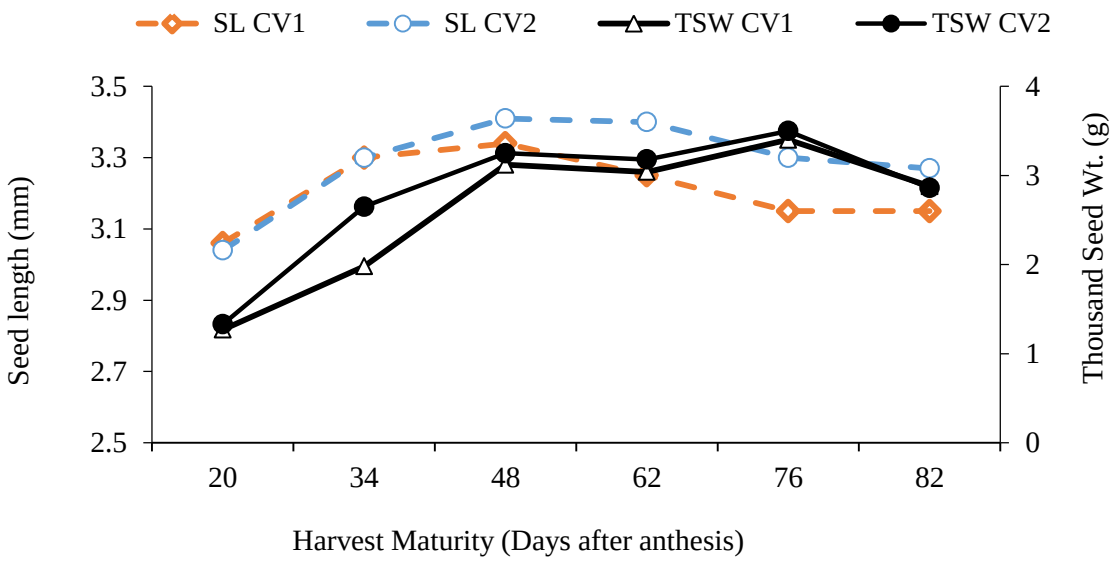


Figure 6: Mean Seed length (SL) and thousand seed weight (TSW) during seed development of African eggplant cv. *Oforiwa* (CV1) and cv. *Kpando* (CV2) under both tropical (TCC) and temperate oceanic climates (TOC).

Seed length and thousand seed weight interatively differed significantly ($p \leq 0.001$) among the two cultivars and under the different production environments. The means of both seed length and thousand seed weight at each harvest maturity stage for both seed production environments is presented in Figure 6, illustrating the changes in seed length and thousand seed weight of developing seeds.

Seed length (mm) and thousand seed weight (TSW, g) of African eggplant followed similar trend in during development for both cultivars under both growing environments. Seed length and thousand seed weight increased from the 20th day after anthesis, reaching a maximum at 48 DAA and 76 DAA respectively (Fig. 6).

Subsequently, both seed length and thousand seed weight declined marginally, reflecting the rapid loss of moisture that occurs after physiological maturity is reached. The linear increase of seed size and seed weight (thousand seed weight) with maturation is attributed to the accumulation of nutrients and moisture content as the fruit matures and remains unchanged declines after physiological maturity when water content is low and maximum dry matter content has been achieved (Bewley *et al*. 2013).

Thus, considering these three factors; seed moisture, dry seed weight and thousand seed weight, it can be reported that African eggplant seeds attains physiologically maturity at 48 or 62 DAA for cv. *Oforiwa* under tropical and temperate oceanic climates respectively and 76 DAA for cv. *Kpando* regardless of the production environment. The differences observed for the two cultivars regarding the maturity stage to physiological maturity could be attributed to their genetic differences. Days to first flowering and fruit set in cv. *Kpando* takes 10-12 and 5-7 more days respectively compared to cv. *Oforiwa* (Bortey, 2019, unpublished data). Vidigal *et al*., (2011) also reported that mass maturity or physiological maturity of sweet pepper (*Capsicuum annuum)* seeds occurred at 75 DAA. This results concurs with a report by Demir *et al.*, (2002), when maximum mass maturity varied 2 days in two growing seasons for eggplant (*Solanum melongena* L.).

4.4.2 Relationship of seed moisture content on percentage emergence as influence by harvest maturity

The percentage seedling emergence was significantly different ($p \leq 0.01$) at harvest maturity stage and cultivar levels. The relationship of seed moisture content on seedling emergence during fruit development is presented in Figure 7. At second harvests (34

DAA), although seeds had acquired ability to germinate (<10%), emergence was very low and seed moisture content was between 51 to 61.7%. As harvest was delayed, emergence percentage increased (Fig. 7) and seed moisture accordingly decreased. Emergence percentage was highest at 76 DAA for both cv. *Oforiwa* and *Kpando* when seed moisture content was 42.5% and 46% respectively.

It was however, observed that the increase in emergence percentage was significantly ($p < 0.01$) higher in cv. *Oforiwa* than in cv. *Kpando* between the development phase of 34 DAA and 76 DAA'.

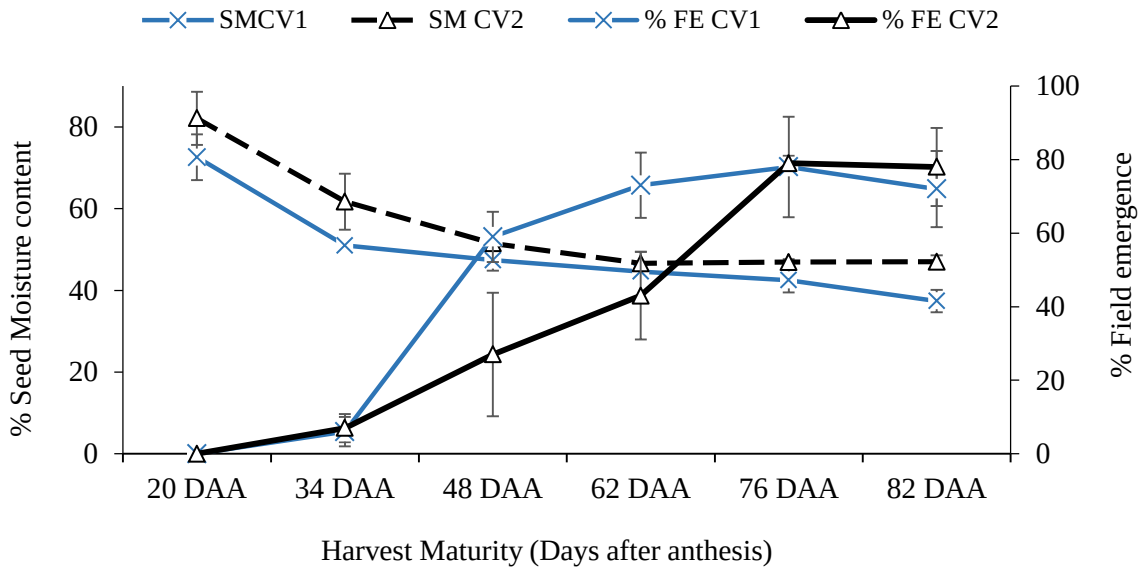


Figure 7: Relationship of seed moisture content and Field emergence of cv. *Oforiwa*) and cv. *Kpando*) at different maturity stage (vertical bars are ± standard deviation of means). SMCV1: Seed moisture content of cv. *Oforiwa*; SMC2: Seed moisture content of cv. *Kpando*; % FECV1: Percentage field emergence of cv. *Oforiwa*; % FECV2: Percentage field emergence of cv. *Kpando*.

4.4.3 The effect of harvest maturity on seed germination under both tropical and temperate climates

Percentage seed germination and field emergence at different harvest maturity stages is presented for seeds produced under both tropical and temperate oceanic climates (Figure 8 and 9 respectively. The independent effect of harvest maturity, cultivar and climate was significantly different ($p \leq 0.01$) for percentage germination. The two way interaction of cultivar x harvest maturity and climate x harvest maturity were also significant ($p \leq 0.01$) for germination percentage. The interaction of climate x cultivar x harvest maturity significantly ($p \leq 0.01$) influenced percentage germination.

In the early stages of development (20 DAA), no germination occurred for seeds produced under a tropical climate (A) for cv. *Kpando* while <10% germination was recorded for cv. *Oforiwa* under tropical conditions (Fig. 8A). Germination then increased sharply in fruits harvested from 48 DAA and was maximum (100%) for both cultivars at 76 DAA and maintained or marginally declined thereafter (Fig. 8A). Fruits harvested at early stages (20 and 34 DAA) from the temperate oceanic climate (Fig. 8B), however failed to germinate for both cultivars until the third stage of harvest (48 DAA). Later harvests recorded a steady increase in percentage germination until it reached a peak of 98% at 76 DAA and slightly declined afterwards (Fig. 8B). The maximum percentage germination for cv. *Kpando* coincided with the physiological maturity (PM) at 76 DAA. The maximum germination for cv. *Oforiwa* also occurred 76 DAA but some time after PM (48 and 62 DAA).

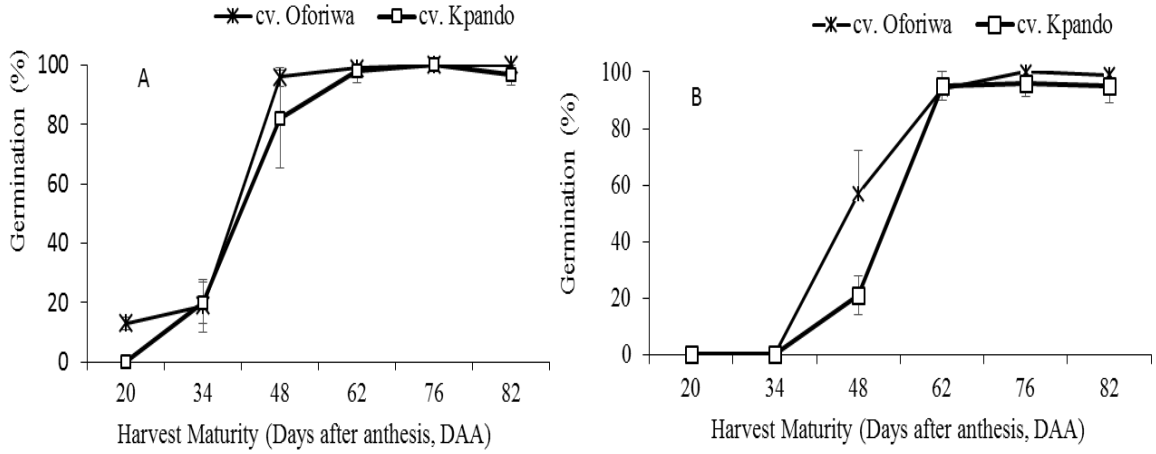


Figure 8: Changes in percentage seed germination of cv. *Oforiwa* and cv. *Kpando* extracted from fruits harvested at different maturity stages and produced under tropical climate (A) and temperate oceanic climate (B) (vertical bars are ± standard deviation of means).

4.4.4 Effect of harvest maturity on percentage field emergence under tropical and temperate climates

Seedling emergence tests were performed on the two cultivars produced under the two varied climatic conditions (Figure 9). The data showed a significant effect for independent factors of cultivar, climate and harvest maturity ($p \leq 0.01$). The interactive effect of climate x harvest maturity and cultivar x harvest maturity were significantly different ($p \leq 0.01$). The three way interactive effect of climate x cultivar x harvest maturity was however not significant ($p > 0.05$).

From the tests, it was shown that at early harvests, although seeds had acquired the ability to germinate (Fig. 8), percent emergence was very low regardless of the seed production environment. In particular, seeds of cv. *Kpando* obtained from the temperate oceanic climatic (TOC) conditions (Figure 9B), did not emerge until third harvest (48 DAA) and increased gradually thereafter until it reached maximum emergence of 72% 76 days after anthesis and marginally declined after. The pattern of seed emergence was however significantly higher in cv. *Oforiwa* than cv. *Kpando* between 48 and 76 DAA under both environments. Seeds produced under the tropical conditions (TCC) (Figure 9A), however emerged after 34 DAA and increased up to 76 DAA and declined slightly. At both production environments and among the cultivars, emergence percentage was highest when fruits were harvested 76 days after anthesis.

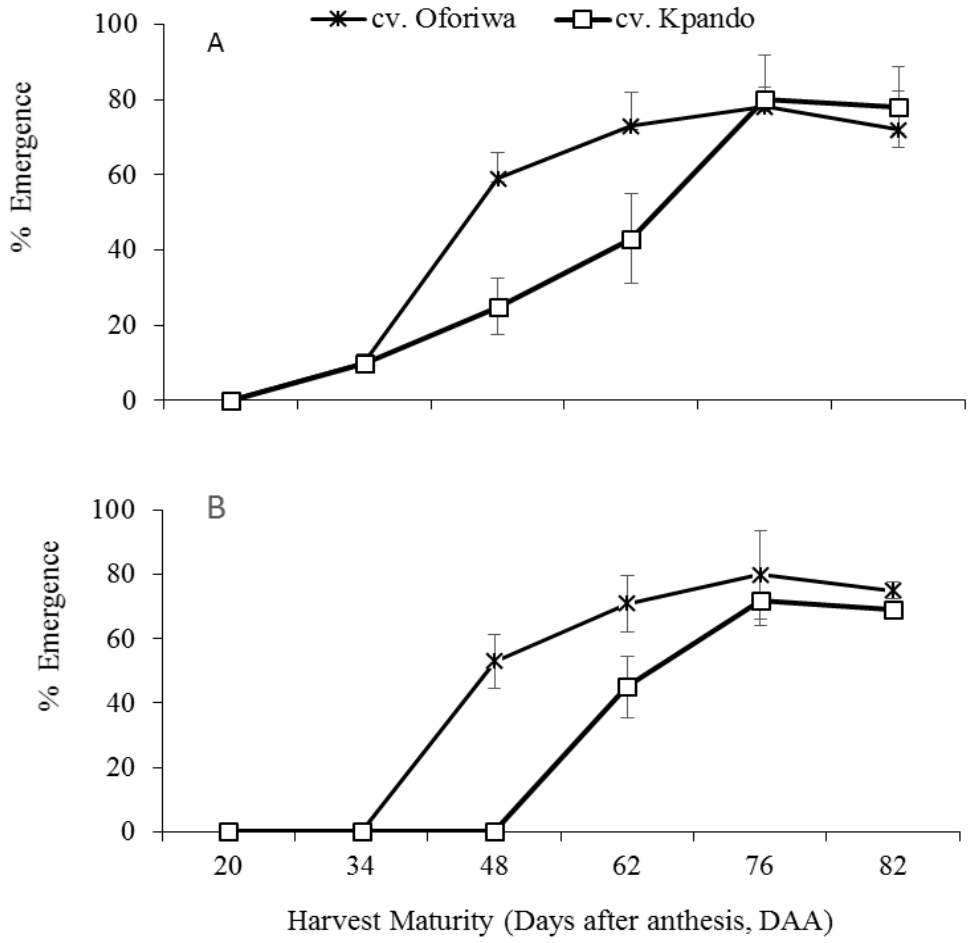


Figure 9: Effects of time of harvest on emergence percentage of African eggplant seeds for cv. *Oforiwa* and cv. *Kpando* grown at a tropical climate (TCC, A) and temperate oceanic climate (TOC, B) (vertical bars are ± standard deviations of means).

Seeds harvested early (20 and 34 DAA) depending on the production environment had already presented germination capacity, although in low percentages. Percent germination however increased from 48 DAA consistent with results by Martins *et al*., (2012) for eggplant and *Capsicum baccatuum* (Silva *et al.,* 2015), although percentage emergence was low in our results. However, in Okra, seed germination commenced as early as 30 DAA recording 44% (Santos *et al.*, 2020).

Harrington (1972) had hypothesized and later supported by Ellis (2019) that maximum seed quality was attained at physiological maturity (PM), after which deterioration started and seed germination and vigour declines. This view has been observed for okra (Demir & Ermis, 2005; Bortey & Dzomeku, 2016), selected *Solanum aethiopicum* spp. (Tetteh *et al*., 2021) and *Allophylus edulis* fruits (Kaiser *et al.,* 2016). In Okra (Demir and Ermis, 2005; Bortey and Dzomeku, 2016), maximum germination and percentage emergence occurred at 30 DAA and coincided with maximum seed dry weight. In the case of *Solanum* accessions, Tetteh *et al.* (2021) observed maximum seed germination (86 – 94%) when 100 seed weights were maximum (0.284g – 0.62g). Thus, the present results for cv. *Kpando* was consistent with the hypothesis that maximum seed quality is reached at physiological maturity (maximum seed dry matter) which in this case occurred at 76 DAA.

In contrast, cv. *Oforiwa* did not attain maximum germinability until after 14 days under tropical climates (Fig. 8A) and 28 days under temperate climate (Fig. 8B) after physiological maturity. This suggests that maximum dry seed weight for cv. *Oforiwa* did not coincide with maximum physiological quality. This phenomenon of maximum seed germination and PM not coinciding is also consistent with the views by (Martins *et al*., 2012; Santos *et al.,* 2020) and consistent with the new hypothesis by Ellis and Pieta Filho, (1992) that some seeds do not attain maximum germinability until sometime after mass maturity (physiological maturity) or the end of the seed-filling phase. One probable cause could be the difference in seed weight at maturity. The cultivar *Kpando* had heavier seeds compared to cv. *Oforiwa*, hence had both a greater rate and greater duration of seed filling than cv. *Oforiwa.* Consequently, there is likely to be more food reserves

accumulation in cv. *Kpando* and more time to attain physiological maturity which synchronizes with maximum germination and emergence. The explanation concurs with the views of Zanakis *et al*., (1994), who observed similar cultivar difference in attainment of physiological maturity among three soybean.

It can however be reported that, regardless of the seed production environment and cultivar, the African eggplant seeds attain maximum germinability (i.e. percent germination and emergence) when seeds were harvested 76 days after anthesis. Silva *et al*., (2015) and Santos *et al*. (2020) obtained similar results, in a study with seeds of *Capscium baccatum* L. and Okra respectively, that best seed quality was found for fruits harvested between 60 and 70 DAA. In tomato, Borges *et al*., (2019) concluded harvesting at 70 DAA gave highest germination quality after attaining PM at 60 DAA. Ruiz and Parera (2017) reported maximum germination for pepper at 9 weeks after anthesis (WAA) when PM had already occurred at 8 WAA. In the case of Safflower, Ramos *et al*. (2021) observed just 3 day interval between (36 - 39 days after flowering) between maximum seed germination and occurrence of PM. Recently, Santos *et al.,* (2020), reported that okra attains PM at 30 DAA but maximum germination occurs 20 days after (50 DAA). In contrast with the hypothesis of Harrington (1972) and in agreement with Demir & Ellis, (1992a), the present study did not provide evidence for sharp decline in percentage seed germination after attainment of maximum quality.

4.4.5 Relationship of harvest maturity on time to reach 50% germination and emergence

The time to reach 50 percentage seed germination (T_{50}, days) under controlled conditions significantly differed ($p \leq 0.01$) from those under field conditions and during seed development (Fig. 10A & B). The trend was similar for seeds produced under both tropical and temperate oceanic conditions. Under standard germination test conditions, seeds harvested for cv. *Oforiwa* 34 DAA took 9.9 days to attain 50% seed germination (T_{50} Germ) and declined linearly to 5 days at 76 DAA (Fig. 10 A), where percentage seed germination and emergence were maximum (Figures 8A, B and 9A, B) respectively. It however, took 9 more days (18.6 days) for the seeds of the same maturity stage (34 DAA) to attain 50% emergence (T_{50} Emer.) (Figure 10A and B).

Similar observations were made for cv. *Kpando* under both climatic conditions (Fig. 10A and B). Under both climatic conditions, cv. *Kpando* however, took between 1-3 days more to reach 50% emergence compared to cv. *Oforiwa* (Fig. 10A and B).

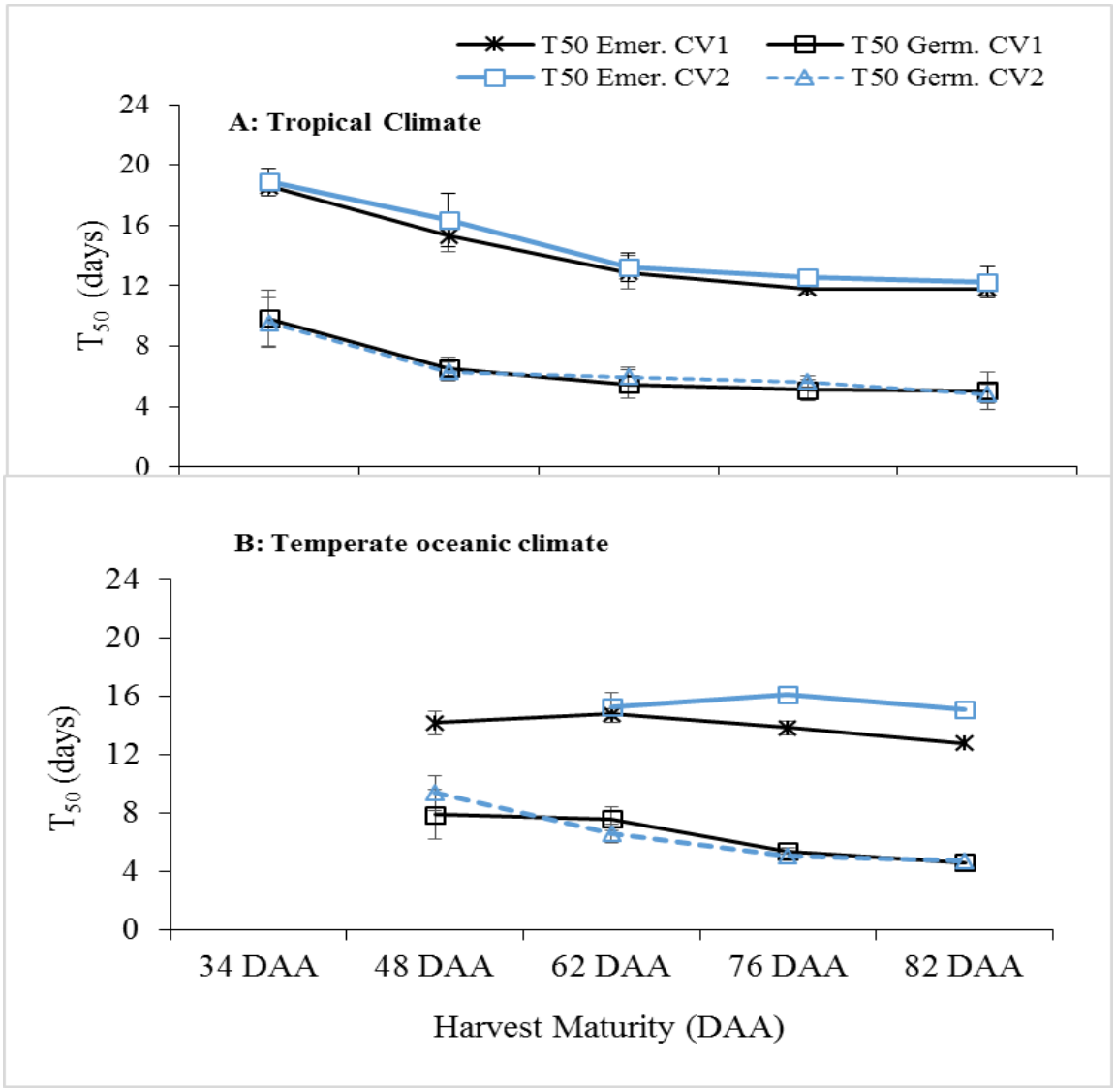


Figure 10: Relationship of harvest maturity on time to reach 50% (T_{50}) seed germination and emergence for seeds produced under both tropical (A) and temperate oceanic (B) climates for cv. *Oforiwa* (CV1) and cv. *Kpando* (CV2) (vertical bars are ± standard deviation of means).

The longer duration taken for seeds to emerge in comparison with germination under controlled conditions could be attributed to temperature effect and resistance from germinating media. As observed in earlier results on effect of termperature on germination, future emergence test should create a micro climate close to 30/20 °C under greenhouse conditions to ensure optimum emergence.

4.4.6 Changes in fruit morphological characters (diameter, weight and seeds per fruit) during development and maturation

The fruit morphological characteristics such as fruit weight, diameter, length and colour were monitored to investigate the association with seed physiological quality of the African eggplant (Figure 11). The fruit weight and diameter between the cultivars as well as the environment varied significantly with harvest maturity, cultivar and their interactions ($p \leq 0.01$).

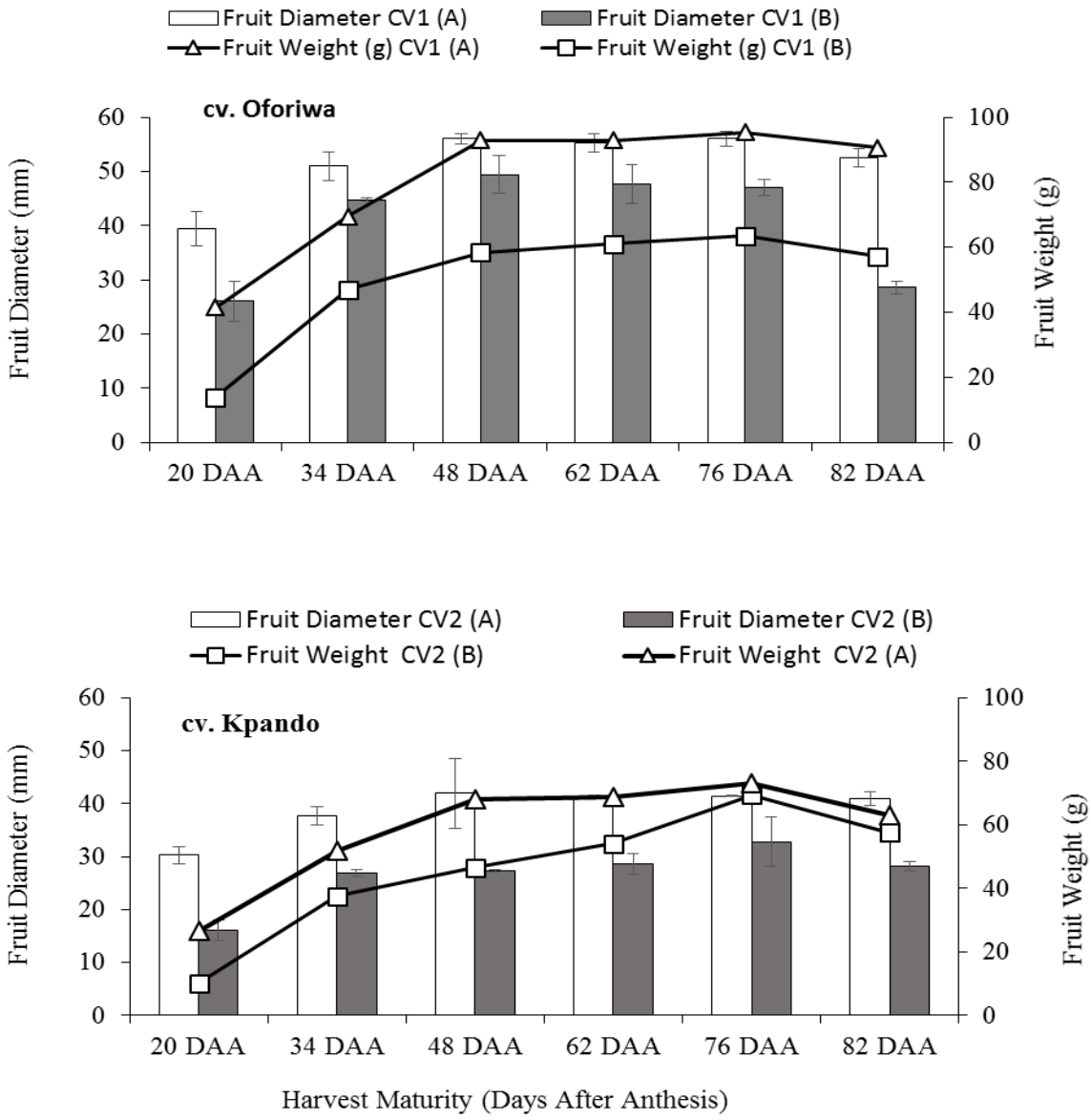


Figure 11: Effects of harvest maturity stage on fruit weight and diameter of cv. *Oforiwa* and cv. *Kpando* produced under tropical and temperate oceanic climates. CV1 (A): cv. *Oforiwa* produced under tropical climates; CV2 (B): cv. *Kpando* produced under temperate oceanic climates. Error bars represent standard deviation.

During fruit development and maturation, fruit width/diameter and fruit weight increased until 48 DAA and 76 DAA respectively and thereafter declined steadily for both cultivars and under both climates (Fig. 11). For cv. *Oforiwa,* fruit diameter was maximum after day 48 when seeds were physiological matured and maintain same up to 76 DAA before declining. Fruit weight, in the other hand reached maximum at 76 DAA and declined slightly thereafter at both climates (Fig. 11 (A) and (B). It was however, observed that both fruit diameter and weight for cv. *Oforiwa* produced under the tropical climate (A) were significantly ($p \leq 0.01$) bigger and heavier than those produced under the temperate oceanic conditions (B). The fruit weight and diameter also significantly differed ($p \leq 0.01$) for both cultivars at different maturity stages and seed production environments (Fig. 11). Fruits produced under the tropical climates (Fig. 11: CV1 A and CV2 A) had heavier fruit weights and larger width compared to those produced under the temperate climates (Fig. 11: CV1 B and CV2 B) for both cultivars.

Closely coinciding with the maximum fruit weight and width was the number of seeds per fruit (Fig. 12 A and B). Larger and heavier fruits had significantly more seeds per fruit (cv. *Oforiwa*) compared to fruits of cv. *Kpando* (Fig. 12 A and B) under both production environments. It was observed that significantly ($p \leq 0.01$) more seeds per fruits was recorded under tropical climates (Fig. 12A) than under temperate climates (Fig. 12B).

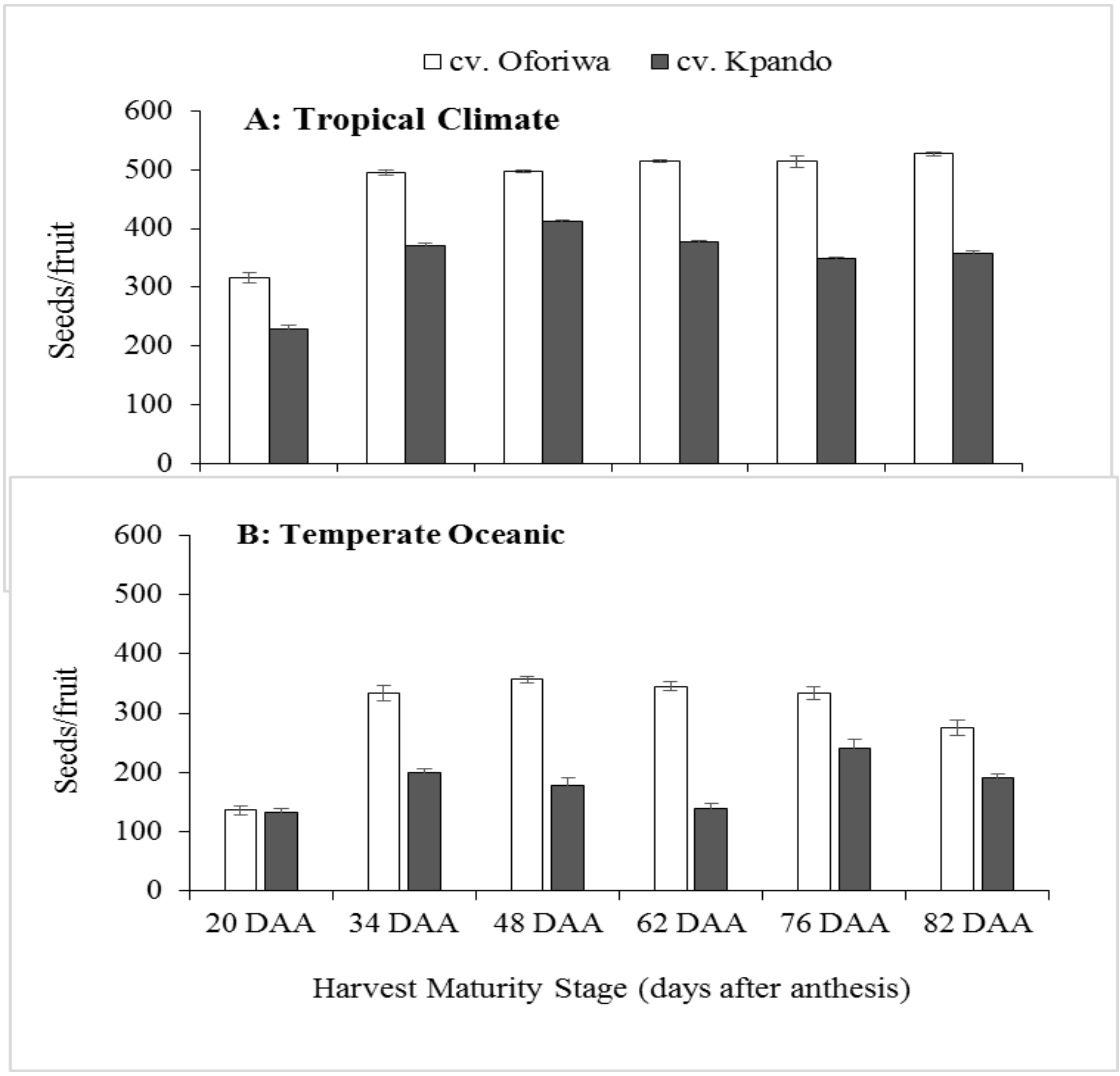


Figure 12: Number of seeds per fruit differed between cv. *Oforiwa* and cv. *Kpando* and under both tropical and temperate oceanic climates. Error bars represent standard deviation.

All fruit attributes (i.e. diameter, weight and length) recorded significant increases ($p \leq 0.01$) between the first harvest stage (20 DAA) and the third harvest stage 48 DAA, when fruit diameter was maximum for both cultivars. While fruit width/diameter maintained up to 76 DAA and thereafter declined, fruit weight continued to increase marginally and peaked at 76 DAA under both conditions.

The increase in values recorded for fruit diameter and weight as maturity progresses could be an indication of increase in the accumulation of assimilates during fruit maturation. This is in agreement with reports by Raz *et al*. (2001) and Bentsink and Koornneef (2008) that as an embryo undergoes maturation, there is food reserve accumulation resulting in fruit size and weight increase. While it can be said that the maximum fruit size for both cultivars was attained at 48 DAA as no further increase was observed in diameter, fruit weight however increased until 76 DAA. This could indicate dry matter accumulation which resulted only in weight increase from this stage. This further points to the fact that there is usually an increase in sink strength of the fruit at this stage of development resulting in an enhanced transport of nutrients towards the fruit until maturation (Tanksley, 2004). Recent studies by Meng *et al.,* (2020), has reported a linkage of increase cell numbers and endogenous IAA content to fruit size of cucumber during development. However, same cannot be justified in the present study.

The observed decline in fruit weight after 76 DAA reflects a possible rapid loss of moisture that occurs after this stage when fruits are fully matured. These figures show that both fruit weight and size were reduced with the maturation process as a function of moisture reduction resulting from shriveling of fruits during the latter stage of maturity when the rains had ceased. Similar results were reported by Silva (2002) with the fruit size of *Cnidosculus phyllacantus* reaching maximum values 53 days after flowering (DAF) while *Physalis angulata* L. from the Solanaceae family also reached maximum fruit weight and diameter at 35 DAA (Santiago *et al*., 2019). In *Solanum melongena* L., Kortse *et al.*, (2017) recorded maximum fruit diameter and weight at 35 days after anthesis.

4.4.7 Seed quantitative characteristics as a function of harvest maturity stages for seed lots produced under tropical and temperature oceanic climates

4.4.7.1 Measurement of Germination rate of seed lots.

The measurements of germination rate are quantitative traits that can inform the dynamics of the germination process among seed lots in relation to seed vigour and its ability to develop into a normal seedling. The results showed a significant difference ($p \leq 0.01$) in harvest maturity stage on the parameters characterizing germination rate of seed lots from both climates (Table 5 and 6). With the exception of germination index (GI), all other parameters measured were however not significantly different between the two cultivars and their interactions with harvest maturity at $p > 0.05$ significance level. Thus, the results presented and discussed is means of the two cultivars and how seeds extracted at different maturity stage influenced these quantitative traits.

Table 5: Parameters characterising germination rate of seeds lots as influenced by harvest maturity stage and produced under tropical climate.

Harvest Maturity	MGR (day^{-1})	MDG	CVG (%)	GI	GV	PV (day^{-1})
34 DAA	0.105 c	1.02 c	10.51 c	0.63 c	2.10 c	1.83 c
48 DAA	0.139 b	4.23 b	13.94 b	3.32 b	40.51 b	9.42 b
62 DAA	0.156 ab	4.68 a	15.63 ab	4.09 ab	54.76 a	11.65 ab
76 DAA	0.164 ab	4.76 a	16.45 ab	4.40 a	58.87 a	12.36 a
82 DAA	0.180 a	4.68 a	18.09 a	4.83 a	64.08 a	13.64 a
Mean	0.149	3.87	14.92	3.45	44.06	9.78
SEM	0.05	0.1	0.72	0.18	3.41	0.66

Note: MGR: Mean germination rate; MDG: Mean daily germination; CVG: Coefficient of velocity of germination; GI: Germination index; GV: Germination value; PV: Peak value; SEM: standard error of means. Means followed by same letters within each column are not significantly different according to Tukey's HSD test at 5% significant level.

Table 6: Parameters characterising germination rate of seeds lots as influenced by harvest maturity stage and produced under temperate oceanic climate.

Harvest Maturity	MGR (day^{-1})	DG	CVG (%)	GI	GV	PV(day^{-1})
48 DAA	0.109 b	2.78 b	10.96 b	1.24 c	13.15 c	3.55 c
62 DAA	0.129 b	6.75 a	12.94 b	3.34 b	61.23 b	9.01 b
76 DAA	0.166 a	6.92 a	16.64 a	4.48 a	84.94 a	12.14 a
82 DAA	0.179 a	6.99 a	17.95 a	4.72 a	93.46 a	13.46 a
Mean	0.146	5.86	14.62	3.45	63.19	9.54
SEM	0.07	0.16	0.54	0.17	3.97	0.51

Note: MGR: Mean germination rate; MDG: Mean daily germination; CVG: Coefficient of velocity of germination; GI: Germination index; GV: Germination value; PV: Peak value; SEM: standard error of means. Means followed by same letters in each column are not significantly different based on the Tukey's HSD test at 5% significant level.

Although, maximum germinability as influenced by harvest maturity occurred 76 DAA, the maximum values for germination rate parameters as an indication of seed vigour were observed 7 days after (82 DAA) as shown in Tables 5 and 6. The values were however, not statistically different from the mean values at 76 DAA.

Mean germination rate (MGR) increased with seed maturation with the highest value (0.180 and 0.179) occurring 82 DAA and did not differ under both climates. Since, MGR measures the speed of germination and quantifies the seedling vigour (Czabator, 1962), the results show that higher seed vigour is obtained from seeds extracted from latter harvests (76 or 82 DAA) than earlier harvests. It is worth noting that this measure did not vary regardless of the seed production environment.

Mean daily germination (MDG) gives an indication of number of seeds germinating per day. More seeds (4 to 6) seeds were germinating per day from seed lots obtained at PM.

This suggests that as seeds matures, the number of seeds germinating per day increases, which is another good indicator of seed vigour.

Coefficient of velocity of germination (CVG) according to Jones and Sanders (1987) gives an indication of rapidity of germination. CVG values increases when the number of germinated seeds increases and the time required to for germination decreases. From the present results, it has been established that CVG is influenced by seed maturation, suggesting that as seed matures, less time is required for it to germinate but at a faster rate. This is due to the increase in seed dry matter content which gives the seed more energy. Martins *et al.,* (2012) observed similar results for eggplant where seeds harvested between 63 and 70 days after pollination germinated faster than earlier harvests. This measure is a good indicator of both seed vigour and germinability of a seed lot.

Germination index (GI) increased with maturity stage and the highest index (4.83) occurred from seeds extracted from fruits harvested 82 DAA but not significantly different from those harvested 76 DAA. The lowest index (0.63) was observed in seeds extracted from earlier harvests (34 DAA). GI combines both germination percentage and speed of germination (Berch-Arnold *et al.,* 1991; Kader, 2005) and thus serve as a good measure of seed vigour and germinability of a seed lot. Vidigal *et al.,* (2011) using speed emergence index (SEI) as an indicative measure of vigour reported maximum SEI values when seeds were extracted from pepper fruits harvested from 60 DAA to 75 DAA.

Germination value (GV) increased with seed maturation with the highest value (64.08) occurring at 82 DAA for seed lots produced under tropical climate (Table 5) and 93.46 for seed lots produced under temperate conditions (Table 6). GV as proposed by Czabator (1962) provides a single measure to capture bot speed and completeness of germination.

This suggest that GV is also a good measure to predict the seed vigour of the seed lots, taking into account the speed and ability to complete the germination process.

Peak Value (PV) was significantly influenced by harvest maturity as it increases with maturation. Higher peak values indicates seed lots with higher speed and germinability and this was observed in seeds extracted at 76 and 82 DAA. Peak value (PV) characterizes the ‘maximum cumulative germination percentage against the number of days taken to reach this percentage’ (Brown and Mayer, 1988).

The results of the present study showed that MGR, MDG, CVG, GI, GV and PV are good parameters to classify the vigour and germinability of seed lots. The observation of seed vigour parameters occurring after physiological maturity (76 DAA) is consisted with the reports by Tekrony and Egli (1997). In their study, maximum seed vigour for pepper (*Capsicum spp*.) and tomato (*Lycopersicon esculentum* Mill.) occurred 6 and 10 days respectively after PM. GI and CVG measurements give maximum weights to the time when majority of seeds in a lot germinates, hence, these measurements can further assist in estimating the timing of cultural practices following sowing (Kaders (Al-Mudaris), 1998) and the stress resistance of the seed lots (Kader and Jutzi, 2002).

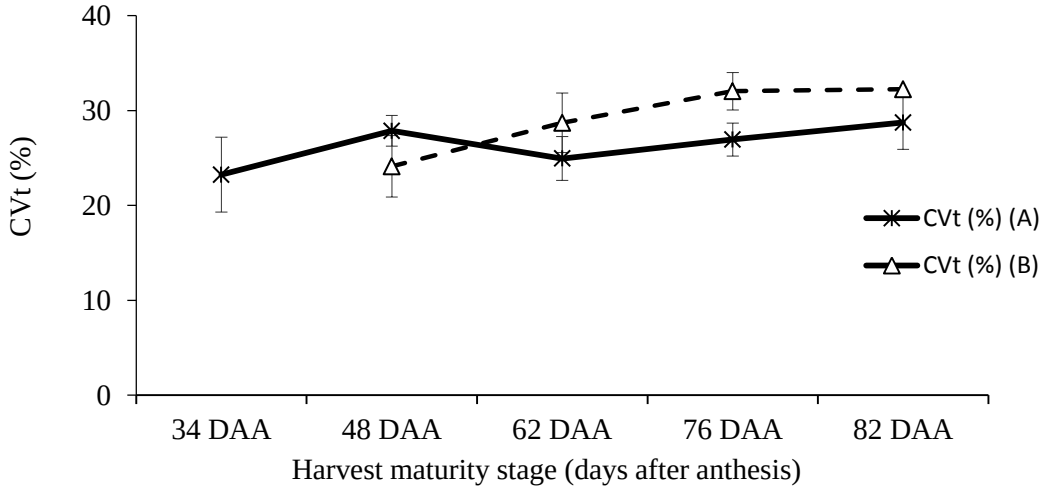


Figure 13: Effect of harvest maturity stage on Coefficient of variation of germination time (CVt) as a measure of uniformity of seed germination of seed lots produced under tropical (CVt, A) and temperate oceanic (CVt, B) climates. Bars represent standard error of means (SEM).

The germination dispersion over time expressed by the coefficient of variation of time (CVt) was not significantly different for both cultivar and harvest maturity levels (Fig. 13). It however, varied between the seed production environments (Fig. 13).

The CVt measurement was proposed to assess the germination uniformity or variability of seeds in relation to mean germination time (Dorneles *et al.*, 2005; Ranal *et al.*, 2006). The low values of CVt (23.2 to 32 %) recorded among the seed lots showed that the germination of seeds was homogeneous. Similar CVt values were observed in *Baccharis trimera* (Less.) var. CPQBA-1 seeds (Moreno-Pizani *et al.*, 2019).

Table 7: Parameters characterizing germination synchrony of seeds lots as influenced by harvest maturity stage

	Tropical climate		Temperate climate	
Harvest Maturity	U (bit)	Z	U (bit)	Z
34 DAA	1.74	0.16	**	**
48 DAA	2.24	0.24	2.04 b	0.16 ab
62 DAA	2.27	0.21	2.64 a	0.14 b
76 DAA	2.24	0.20	2.38 ab	0.19 ab
82 DAA	2.14	0.25	2.19 b	0.22 a
Mean	2.13*	0.21*	2.31	0.18
SEM	0.13	0.02	0.09	0.03

Note: U: Synchronization index; Z: degree of germination overlaps, SEM: standard error of means; *: means were not significantly different among the harvest maturity stages. **: No germination occurred at maturity stage.

The parameters measured to characterize the germination synchronization were not significantly different among the different harvest maturity stages ($p > 0.05$) for seed lots produced under tropical climates. There was significant difference ($p \leq 0.05$) among seed lots from the temperate climates (Table 7). The mean of the uncertainty (U) value was far from zero ($U \geq 2.13$) and less than one (≤ 0.21) indicate that seed germination occurred well-distributed in time. It was however, observed that seeds extracted from earlier harvests (34 DAA under tropical climate and 48 DAA under temperate climate) were more synchronous (low synchronization index) and a low degree of germination overlaps indication homogeneity. Later harvests seed lots had higher synchronization index (U) and higher values for germination overlaps, suggesting that as seeds of *Solanum aethiopicum* matures, they tend to be more asynchronous. That is showing physiological heterogeneity and this could explain their erratic seed germination behaviour.

4.4.8 Relationship of fruit morphological traits (fruit weight, size, colour) and seed physiological quality.

Table 8: Correlation matrix for fruit morphometric and seed germination metrics of cv. *Oforiwa* produced under tropical climate.

	Fwt (g)	*FD (mm)*	*FL (mm)*	*Seed Size*	*%SG*	*MGT*	*T_{50}*	*GI*	*TSW*
Fwt (g)	1								
FD	0.969*	1							
FL	0.839*	0.918*	1						
Seed Size	0.483	0.642	0.834*	1					
%SG	0.924*	0.813*	0.595	0.216	1				
MGT	-0.903*	-.781*	-0.538	-0.071	-0.967*	1			
T_{50}	-0.926*	-.809*	-0.583	-0.141	-0.983*	0.996*	1		
GI	0.889	0.753	0.518	0.061	0.977*	-0.996*	-0.996*	1	
TSW	0.980*	0.936*	0.742	0.379	0.950*	-0.928*	-0.943*	0.913*	1

Note: *Significant at $p < 0.05$ Fwt: Fruit weight, FD: Fruit width/diameter, FL: Fruit length, %SG: Seed germination; MGT: Meant Germination Time, T_{50}: Time to 50% seed germination; GI: Germination index; TSW: Thousand seed weight

Table 8 illustrates a multiple correlation to evaluate the degree of association among the fruit morphological traits and seed germination parameters of African eggplant at different maturity stages produced under both tropical and temperate climates. Strong positive correlations were found between fruit weight and percentage seed germination (r = 0.92), between fruit diameter (size) and seed germination (r = 0.81) and between seed weight (TSW) and seed germination (r = 0.95). There was however, a moderate positive correlation (r = 0.59) between fruit length and seed germination. Mean germination time (MGT) and time to 50% (T_{50}) germination were negative and significantly related to seed germination (r = -0.96) and (r = -0.98) respectively seed germination, suggesting that seed takes less time to germinate as it seed matures.

The present study further established a strong positive correlation between the fruit morphological metrics and seed physiological traits (Table 8). A strong correlation between fruit weight, diameter (size) and percentage seed germination and thousand seed weight was found to be statistically significant ($p \leq 0.05$).

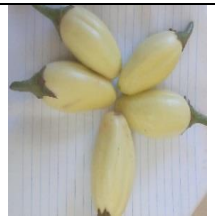
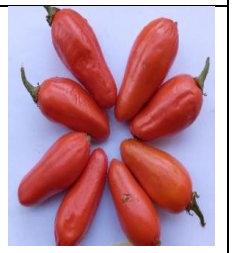
This observation suggests that the size and weights of fruit can be related to the seed weight (mass) resulting from the accumulation of food reserves as there is enhanced transport of nutrients towards the fruit and subsequent transfer to the seed during maturation (Tanksley, 2004). Consequently, seeds with heavier weights (mass) are likely to possess enhanced seed vigour and germinability. It is been reported that distinct seed sizes (mass) have different levels of starch and other energy reserves which influences the expression of germination and initial growth of seedlings (Shahi *et al*., 2015). Thus, germination depends on the capacity of the seed to mobilize and efficiently utilize its reserves more efficiently (Bewley *et al.,* 2013).

Table 9: Relationship of Fruit Colour Changes during development and maturation of African eggplant, cv. *Oforiwa* and percentage seed germination 'produced under a tropical (A) and temperate oceanic (B) climates).

Fruit Colour (RHS Chart)		RHS2015 4C	RHS2015 14B	RHS2015 N25B	RHS2015 N30C	RHS2015 N30C	RHS2015 N30B
cv. *Oforiwa from TC Seed quality*	A						
	% G	13	19	96	99	100	100
cv. *Oforiwa from TOC*	B						
Seed Quality		RHS2015 157A	RHS2015 4C	RHS2015 14B	RHS2015 N25B	RHS2015 N30C	RHS2015 N30B
	% G	0	0	51	94	100	99

Colour code was according to Royal Horticultural Society Colour chart 2015 6th edition. %G: percentage Germinability; A: Tropical climate (TC); B: Temperate Oceanic climate (TOC), DAA: Days after anthesis.

Table 10: Relationship of Fruit Colour during development and maturation of African eggplant, cv. *Kpando* and percentage seed germination 'produced under a tropical (A) and temperate oceanic (B) climates.

Fruit Colour (RHS Chart)		RHS2015 157A	RHS2015 14B	RHS2015 N25C	RHS2015 N30C	RHS2015 N30C	RHS2015 N30C
cv. *Kpando from TC Seed quality*	A						
	% G	0	20	82	98	100	97
cv. *Kpando from TOC*	B						
Seed Quality	% G	RHS2015 157A	RHS2015 157A	RHS2015 14A	RHS2015 N25A	RHS2015 N30C	RHS2015 N30A
		0	0	21	95	96	95

Colour code was according to Royal Horticultural Society Colour chart 2015 6th edition. %G: percentage Germinability; A: Tropical climate (TC); B: Temperate Oceanic climate (TOC); DAA: Days after anthesis

Tables 9 and 10 show the relationship of fruit colour changes during development to seed germinability ‘under both tropical monsoon (A) and temperate oceanic (B) climates. The study revealed that the environment under which fruits are produced influences the rate of changes in colour during development. For both cultivars, it took 14 for fruits harvested at 20 DAA to change colour from RHS2015 4C to RHS2015 14B under tropical conditions (A), while it took 28 days (48 DAA) under a temperate climate (B) (Tables 9 and 10). The results further showed that fruit colour can be a suitable indicator of seed germination quality, irrespective of the environment (Tables 9 and 10). Under both tropical and temperate climates and cultivars, fruits within the Green-White colour group (RHS2015 157A) recorded no seed germination, yellow-green colour group (RHS2015 4C) also recorded no or low germination and yellow-orange colour group (RHS2015 14A and B) recorded lower seed germination (19-51%). However, all fruits harvested within the orange colour group (N25A-C) and Orange-red colour group (N30A-C) recorded high percentage seed germination (82 -100%) irrespective of the cultivar and environment (Tables 9 and 10). The maximum seed germinability also coincided with the harvest maturity stage of 76 DAA for all cultivars and under both production environments.

It must be acknowledged that monitoring the number of days after anthesis/flowering to determine the ideal time of harvest for the purpose of obtaining high quality seed can be challenging for seed producers or farmers under open field cultivation. Thus, the criterion for associating fruit colour changes to seed physiological quality could be a suitable alternative.

It was further observed that the environment under which fruits are produced influences the changes in colour during development. This was however, the case in the early harvest stages from 20 to 34 DAA. The colour changes in the tropical climate initiated weeks earlier than in the temperate oceanic climates for both cultivars (Tables 9 and 10).

The present study showed that fruit colour can be a suitable indicator of seed physiological quality (germinability). Seed germination was initiated when fruit colour change commenced at 34 DAA under tropical climate and 48 DAA under temperate climates for both cultivars. All fruits harvested with strong orange and vivid reddish orange colours, corresponding to the orange colour group (N25A-C) and or Orange-red colour group (N30A-C) respectively, had high percentage seed germination (82 -100%) irrespective of the cultivar and environment at latter stages of harvest (Tables 9 and 10).
In an earlier report, Sena and Gariglio (1998) and Germaque *et al.* (2002) had suggested that the changes in fruit colour during development is a suitable indicator to assist in determining the optimum harvest stage of several species. This present results is consistent with other reports for other crops that used colour to associate seed physiological quality. In *Jatropha Curcas*, Aquino *et al.,* (2009) confirmed that fruit colour changes is one of the indicators that have been well associated to seed physiological quality. In their study, maximum germination of 96 % germination was observed for fruits harvested at yellow green colour, higher than fruits harvested at green stage (83%) or black colour stage (86 %). In paprika chili (*Capsicum annuum* L.), harvesting at red ripe stage produced the highest values for the seed quality parameters including 1000 seed weight, germination percentage, shoot length, root length, seedling vigor index and seedling dry weight (Shamsheerahmed *et al*., 2008).

In a study of *Jatopha curcas*, Kaushik (2003) observed maximum seed germination (89%) in yellow fruits, while Dranski *et al.*, (2010) recorded high germination in *Jatropha curcas* seeds harvested when fruits had turned brown corresponding to 7 YR 4/2 according to the Munsell colour chart.

In Solanum spp., Sukprakarn *et al.*, (2005) suggested harvesting fully ripe fruits (brownish colour) of purple variety of brinjal (*Solanum melongena* L.) for the production of high quality seeds. Demir *et al.*, (2002) and Demir and Mavi (2003) observed similar colour changes in *Solanum melongena* L., which was associated with seed germinability. Other similar observations have been made for tomato (Demir & Samit, 2001a) and pepper (Demir & Ellis, 1992b).

For the differences in fruit colour initiation observed under the two climatic conditions, this could be attributed to temperature differences in the production environments as higher temperatures is reported to advance fruit colour significantly compared to lower temperatures (Gross, 1987).

Although, such a comparative study of this crop or related species has not been reported to establish the real impact of temperature under field conditions on fruit colour, Gross (1987) however reported that higher temperatures under storage conditions can affect pigment concentrations in different fruits by influencing chlorophyll degradation. Manolopoulou and Varzakas (2016) also indicated that as storage temperature increases, the chlorophyll degradation rate increases, resulting in colour changes and ripening. The present results therefore suggest that seed germination quality of African eggplant (*Solanum aethiopicum* L.) is associated with changes in fruit colour and can be a suitable indicator of seed physiological quality in addition to days after anthesis.

4.5 Conclusion

Fruit harvesting maturity stage influenced both 'seed physical and physiological quality with a strong relationship between fruit morphological traits' (size, weight, and colour) and seed physiological characteristics of African eggplant (*Solanum aethiopicum* L.)

4.6 Recommendations

i. The suitable harvest maturity stage for 'maximum seed quality (i.e. germination, emergence, thousand seed weight and mean germination time)' is "76 days after anthesis (DAA) for both cultivars irrespective of the seed production environment".

ii. For maximum seed quality, bigger, heavier and fruits with strong orange and vivid reddish-orange colours corresponding to N25A-C and N30A-C of the RHS colour chart should be harvested.

4.7 Suggestions for further research

There is need to evaluate and establish the suitable harvest maturity stage for other *Solanum aethiopicum* L. crops for the purposes of quality seed production.

CHAPTER FIVE

DETERMINATION OF THE EFFECT OF SEED MATURITY STAGE ON THE BIOCHEMICAL CHARACTERISTICS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.) AND ITS RELATIONSHIP WITH SEED PHYSIOLOGICAL QUALITY

Abstract

Several biochemical changes occur during the development and maturation of seeds with its associated influence on seed quality, especially viability and vigour attributes. The objective of this study was determine the effect of seed maturity stage on the biochemical characteristics of the African eggplant (*Solanum aethiopicum* L.) and to establish the biochemical component that significantly play a role in maintenance of seed physiological quality. The seed physiological quality and biochemical components were evaluated at 20, 34, 48, 62, 76 and 82 days after anthesis (seed maturity stages). Seeds were subjected to viability tests and vigour was assessed by germination index, mean daily germination and mean germination time. Biochemical characteristics profiled included total protein, crude fat, sucrose, glucose, fructose, tannin and antioxidant activity. Data were subjected to analysis of variance and means were compared by the Tukey's test at 5% significant level. The seed biochemical components and physiological quality were correlated using multivariate statistics. Seed physiological quality improved as a function of seed maturity and attained maximum viability 14 days after PM. Seed maturity stage significantly influenced the biochemical characteritics of African eggplant seeds. Seed protein, sugars (sucrose, glucose, fructose) did not significantly contribute to seed germination improvement. Antiodixant activity, crude fat and tannin content correlated significantly in maintaining seed viability. Among the biochemical characteristics, tannin content can serve as a suitable biochemical marker for seed germination and vigour maintenance in African eggplant seeds.

Introduction

During the development and maturation of seeds, myriads of physiological and biochemical changes occur. Most of these physiological changes include seed dry weight and moisture content, germination percentage and emergence rate, aging, fruit and seed colour and their relationship with seed quality aspects have been studied (Samarah and Abu-Yahya, 2008; Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009; Vidigal *et al.,* 2011; Eskandari, 2012).

However, other biochemical techniques have also been demonstrated as effective tools for assessing seed quality. Ramya *et al*. (2012) determined the activity of enzymes involved in cell respiration during seed development and maturation phases of onion (*Allium cepa* L.) while Oliveira *et al.,* (2013) and Silva *et al.,* (2015) studied amylase enzyme expression in maize seeds and reserve mobilization and metabolites accumulation during development of *Capsicum baccatum* respectively. These numerous cellular and biochemical events that occur during seed development are reported to be associated with the acquisition of desiccation tolerance in seeds especially orthodox (Leprince *et al.,* 1993; Bewley *et al.,* 2013) with influence on seed physiological quality. These changes include activation of anti-oxidation defenses (Leprince *et al.,* 1993), accumulation of storage reserves and metabolites such as structural proteins, carbohydrates, lipids among others, which are directly or indirectly related to the integrity of their cell membranes and by implication confers seed vigour and storability (Weber *et al.,* 2005; Carvalho *et al.,* 2009).

Jarret *et al*. (2016) reported that some *Solanum* species possess fatty acid levels in seeds ranging from 57% to 25.2% for linoleic and oleic acids respectively and lipid peroxidation activities. Silva *et al.,* (2015) also observed that neutral lipids accummulated

14-times between 10 and 30 days after anthesis then decreased about 78% from 30 to 60 DAA. Thereafter, there was a doubling of lipid contents between 60 to 80 days after anthesis in *Capsicum baccatum* seed development (Silva *et al.,* 2015). The increase in the latter stage of maturation as they play critical role in cell membrane composition for the purpose of maintaining cell membrane integrity. Fatty acids and their effects on seed viability and vigour has been reported (Kumar *et al*., 2015).

Silveira *et al.,* (2004), observed that buffer-soluble protein contents and dry matter increased progressively during development, reaching their maximum values at the matured stage of *Pinus taeda*, a conifer. In studying the seed storage proteins accumulation in *Cleome gynandra* L. and *Brassica kaber* L., Ochuodho *et al.,* (2006) observed that the content of seed proteins increased as the seeds of Cleome matured. The authors further reported that green seeds (immature) extracted from green pods showed the least number of protein bands using SDS-PAGE profile. Silva *et al.,* (2015) also reported that soluble protein content in *Capsicum baccatum* seeds increased 66% between 10 and 40 days after anthesis and remained almost steadily until the last harvest at 80 days after anthesis. Total soluble sugars (TSS) and total free amino acids (TFAA) contents on the other hand decreased sharply during *Capsicum baccatum* seed development (Silva *et al.,* 2015). Both declined drastically by 80 % and 60 % respectively when seeds were precociously between 10 and 30 days after anthesis (Silva *et al.,* 2015). The decrease in TSS and TFAA parallel with the accumulation of neutral lipids indicating their utilization as precursors of carbon and nitrogen respectively (Silva *et al*., 2015).

Oluwanyi *et al.* (2017), evaluated the proximate and antioxidant activities of some *Solanum aethiopicum* and *Solanum macrocarpon* and reported the presence of some antioxidants in the fruits of these species. It is however, not known and reported whether these reserve metabolites and antioxidants activities, which are vital to enhancing rapid and uniform germination as well as maintaining the viability of this crop during maturation are found in the seed. We therefore investigated in this study the biochemical changes that occur during development and maturation of the African eggplant (*Solanum aethiopicum* L.) and evaluated the association of the biochemical parameters with seed physiological quality. Specifically, the changes studied were limited to seed protein, fat content, antioxidant activity, tannins and sugars (sucrose, glucose and fructose).

5.1 Objectives of Study

To determine the effect of seed maturity stage on biochemical characteristics of African eggplant (*Solanum aethiopicum* L.) and its relationship with seed physiological quality.

5.2 Research Questions

i. What is the accumulation pattern of biochemical components such as total protein, fats, soluble sugars, antioxidant activity and tannin during seed evelopment of African eggplant (*Solanum aethiopicum* L.) and how does it affects seed physiological quality?

ii. Which of the biochemical characteristics identified in (i) have the greatest contribution to the maintenance of physiological quality of African eggplant seeds?

5.3 Materials and Methods

5.3.1 Plant Materials

Seeds of African eggplant (*Solanum aethiopicum* L.) used for this experiment were obtained from the previous study as described in chapter four (4). Only cv. *Oforiwa* was used for this experiment because there was adequate quantities of seeds to run all the biochemical characteristics for the different seed maturity stages.

5.3.1.1 Sample preparation

Seeds were extracted manually from fruits harvested at 6 maturity stages (20, 34, 48, 62, 76 and 82 days after anthesis). After drying seeds under shade for 48 hours to a moisture level of between 7-8%, seeds were put in an air-tight aluminium foil and stored at freezing temperature until the biochemical determination were conducted. All analysis were conducted on dry seeds. Seed moisture levels were all adjusted to 10 ± 0.56 before subjecting to various biochemical tests.

5.3.2 Data collected

i. **Seed Germination:** This was conducted according to the methods previously described in chapter five (5).

ii. **Seed vigour:** The seed vigour was measured based the following quantitative parameters that gives an indication of seed vigour namely germination index (GI), mean daily germination (MDG) and Mean germination rate (MGR) were calculated using the formulas as previously described in chapter five (5).

5.3.3 Biochemical Quantification

All biochemical studies were conducted at the Mycotoxin and Nutrition Platform (laboratory) located at the International Livestock Research Institute (ILRI), Nairobi, Kenya. All biochemical analysis were determined on dry seed samples.

Determination of Crude protein: The content of crude protein in the seeds in the cause of development and maturation was determined by Kjeldahl Method according to Puwastien *et al*., (2011) and Latimer, (2012). Samples of 200 mg of dry seeds were blended until homogenous. The Analysis-Blank used included two reagent blanks (containing all reagents used in nitrogen analysis except the sample) in every batch of analysis to subtract reagent nitrogen from the sample nitrogen. The test sample was thawed to room temperature and mix the sample thoroughly. Duplicates of 0.5 g was weighed into the digestion tube. 1 table catalyst and 10 mL sulfuric acid was added and placed the digestion tube in the digester. The digest mixture initially at low temperature to prevent frothing and boil briskly until the solution is clear and is free of carbon or until oxidation is complete. After this, a 250-500 mL Erlenmeyer flask containing 50 mL of 4% boric acid with indicator as receiver on the distillation unit was added. Further, 100 mL of water and 70 mL of 50% sodium hydroxide was added to the digests and start distillation. Distillation was done until all ammonia has been released and capture all distilled ammonia. (This is an automatic process in the distillation unit). Lastly, the distillate titrated with the standardized 0.1 N Sulphuric acid until the first appearance of the pink colour and record the volume of acid used to the nearest 0.05 mL.

Calculation and expression of results: N (g %) = (mL 0.1N HCL sample-mL 0.1N HCL blank) x 0.0014 x N H2S04 x 100)/ (Weight of sample). Protein (g/100g) = % total nitrogen x appropriate nitrogen conversion factor (6.25).

Crude fat determination: The crude fat content of the seed samples at the various stages of maturity was determined according to AOAC official method 2003.06 Crude fat in feeds, cereal grains and forages.

Determination of Antioxidant Activity: The determination of antioxidant activity was determined according to the procedures prescribed by Molyneux (2004) and Shalaby & Shanab (2013). Seed samples were milled, and 0.1-0.5 g of the sample put into clean propylene tubes. Add 10 ml of the 80 % methanol was added to each sample. Samples were shook on a mechanical shaker at 25 °C for 24 hours and later centrifuged at 4,000 revolotions per minute (rpm) for 10 min. The supernatant aliquot for determination of the total antioxidant activity was then taken out for determination of total antioxidant activity. Blank, standards and samples were pipetted into their respective wells in a microtiter plate. 50 µl of each standard and unknown sample or appropriately diluted sample was pipetted into microtiter. 50 µl of DPPH was added.

Using a plate shaker/incubator, the plates were shook well and incubated in the dark for an appropriately optimized incubation time. The absorbance was read at 515 nm in a Microtiter plate spectrophotometer reader and plotted a standard calibration of Trolox. The results of total antioxidant content was expressed as mg of Trolox Equivalents per 100 g of dry sample.

Total antioxidant activity as mg Trolox Equivalents per g of dry sample was calculated and expressed as below:

$$\textbf{\textit{Total antioxidant activity in mg of Trolox equivalent per 100 g of dry sample}} = \frac{\boldsymbol{C\ XDFX100}}{\boldsymbol{WX1000}}$$

Where,

C = Concentration obtained from the calibration in µg/ml

DF = Total dilution factor

100 = Conversion factor to report results in mg/100g

W = weight of the sample in grams

= conversion from µg/ml to mg/ml.

Determination of Total Tannins: The determination tannins content was determined by Folin-Denis Method Using a Microplate Reader and procedures according to Price & Butler (1977) and modifications by Terrill, *et al*., (1992) and Saxena *et al.,* (2013). 0.25g of the sample was weighed into clean conical flask. 37.5ml of deionized distilled water was added. 0.25g of the sample was weighed into clean conical flask. 37.5ml of deionized distilled water was added. The flask was heated gently and boiled for 30 minutes. After allowing the mixture to cool and transferred into a clean 50ml falcon tube, making up to 50 ml with deionized distilled water. The mixture was centrifuged at 3,500 rpm for 15 minutes. The supernatant aliquot was then taken out for determination of the tannins content.

The tannin content is determined using the modified Folin-Denis procedure. Blank, standards and samples were pipetted into their respective wells in a microtiter plate. Upon adding the samples/blank/standards and Folin-Denis reagent, the mixture was mix gently

by priming and after 5 minutes and 7 % Na_2CO_3 added and prime gently. The absorbance was read at 700 nm in a microtiter plate spectrophotometer reader and plotted against a standard calibration (0.01- 0.02 – 0.04 - 0.06 – 0.08 – 0.1 mg/ml) versus absorbance readings of Tannic acid in deionized water. The results of tannin content was expressed as mg Tannic Acid Equivalents (TAE) per gram of dry sample. The tannin content of the samples was calculated as tannic acid equivalents from the standard graph according to the formula below:

$$Tannic\ content\ in\ \text{mg TAE per g of dry sample} = \frac{\text{C XDFX100}}{\text{WX1000}}$$

Where;

C = Concentration obtained from the calibration in µg/ml

DF = Total dilution factor

W = weight of the sample in grams

100 = Conversion factor to report results in mg/100g

1000 = conversion from µg/ml to g/ml.

Analysis of Sugars (Sucrose, Glucose and Fructose)

Since the initial analysis of samples for protein and fat showed high levels, the following procedure as described below was used to determine the individual sugars studied.

a. 1g finely ground sample was weigh accurately into 50 mL centrifuge tube, 30 mL petroleum ether was added and vortexed. The petroleum ether was discarded. This process of extraction was repeated 3 times.
b. Sample was dried in oven 60 °C for 1 hour to remove any residual petroleum ether then transferred defatted sample into 25 mL volumetric flask.

c. 1.25 mL 15% potassium hexacyanoferrate and 1.25 mL 30% zinc sulphate were pipetted and mixed well. After which it was made to stand for 15 minutes, 5 mL acetonitrile added and make to volume with distilled water, mixed well and allowed to stand overnight.

b. Approximately 1.5 ml of the solution was filtered through a 0.2µm nylon filter. At this stage the sample is ready for injection using the Shimadzu Nexera Liquid chromatograph. The instrumental parameters is show below:

Instrument	Shimadzu Nexera Liquid chromatograph			
Work station	Lab Solutions software			
Sample Management	Nexera Auto sampler SIL-30AC			
Detector parameters	Evaporative Light Scattering Detector parameters			
	Temperature		70	
	Nebulizing gas flow rate		350kPA	
Column	Lichrospher NH_2-5 (150 x 4.6 mm,5 µM)			
Chromatographic conditions	Run Time	Oven temp	MP: A (ACN)	MP: B(H20)
	15 Min	40 ^oC	82%	18%
Flow Rate	1.2ml/min			
Injection volume	10 µL,			

Calculation and Expression of Results:

The Lab solutions data analysis software was used to establish a standard calibration curve from a plot of peak areas against the known concentration of the injected series of standards. The analyte of interest was identified by the retention time of the

chromatographic peak of the target compound in the test sample and that of the corresponding standard chromatographic peak. The concentration of the test solution was determined from the calibration curve. The values should be within the linear range of the standard curve, if the lie outside of the linear range, the samples should be diluted, loaded into the UPLC auto sampler and re-analyzed.

The content of each individual sugar in the test sample is calculated according to formula:

$$Totalamount\ of\ each\ sugar(g/100g) = \frac{\mathrm{C\ XDFX100}}{\mathrm{WX1000}}$$

Where;

C = Concentration obtained from the calibration in μg/ml

DF = Total dilution factor

W = weight of the sample in grams

100 = Conversion factor to report results in mg/100g

1000 = conversion from mg/ml to g/ml

5.3.4 Experimental design and data analysis

This experiment was laid out as a single factor experiment in a completed randomized design (CRD) with 6 levels of seed maturity stages. All seed physiological quality tests were conducted with four replications while biochemical analysis were in triplicates. All laboratory analysis was conducted as a complete randomized design.

Statistical analysis were implemented using Statistical package for social sciences (SPSS version 2016) software. Data were represented as mean ± standard deviation. Data were subjected to analysis of variance and means were compared by Tukey's HSD at 5% probability ($p < 0.05$).

A multivariate correlation matrix was analyzed to evaluate the relationship between the biochemical components and physiological quality data of the seeds obtained from different maturity stages. As a confirmatory analysis, a principal component analysis (PCA) was used to establish the relationship between the biochemical components and the main variables that contribute to sample similarity. In addition, a Partial Least Squares Regression (PLS-R) analysis was employed to identify the biochemical variable that significantly influenced the seed physiogical variables studied.

5.4 Results and Discussion

5.4.1 Seed physiological quality at different maturity stages for cv. *Oforiwa*.

The seed germination percentage and seed vigour changes during the development of the African eggplant cv. *Oforiwa* is present in Figure 18.

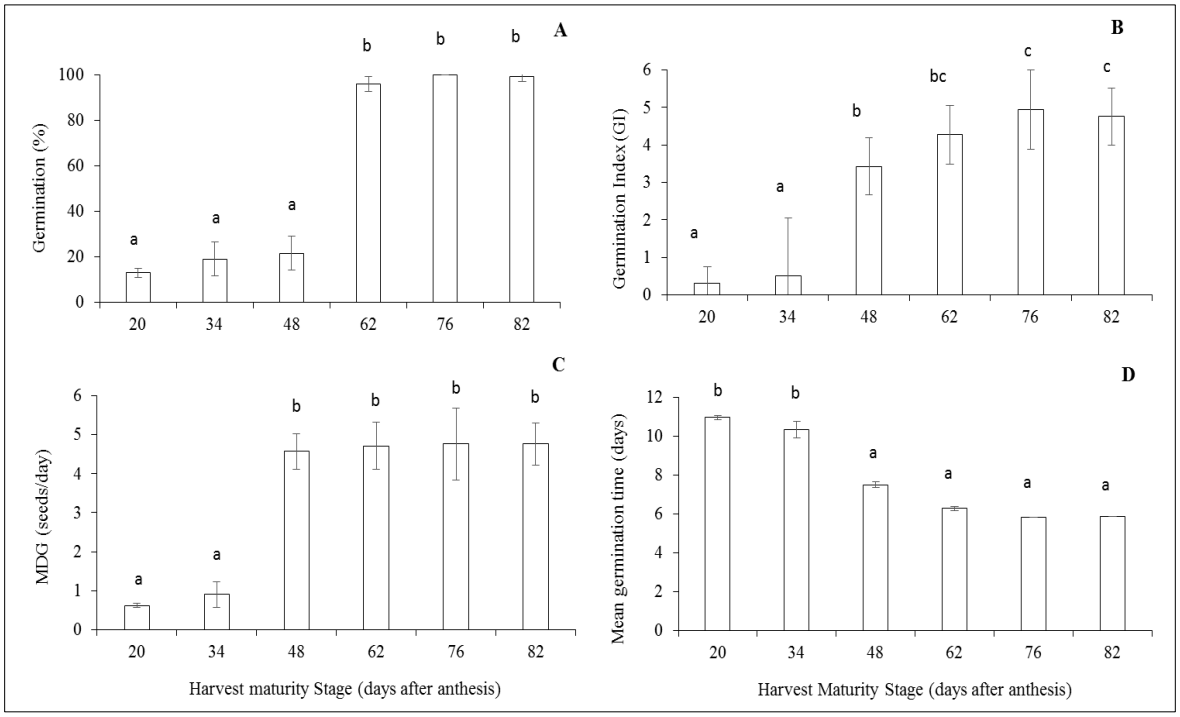


Figure 14: Means for seed germination (A), germination index (B), mean daily germination (C) and mean germination time (D) of African eggplant cv. *Oforiwa* harvested at six maturity stages. The means were separated by Tukey's HSD test at 5% probability and vertical bars indicate ± standard deviation for n = 4 replications. Error *bar* of standard deviation, if not visible, is smaller than the symbol.

Seed germination capacity and seed vigour, measured by germination index and mean daily germination and mean germination time significantly ($p \leq 0.001$) improved as seeds of African eggplant matures (Fig. 14).

Seed germination were < 20 % when seeds were extracted from 20 to 48 days after anthesis (DAA) fruits. Germination sharply increased afterwards, from fruits harvested 62 DAA and peaked at 76 DAA with 100 % germination (Fig. 14A). Thereafter, seed germination marginally declined albeit insignificantly. As harvest delayed, seed vigour also improved significantly ($p \leq 0.001$). Germination index (GI) and mean daily germination (MDG) increased from 48 DAA and were maximum at 76 DAA, coinciding with maximum seed germination (Fig. 14 B & C). Highest GI value was 4.96 at 76 DAA while 4.76 seeds were germinating per day when seeds were harvested 76 DAA. As expected, there was an inverse relationship of seed maturation with mean germination time (MGT) (Fig. 14 D). Seeds harvested precociously took more time (10 – 11 days) to complete the germination process evidenced by radicle protrusion while this time reduced drastically to 5.8 days when seeds had matured in latter harvests (Fig. 14 D). This indicates that seed vigour improves with maturation.

The acquisition of germination capability occurs at the early stages of seed development (precocious harvests) for some species (Borges *et al.,* 2006; Lamara *et al.,* 2013; Silva *et al.,* 2015), while others happen later in the development stage. In the present study, the seeds acquired germination capability in the early stages of development (20 DAA) albeit at low germination percentage. Seeds harvested precociously may fail to germinate due to immature embryos at this stage. Seed germination capacity however, improved significantly after 48 DAA with maximum germination capacity at 76 DAA. This results is consistent with other reports on tomato (Dias *et al.,* 2006), pepper (Vidigal *et al*., 2011) and eggplant (Demir *et al.,* 2002). This behaviour corroborates the hypothesis that some

seeds borne in fleshy fruits obtain maximum germination capacity after seed-filling stage or physiological maturity (Demir and Ellis, 1992a; Oliviera *et al.,* 1999).

Seed vigour as assessed by germination index and mean daily germination proved to be good seed quality measures. GI measures both germination percentage and speed of germination. Higher values of GI denotes a higher seed vigour while a lower value indicates low seed vigour (Bench-Arnold *et al.,* 1991). Mean daily germination on the other hand measures the number of seeds germinating per day, which is indicative of seed vigour. The higher the number of seeds germination per day, the higher the seed vigour of the sample or seedlot. The present results showed that, seed vigour coincided with maximum seed germination at 76 DAA. An earlier report according to Kwon and Bradford (1987) indicated that maximum germination and vigour for tomatoes occurred 15 days after attaining maximum seed dry weight and coincided with maximum germination as observed in this study. This observation was later validated according to a study on tomatoes (Demir & Ellis, 1992a), that the highest germination percentage in tomato is achieved when fruits are harvested 70 days after anthesis (DAA). Vidigal *et al*., (2009) also reported maximum seed vigour measured by first germination count in pepper seeds to occur at 70 DAA, which is consistent with the present results.

As seed matures, the time required to complete the germination process reduces; which is an indication of improved vigour. This is attributable to the fact that the energy source required for initiation of the process increases with maturity. The lowest time required for maximum seed germination occurred at 76 DAA. This results confirm the earlier observation that maximum seed quality of the African eggplant is obtained when seeds are harvested 70-76 DAA.

5.4.2 Biochemical changes during seed development and maturation of African eggplant seeds

Figure 15 illustrates the changes in protein and fat content during seed maturation of African eggplant cv. *Oforiwa*. Crude protein accumulation was not significantly different ($p > 0.05$) while crude fat was highly significant ($p \leq 0.001$). It was observed that protein and fat accumulation during seed maturation were parallel. Protein and fat reserves increased from 15 mg/100 and 16.56 mg/100 respectively from early stages of development (20 DAA) and increased marginally and steadily thereafter. There was a parallel decline for both reserves at 62 DAA and increased again until last harvest at 82 DAA. The content of crude fat was observed to be significantly higher than protein from 34 days after anthesis till the last harvest (Fig. 15).

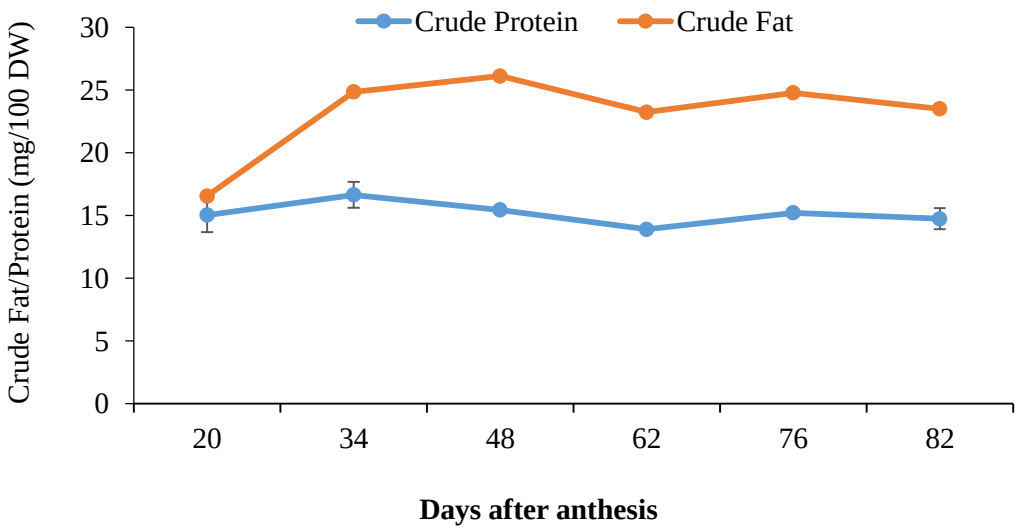


Figure 15: Content of crude protein and crude fat during seed development in African eggplant cv. *Oforiwa*. The means were separated by Tukey's HSD test at 5% probability and vertical bars indicate ± standard deviation for n = 3 replications. Error *bar* of standard deviation, if not visible, is smaller than the symbol. DW: dry weight.

The results regarding the protein and fat accumulation patterns is consistent with Silva *et al*. (2015) for *Capsicum baccatum* seeds, where there was parallel accumulation of these two reserves during seed development. For *Capsicum baccatum* seeds, protein increased between 10 to 40 DAA and remained unchanged thereafter (Silva *et al*., 2015), similar to the current results except that there was a marginal decline after 34 DAA before remaining unchanged until last harvest at 82 DAA.

From the biochemical perspective, seed vigour has been associated with soluble protein, starch and soluble sugar contents (Henning *et al*., 2010). Protein accumulation during maturation is also reported to be involved in the synthesis of genetic material and the enzymatic reactions, which are essential to cellular metabolism (De Souza *et al*., 2018) in addition to its protective role. According to Henning *et al*., (2010) and Han *et al.,* (2017) variations in protein accummulation in seeds as they develop may provide efficient indicators for monitoring biochemical processes associated with seed vigour.

In this study, the correlation matrix showed a positive correlation (r = 0.501) of protein content with mean germination time, which is a measure of the time required to complete the germination process (Table 11), which is indicative of the seed vigour. However, protein content was negatively correlated (r = -0.478) with seed germination suggesting that the mere presence and levels of protein content cannot be good indicator for seed germinability.

Fats or lipids in seeds serve as a source of energy reserve during germination and as assimilates for young seedlings. This could explain the positive correlation (r = 0.51) observed between fat and germination index, which is indicative of speed of germination

and indicative of seed vigour. Further, the relatively high total fat content observed in African eggplant seeds corroborates with the results of Jarret *et al.*, (2016) that seed oil content of *Solanum aethiopicum* is high, with linoleic acid at about 57%. This may add to the knowledge on the possible extraction of seed oil from African eggplant to diversify the sources of lipids eaten by Africans where this crop is largely cultivated.

5.4.3 Antioxidant activity and Tannin content during seed development and maturation of cv. *Oforiwa*.

Figure 16 illustrates the antioxidant activity and tannin content accumulation during seed development and maturation. There was significant difference in both antioxidant activity and tannin content at the various seed maturity stages ($p \leq 0.001$). Antioxidant activity at early seed development stage (20 DAA) was extremely high (289 TE/100g) and reduced drastically about 71.6 % at 34 DAA. It maintained a relatively same level from 34 DAA to 76 DAA and increased marginally to 106 TE/100g at last harvest (82 DAA) (Fig. 16). Tannin content was also high throughout seed development. It increased from initial early seed development from 696.98 TAE/100 g and peaked at 62 DAA and declined marginally afterwards (Fig. 16).

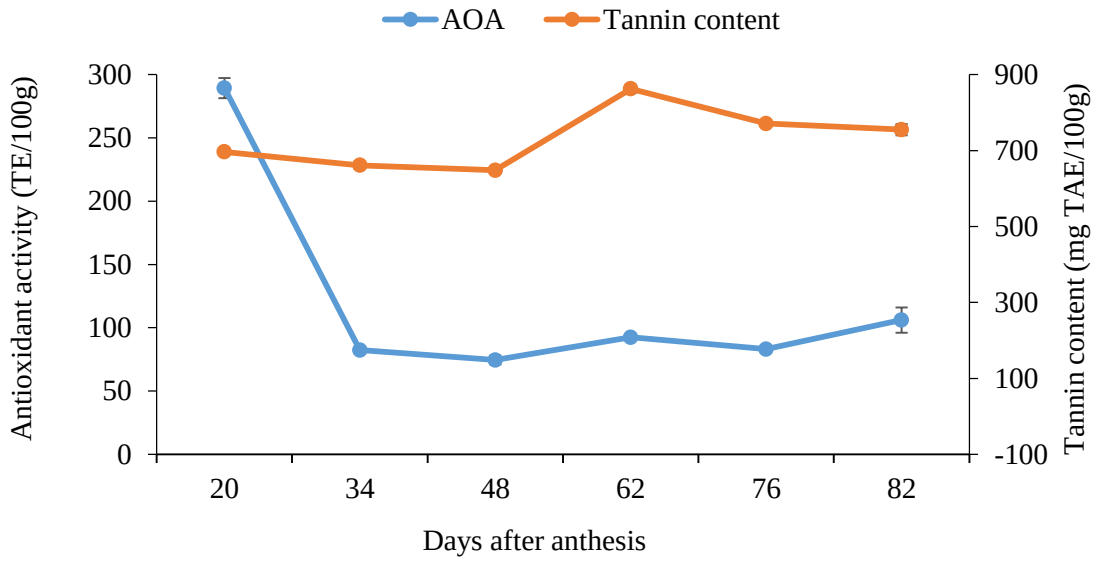


Figure 16: Means of Antioxidant activity (AOA) and Tannin content during seed development in African eggplant cv. *Oforiwa*. Means were separated by Tukey's HSD test at 5% probability and vertical bars indicate ± standard deviation for three replications. Error *bar* of standard deviation, if not visible, is smaller than the symbol. TE: Trolox equivalent; TAE: Tannic Acid equivalent.

Antioxidant activity refers to the activity of antioxidant enzymes such as catalase, superoxide dismutase (SOD), peroxidase, ascorbate peroxidase activity (APX) among others (Pehlivan, 2017). Antioxidant systems play an important role in embrogenenis and cell growth (Foyer and Noctor, 2005; Halliwell, 2006). The higher levels of antioxidant activity observed in the early stages of seed development could be attributed to the high seed moisture contents as there is lot of metabolic reactions associated with histodifferentiation in the early stage. This assertion is corroborated by the views of Gomes and Garcia (2013). The high antioxidant activity at the early and final stages of seed development and maturation pattern is consistent with the reports of De Souza *et al*., (2018) for *Hevea brasiliensis* L. The authors observed high antioxidant activity, particularly SOD and APX in early development stage, declined and then increased at the end of the maturation phase. Antioxidants have been reported to play an important

physiological role of controlling intracellular concentrations of reactive oxygen species (ROS) (Kapur *et al*., 2015) and thereby scavenging and deactivating oxidative agents by converting the toxic radicals to less reactive species (Apel & Hirt, 2004; Halliwell, 2006). The increase in antioxidant activity at the final stage of maturation in this study could be linked to the activation of the scavenging role played by antioxidants. ROS are reported to be involved mostly at the final stage of seed development whereby there is dramatic loss of water due to maturation drying (Pammenter & Berjak, 1999). At this stage, it is expected that antioxidant activity is elevated to counter the activities of ROS, thereby preventing seed deterioration as a reaction of cell damage.

Tannin, a type of flavonoids is an antioxidant reported to accumulate in seed coats (Demonsais *et al.*, 2020). It was observed that its accumulation started in the early stage of seed development and maintained at relatively high levels, peaking at 62 days after anthesis. This is consistent with the reports on tannin accumulation pattern that at early seed developmental stages, differentiating alongside the developing endosperm, begin to accumulate proanthocyanidins, a type of oligomeric flavonoids also known as condensed tannins (Kitamura *et al*., 2004; Demonsais *et al*., 2020). According to Debeau-jon *et al*., (2000), Arabidopsis transparent testa (tt) mutants that were deficient of flavonoid synthesis had poor viability. This could explain why tannin content was strongly and positively correlated with germination percentage ($r = 0.83$) and seed vigour measured by germination index ($r = 0.62$) and mean daily germination ($r = 0.55$) in the current study. At 62 days after anthesis, the maximum levels of tannin content was observed (862 mg TAE/100g) and declined thereafter. This stage coincided with the mass maturity or

physiological maturity stage of cv. *Oforiwa*, signaling the end of seed–filling stage and transitioning to desiccation tolerance phase.

It is reported that tannins impregnate into cell walls, induce cell wall hardiness of seed coat, thus reducing the risk of degradation by improving seed solidity and seed coat impermeability (Debeau-jon *et al.*, 2007; Loubery *et al.*, 2018). The gradual increase in tannins contents and peaking at PM (62 DAA) could further suggest a relationship with seed colour as seed matures. Young immature seeds at early harvests were white until PM where seeds attained their typical colour.

5.4.4 Sugar content accumulation during seed development and maturation in African eggplant cv. *Oforiwa.*

Figure 17 shows the changes in soluble sugars during seed development of African eggplant. Soluble sugars (sucrose, fructose and glucose) were measured throughout the development and maturation with notable changes ($p \leq 0.001$) at the various stages of seed development (Fig. 17). All sugars were high at early stages of seed development (20 DAA). Sucrose content was 28.33 mg/100g DW, higher than glucose and fructose (12 and 11 mg/100g) respectively. There was a dramatic reduction from this stage to 34 DAA before in began a gradual increase again. Both glucose and fructose accumulation pattern was similar and parallel throughout seed development (Fig. 17).

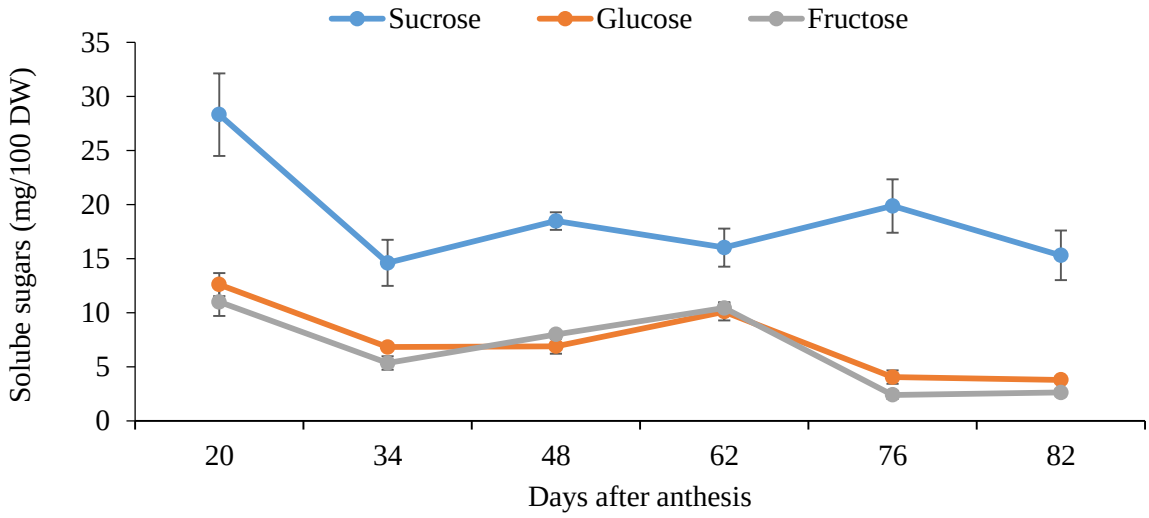


Figure 17: Changes in soluble sugar contents (sucrose, glucose and fructose) of African eggplant cv. *Oforiwa* seeds during development. Each data point is the mean of 3 independent measurements. The means were separated by Tukey's HSD test at 5% probability. Vertical bars indicate ± standard deviation. Error *bar* of standard deviation, if not visible, is smaller than the symbol. DW: dry weight.

It can be inferred from the results shown in figure 20 that the increased sucrose levels may have contributed to the maxiumum germination and vigour observed at 76 days after anthesis as suggested by Lin & Huang, (1994) and later by Hanning *et al.*, (2010). However, the correlation matrix analysis showed a negative relationship (Table 11). Any possible influence will be related to its contribution to the mean germination time which had a positive correlation with all the sugars (r = 0.43 to 0.56) (Table 11).

The hexose (glucose and fructose) are related to cell growth and mitotic activity (Weber *et al.,* 2005) and this could explain their relatively high levels at early development stage of seed development. The drastic decline in sugar levels could be linked to the reduction in seed moisture content, allowing dry matter accumulation. Silva *et al.*, (2015) also

observed a 67% decrease in starch content from first harvest between 14 to 30 DAA for *Capsicum baccatum* seeds.

Sucrose has a dual function in seed development. It serves as a transport for nutrient sugars and as a signal for triggering storage-associated processes (Koch, 2004), that is transitioning from metabolic sink to a storage sink. Bewley *et al*. (2013) suggested that these sugars may bind to hydrophilic head groups of membrane lipids, thereby replacing water and stabilizing cell membranes as seed undergoes desiccation. The point of interest in this study was to establish the stage at which this transition from a metabolic sink phase to a storage sink stage, then to signal the desiccation tolerance phase occurs in African eggplant. According to Koch (2004), this maturation phase is indicated by a switch from high hexose status to a sucrose-based carbohydrate status. The stage of such transition is 62 days after anthesis according to the current results (Fig. 17). It is not surprising that this stage also coincides with mass maturity or physiological maturity for this cultivar. It can be established that in African eggplant cv. *Oforiwa* seeds, the transition stage for storage-associated differentiation occurs at 62 DAA or mass maturity.

5.4.5 Correlation matrix of biochemical components and physiological data of cv. *Oforiwa* as a function of seed maturation.

A correlation matrix was ran to assess the correlation of the biochemical components and the seed physiological variables measured. It was shown that soluble sugars (sucrose, glucose and fructose), crude protein content and crude fat correlated negatively with germination, germination index and mean daily germination (Table 11).

Negative correlations of these biochemical components, and germination and vigour variables have been reported for maize seeds (Santos *et al*., 2017; Nerling *et al.,* 2018). In the case of Nerling *et al.,* (2018), the authors measured total soluble sugars and was found a strong positive correlation (r = 0.92) with seed vigour measured by accelerated ageing but negatively associated when seeds were subjected to stress condition in cold test (r = -0.05). Further, soluble sugars and total protein were negatively correlated (r = -0.07; -0.61 respectively to percentage germination (Table 11).

For seed germination, there was negative correlation with all sugars (sucrose, fructose and glucose), protein (Table 11). These results are consistent with what Zhan *et al.,* (2018) found in some grass species where protein (r = -0.09) and starch (r = -0.23) were negatively correlated with seed germination. According to Delgado *et al.,* (2015) the initial content of seed reserve composition were not influential factors in maintaining soybean seed vigour. Santos *et al*. (2017) was of the view that germination and seed vigour could be associated with the efficient metabolism of hydrolysis, its mobilization and utilization of these reserves during the germination process rather than the presence of their high contents at maturity. This hypothesis may be the case in this study as has been reported in other studies. For example Yang *et al.,* (2016) reported that the starch

level in the high-starch seeds of *Sorghum bicolor* was reduced 13.5% after 1–3 days from sowing. In legumes, Benítez *et al.,* (2013) reported total starch decrease of 11.8–35.2% during germination while seeds of *Aniba rosaeodora* showed a 29.5% reduction in starch level during germination (Lima *et al.,* 2008). This suggest that the seeds ability to efficiently mobilize and use these seed reserves during germination is the most important factor to consider and not their mere concentration levels.

In the present study, soluble sugars, fat and protein did not positively influence the seed germination and vigour quality of African eggplant cv. *Oforiwa,* and may not be good indicators of seed physiological seed quality. However, these components of seed quality have strong implications on desiccation tolerance and longevity in storage, both of which were not measured in this study.

Positive correlation was however observed for tannin content with germination and seed vigour measured by germination index and mean daily germination (Table 11). As described in previous chapters, tannins can act as antioxidants and may therefore possess the ability to limit oxygen diffusion in the seed coat (Debeaujon *et al.,* 2007; Pourcel *et al*., 2007) , enhancing seed coat solidity against environmental stresses. In Arabidopsis seeds, the absence of flavonoids such as tannins have been reported to correlate with higher seed permeability to water and oxygen thereby lowering seed dormancy and improving viability (Debeaujon *et al.,* 2000; Chahtane *et al.,* 2017). This suggest that tannin content, which in this study was observed to be strongly correlated with seed germination and vigour could be used as as suitable indicator of physiological quality in African eggplant seeds.

Table 11: Correlation matrix of biochemical components and physiological variables of African eggplant cv. *Oforiwa* seeds

Variables	Sucrose	Glucose	Fructose	Crude Protein	Crude fat	AOA	Tannins	% G	MGT	GI	MDG
Sucrose	**1**	**0.591**	**0.471**	-0.139	**-0.749**	**0.808**	-0.206	-0.366	0.406	-0.401	-0.394
Glucose		**1**	**0.946**	-0.274	**-0.712**	**0.716**	0.002	**-0.488**	**0.568**	**-0.600**	**-0.541**
Fructose			**1**	-0.365	**-0.526**	**0.537**	0.032	**-0.473**	0.434	-0.453	-0.341
Crude Protein				**1**	0.165	-0.101	**-0.692**	**-0.478**	**0.501**	**-0.472**	**-0.494**
Crude fat					**1**	**-0.980**	0.038	0.310	**-0.492**	**0.514**	**0.588**
AOA						**1**	-0.170	-0.422	**0.559**	**-0.571**	**-0.620**
Tannins							**1**	**0.828**	**-0.649**	**0.624**	**0.551**
% G								**1**	**-0.861**	**0.856**	**0.750**
MGT									**1**	**-0.992**	**-0.947**
GI										**1**	**0.964**
MDG											**1**

Values in bold are different from 0 with a significance level alpha = 0.05. % G: germination; MGT: mean germination time; GI: germination index; MDG: mean daily germination; AOA: Antioxidant activity.

5.4.6 The association of biochemical characteristics and physiological response at different maturity stages of cv. *Oforiwa* seeds using principal component analysis

The principal component analysis (PCA) was used to determine the biochemical components that elucidate the maintenance of seed physiological quality in African eggplant (Fig. 18). Components 1 and 2 was able to explain 83.05 % of the variance; 25.92% by the first component (PC1) and 57.13% by the second component (PC2) (Fig. 18).

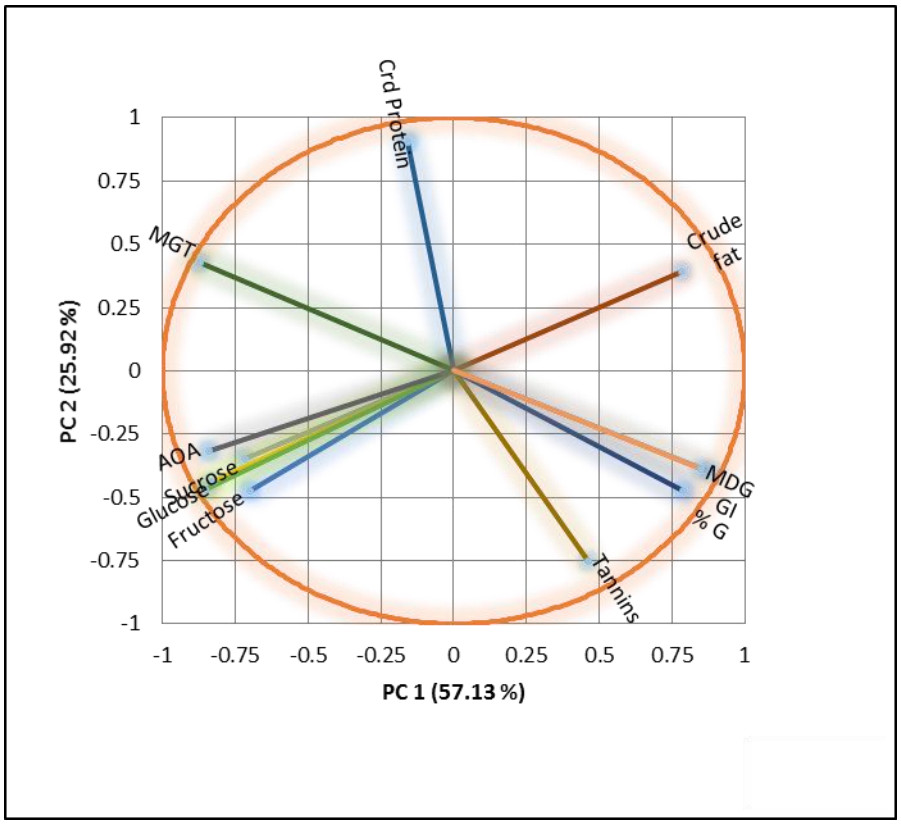


Figure 18: Principal component analysis (PCA) for biochemical components and physiological quality of African eggplant cv. *Oforiwa* seeds. %G: germination; GI: germination index; MDG: mean daily germination; MGT: mean germination time; AOA: Antioxidant activity.

In order to be satisfied that your PCA scatter plot gives a high-dimensional data structure, Varmuza and Filzmoser (2009) suggested that the score plot of the first two principal components should sum to more than 70% of the total variance. In this study, the first two PCs contributed to a total variance of 83.05% (Fig. 18).

The loading values indicated that only crude fat was grouped in principal components (PC1+/PC2+) with no related physiological quality association. In PC1+/PC2-, mean daily germination, germination index and percentage germination were grouped according to their tannin content. In PC1-/PC2+, mean germination time (MGT) was grouped based on protein content. Antioxidant activity was grouped with the soluble sugars in PC1-/PC2- (Fig. 18).

The loading plots indicated the parameters that contributed to discrimination of the objects described in the score plot (Fig. 18). It was observed that the sugars, crude fat and the physiological traits (% G, GI and MDG) were the most important variables that explained the variations in PC1. Crude protein and tannin content were the most important for determining the variations in principal component 2.

The principal component analysis (PCA) indicated that the physiological quality of germination and vigour by germination index and mean daily germination were influenced by biochemical components such as tannin content and crude fat. It further showed that mean germination time was however influenced largely by the soluble sugars and crude protein. More detailed analysis using partial least squares regression analysis (PLS-R) was employed to establish and confirm the effect of tannin content on seed germination and vigour (Fig. 19).

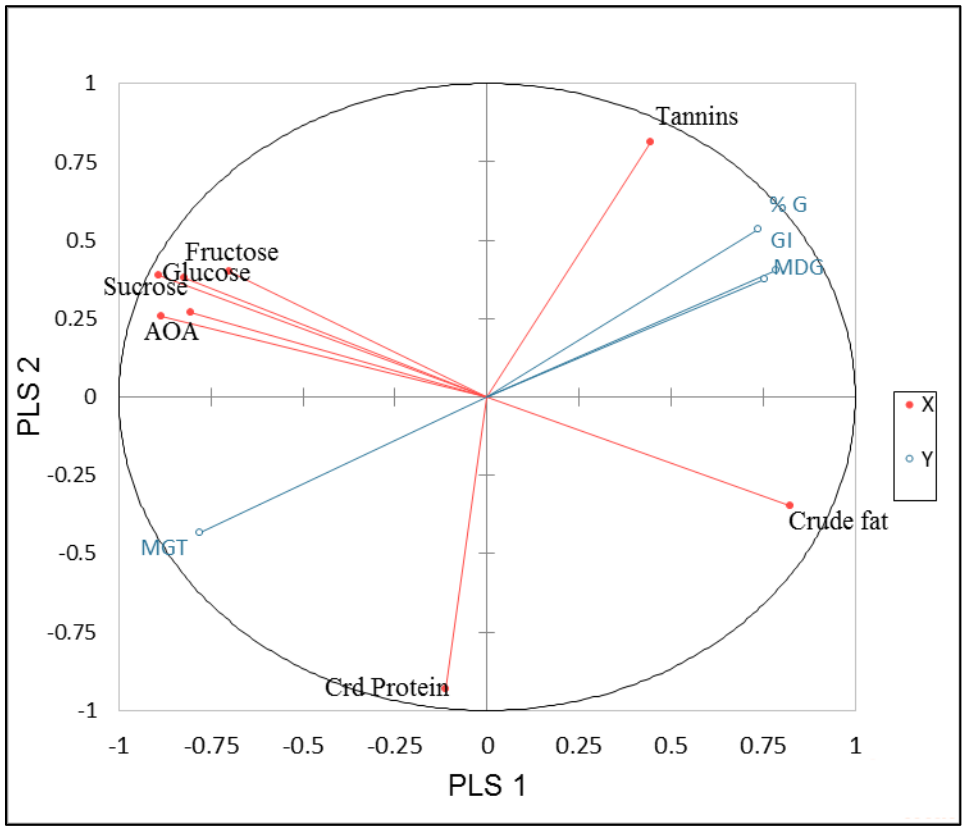


Figure 19: Partial least squares regression analysis (PLS-R) for association of biochemical components with physiological quality of African eggplant seeds. %G: germination; GI: germination index; MDG: mean daily germination; MGT: mean germination time; AOA: Antioxidant activity.

According to the partial least square regression (PLS-R) model, besides tannin content, crude fat content is among the biochemical components that affect both seed germination and vigour indexes such as germination index and mean daily germination (Fig. 19).

This suggest that tannin content plays and important role in the maintenance of the physiological quality of African eggplant cv. *Oforiwa* seeds. This further confirms the strong correlation observed earlier between tannin content and seed germination and vigour parameters. This can be used as an important biochemical marker for the purposes of crop improvement programmes for this crop. The use of the PLS-R model has also

assisted in determining the contribution of each of the biochemical components in seed quality maintenance of this crop.

5.5 Conclusion

Seed maturity stage influence the biochemical characteristics including crude protein, sugars, antioxidant activity and tannin content of African eggplant (*Solanum aethiopicum* L.), with tannin content positely associated with seed physiological quality.

5.6 Recommendation

Tannin content can serve as a suitable biochemical marker for seed quality traits selection in African eggplant improvement programmes.

5.7 Suggestions for further research

i. Studies on the relatively high and steady levels of tannin content during seed development and maturation and its role in seed viability maintenance is required.
ii. Whether the tannin content decrease with time during seed storage and its effect on seed longevity needs further research.

CHAPTER SIX

EVALUATION OF THE EFFECTS OF HARVEST TIME AND AFTER-RIPENING ON SEED PHYSIOLOGICAL CHARACTERISTICS OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.)

Abstract

In the absence of a formal and reliable seed supply for the African eggplant, farmers usually source their seeds from fruits harvested for fresh market (precocious harvests) or from traders who extract seeds from unsold but ripened fruits in storage. Fruits harvested precociously at 20 and 34 days after anthesis do not germinate or record less than 10 percentage germination. This study therefore was conducted to evaluate the effect of harvest time and after-ripening on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.). Fruits were harvested at five maturation stages (30, 40, 50, 60 and 70 days after anthesis) and seeds extracted immediately (0 day) or after-ripened for 5, 10 and 15 days under ambient condition (24 ± 3°C) after each harvest prior to seed extraction. The experiment was laid out in a completely randomized design with split-plot arrangement with four replicaions Main plot constituted the harvest time while the storage periods was allocated to the sub-plot. Seed dry weight, seed moisture content, 1000 seed weight, seed vigour (first count) and germination percentage were determined. It was established that precocious harvest (30 DAA) was not beneficial to physiological seed quality even when subjected to 15 days post-harvest storage. Fruits harvested 40 and 50 DAA did not germinate or had less than 50% germination respectively in both cultivars, some indication of physiological dormancy. However, when seeds were extracted from these fruits ripened for 10 or 15 days in storage, their viability and vigour increased. Seeds extracted from fruits harvested 60 or 70 DAA achieved the maximum germination, varying between 93 to 94% (*cv. Oforiwa*) and 78 to 96% (cv. *Kpando*) independent of after-ripening (post-harvest storage). It is confirmed that after-ripening is inconsequential and not necessary when seeds are harvested at physiological maturity (PM) (60-70 DAA). For the purpose of seed production, seeds should be extracted from fruits harvested 60 or 70 DAA.

Introduction

Studies in determining the suitable fruit harvest for harvesting to in order to obtain high quality seeds is very critical for seed production purposes. Several studies have been conducted to establish the ideal maturity stage for harvesting many fruits whose seeds are borne in berries such as tomato (Demir & Samit, 2001; Dias *et al*., 2006), pepper (Vidigal *et al.,* 2009; 2011), eggplant (Demir *et al*., 2002) and Okra (Bortey and Dzomeku, 2016).

In Ghana, about 90% of farmers extract seeds from harvested fruits of African eggplant purposely meant for frest market and later ripen. Fruits harvested preciously as described, do not germinate or have unpredictable germination. It is observed that most crop species whose seeds are borne in fleshy berries are known to continue to mature after it has been detached from the mother plant. This suggests that the seeds within the harvested fruit may continue to develop and mature (Passam and Karapanos, 2008). This is probably so because some of the seeds harvested may be less mature and result in dormancy due to the insufficiently developed embryo (Demir *et al.,* 2002). However, after-ripening or post-ripening is a technique which has been reported to enhance seed quality and release partial dormancy in such fruits (Yogeesha *et al*., 2006; Iglesias-Fernandes *et al*., 2010). This techniques is also known to promote germination in pepper, eggplant, cucumber, tomato (Edwards and Sundstrom, 1987, Alverenga *et al.,* 1991; Sanchez *et al.,* 1993; Dias *et al*., 2006; Passam *et al.*, 2010a; 2010b).

In studying the eggplant (*Solanum melongena* L.), Passam *et al*., (2010a) harvested fruits 25 days after anthesis (DAA) and stored at 25 °C for 20 days before seed extraction, and reported a significantly higher mean 1000 seed weight compared to seeds extracted without after-ripening. The authors further indicated that after-ripening increased the rate

of germination of seeds from fruit harvested between 45 and 65 days after anthesis. They concluded that storage of prematurely harvested fruit prior to seed extraction allows the seeds of these fruits to after-ripen *in situ* and thereby increases seed size (Passam *et al.*, 2010b) and germination (Passam *et al.*, 2010a). Earlier, Dias *et al*. (2006b) found that allowing a short period of post-harvest fruit storage of tomato improves physiological seed quality.

Similarly, Kortse and Oladiran (2013) observed that after-ripening durations significantly influence 100 seed weight and germination percentage of melon (*Citrullus lanatus* (Thumb) seeds. Shaheb *et al.* (2015) also found the highest germination of French bean seeds to be obtained from the latest harvest than those harvested and extracted earlier. Eggplant (*Solanum melongena*), a close relative of African eggplant had a positive response in relation to fresh and dry seed weights, 100-seed weights and germination percentage when subjected to five to ten days after-ripening (Kortse, *et al*., 2017). Ozden and Demir (2018) in studying Aubergine (*Solanum melongena*) also revealed that after-ripening significantly increase total germination and concluded that aubergine (*Solanum melongena*) seed germination can be increased through after-ripening treatment. There is however, little information reported regarding this phenomenon in African eggplant (*Solanum aethiopicum* L.*)*, particularly the Gilo Group popularly grown and consumed in West Africa.

In this study, fruits were harvested at various stages after anthesis and tested to determine whether the seeds within these fruits could be after-ripened prior to extraction and establish if fruits can be harvested at an earlier stage than at full maturity for maximum seed quality.

6.1 Objective of Study

To evaluate the effects of harvest time and after-ripening on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.).

6.2 Research question

Does seeds within harvested fruits of African eggplant after-ripen (fill and mature) under ambient storage conditions prior to extraction and to what extent does it influence the physiological quality and germination behaviour of African eggplant seeds?

6.3 Materials and Methods

The study location, plant materials and nursery management were as previously described in chapter three.

6.3.1 Field tagging for harvest time and after-ripening treatment

Flowers were tagged at anthesis using different fibre ribbon colours to represent each harvesting time. A minimum of 60 flowers per harvesting stage per plot was randomly tagged, making a total of 240 flowers per harvesting stage. Fruits were harvested 30, 40, 50, 60 or 70 days after anthesis (DAA). Before seed extraction, the fruits from each harvest were extracted immediately (0 day) or after-ripened for 5, 10 or 15 days to reflect farmers' practice under room conditions (24 ± 3 °C and 70-75 % relative humidity). The seeds were then manually extracted under running water at the end of each storage period.

6.3.2 Data Collected

Fresh and Dry Seed Weight (g), seed moisture content (%), thousand seed weight (g) and seed germination percentage were determined according to the procedures and methods described in chapter four (4).

Seed quantitative characteristics determination: Using the Advanced Germination Measurement Tool (Khalid, 2008), other quantitative quality traits of the seeds such as mean germination time (MGT), germination index (GI), mean daily germination (MDG) and time required for the germination of 50% of the total number of seeds to germinate (T_{50}) were calculated.

6.3.3 Experimental design and data analysis

The experiment was laid out in a completely randomized design with a split-plot arrangement. The harvest time comprised 30, 40, 50, 60 and 70 days after anthesis was allocated to the main plot while the after-ripening periods (0, 5, 10 and 15 days) was allocated to sub-plots. All data were collected in four replications.

Data were subjected to Shapiro-Wilk's normality test before Analysis of variance using the Statistical Tool for Agricultural Research (STAR). Mean differences were tested according to Tukey HSD test at 5% significance level. The correlation dynamics among the seed quantitative traits were analyzed by Pearson correlation using Microsoft Excel Office version 2013.

6.4 Results and Discussion

6.4.1 Effect of harvest time and after-ripening on seed moisture content of cv. *Oforiwa* and cv. *Kpando.*

The results showed a significant difference ($p \leq 0.001$) for harvest time, after-ripening periods and their interactive effects on seed moisture for both cultivars (Fig. 20).

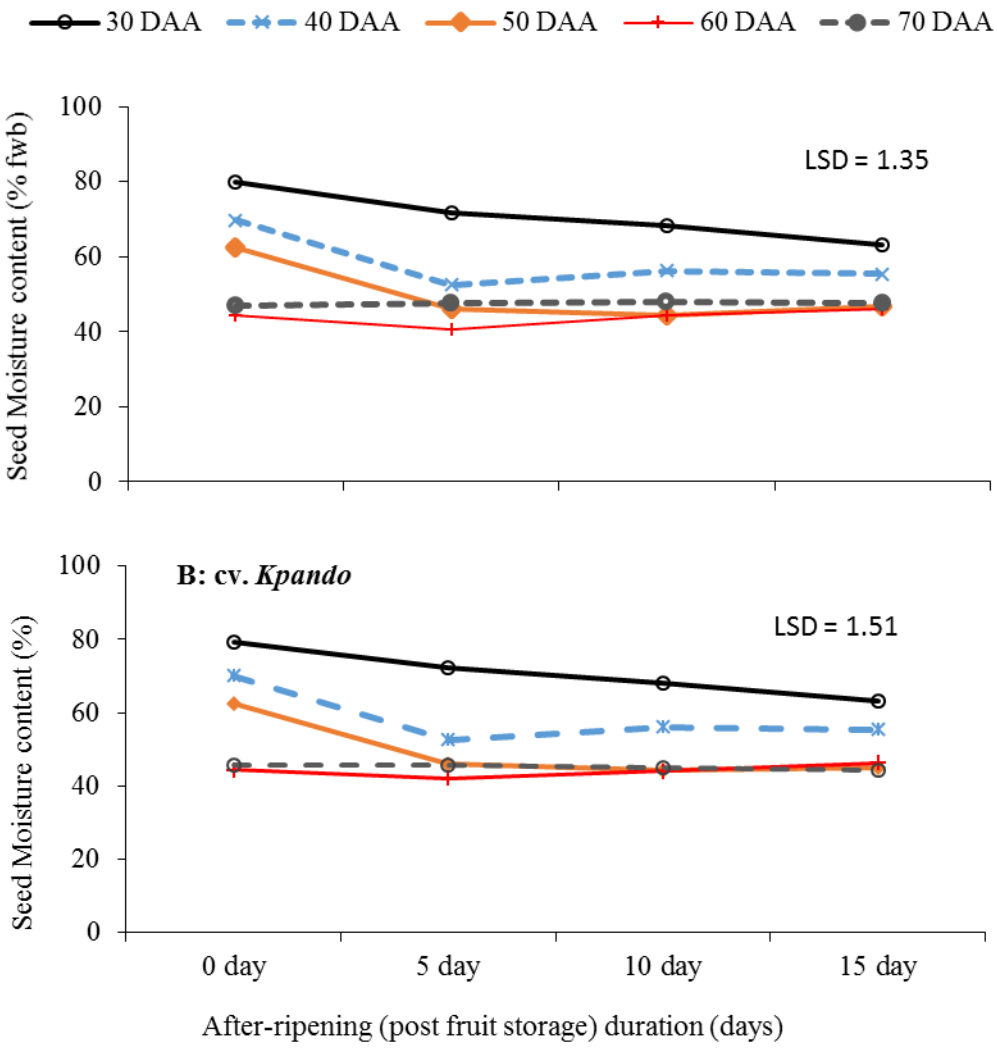


Figure 20: Seed moisture content for African eggplant cultivars harvested at 30, 40, 50, 60 and 70 DAA and after-ripened for 0, 5, 10 and 15 days. LSD: 0.05.

The seed moisture content was high (79.2 - 80 %) at early harvests and declined linearly with maturation (Fig. 2) for both cultivars. The effect of after-ripening was significant in earlier harvests (30 -50 DAA) than in later harvests (60 or 70 DAA).The maximum moisture content, varying between 80 to 63.1% after 15 days storage was found in seeds from fruits harvested 30 DAA, followed by fruits harvested 40 DAA (69.7 to 55.3%) (Fig. 20).

This results is consistent with an observation in peppers (Vidigal *et al.,* 2009) where maximum seed moisture varied between 63.2 to 58.6 % in seeds stored for 15 days prior to extraction from fruits harvested 40 DAA. The seed moisture content decreasing pattern was more as a function of maturation (80 to 47%) from 30 DAA to 70 DAA, than after-ripening treatment. This depicts a typical pattern of orthodox seeds borne in fleshy fruits. The gradual decline in moisture is as a result of dry matter accumulation. The relatively higher seed moisture contents observed at physiological maturity stages (70 DAA) is consistent with vegetable seeds.

Practically, the storage of fruits harvested at 60 or 70 DAA did not affect the seed moisture content which varied between 46.6 and 44.3 % (60 DAA) and between 47 and 47.6% (70 DAA). This observation is consistent in pepper (Vidigal *et al*., 2009). It is generally reported that the moisture content of seeds borne in fleshy fruits have high moisture contents, oscillating between 38 and 45% at maturity and remains high even after accumulation of the maximum dry matter. This corroborates with earlier studies by Demir and Ellis, (1992b; Alan and Eser, 2008; Vidigal *et al*., 2011) for pepper, Dias *et al*., (2006b) for tomato, Demir *et al.,* (2002) for eggplant and recently for wood apple (Murrinie *et al.,* 2019).The present study confirms this observation for African eggplant.

Table 12: The effect of after-ripening for 15 days on dry seed weight and 1000 seed weight of cv. *Oforiwa* extracted from fruits harvested at 30 -70 days after anthesis.

	After-ripening period (AF, days)							
	Dry Seed Weight (mg)				1000 Seed Weight (g)			
Harvest time	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
30 DAA	105.5 e	112.0 e	123.5 d	133.0 e	1.08 e	1.11 e	1.23 d	1.33 e
40 DAA	163.2 d	180.0 d	185.7 c	214.0 d	1.63 d	1.79 d	1.83 c	2.16 d
50 DAA	214.0 c	234.2 b	244.2 a	243.0 b	2.14 c	2.32 b	2.46 a	2.41 b
60 DAA	281.7 a	224.7 c	234.5 b	254.0 a	2.83 a	2.26 c	2.41 ab	2.57 a
70 DAA	241.2 b	240.0 a	237.0 b	223.2 c	2.47 b	2.40 a	2.37 b	2.28 c

Means with the same letter within a column are not significantly different according to Tukey's HSD Test at 5% probability. AF: After-ripening, MS: Maturity stage.

Table 13: The effect of after-ripening for 15 days on dry seed weight and 1000 seed weight of cv. *Kpando* extracted from fruits harvested at 30 -70 days after anthesis.

	After-ripening period (days)							
	Dry Seed Weight (mg)				1000 Seed Weight (g)			
Harvest time	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
30 DAA	102.2 d	146.1 d	145.5 e	140.0 d	1.02 d	1.45 d	1.48 e	1.41 d
40 DAA	133.5 c	172.5 c	265.5 c	271.0 b	1.33 c	1.69 c	2.67 c	2.70 b
50 DAA	258.0 b	254.0 b	296.7 a	296.0 a	2.57 b	2.55 b	3.06 a	3.05 a
60 DAA	260.0 b	273.5 a	289.5 b	257.0 c	2.60 b	2.73 a	2.90 b	2.57 c
70 DAA	272.0 a	273.5 a	251.0 d	257.0 c	2.70 a	2.73 a	2.50 d	2.53 c

Means with the same letter within a column are not significantly different according to Tukey's HSD Test at 5% probability.

6.4.2 Effect of After-ripening on dry seed weight and thousand seed weight at different fruit harvest time for two cultivars of African eggplant

The present experiments showed a highly significant ($p \leq 0.001$) independent and interactive effect of harvest time and after-ripening on seed dry weight and thousand seed weight of both cultivars of African eggplant studied (Tables 12 & 13).

Prior to after-ripening of fruits, the dry seed weight (mg) increased gradually as fruits matures and peaked (281.7 mg) at 60 DAA with a slight decline afterwards in cv. *Oforiwa* (Table 11). On the contrary, the maximum dry seed weight (272 mg) of 100 seeds was observed at 70 DAA in cv. *Kpando* (Table 12) without after-ripening treatment. This observation is consistent with an earlier observations in chapter four of this study that seed physiological maturity for cv. *Oforiwa* and cv. *Kpando* vary at 62 and 76 DAA respectively.

The influence of after-ripening of fruits on dry seed weight was highly significant in earlier harvests (30 – 50 DAA) than in latter harvests (60 -70 DAA) in both cultivars. After 15 days of storage, dry matter content of seeds from fruits harvested earlier (30 -50 DAA) significantly increased in both cultivars. However, seeds extracted from fruits harvested 60 or 70 DAA remained unchanged or decreased marginally (Tables 12 & 13). Seed dry weight increased significantly (296 mg) when fruits of cv. *Kpando* harvested 50 DAA were stored for 15 days, which was higher than without after-ripening (272 mg) (Table 13).

The observed increase of seed dry matter during after-ripening is due to nutrient transfer from the fruits to the seeds (Alvarenga *et al.*, 1991; Barbedo *et al.*, 1994a). The significant effect observed in fruits harvested earlier however suggests that the interval

between anthesis and fruit harvests plays a critical role. It can thus be suggested that the farther the interval from anthesis to harvest, the less influence after-ripen has on seed dry matter content. Alvarenga *et al*., (1991) observed a two fold increase in dry matter content of squash seeds when harvested at 25 DAA and stored for 3 days prior to extraction but decreased when fruits were harvested later at 55 DAA.

On the other hand, the observed decreased or no change in seed dry weight for fruits harvested at 60 or 70 DAA, is due to the fact that the seeds at this stage had already attained physiological maturity, with a completion of the seed filling for *cv. Oforiwa* and *cv. Kpando* respectively. This explains a somewhat stabilized dry matter content of seeds after this maturity stage (Demir *et al.,* 2002).

Further, the observed decline after-ripen for 15 days could be attributed to the fact that seeds borne in fleshy fruits at harvest still possess high moisture content, therefore as they continue to respire by consuming the accumulated reserves the seed dry matter content are likely to reduce (Carvalho and Nakagawa, 2000). The present observation corroborates Vidigal *et al.* (2009) for pepper, Alan and Eser (2008) for hot and iconic peppers where post-harvest storage or after-ripening decreased the seed dry weight. Dias *et al.* (2006b) also reported a slight decline in seed dry matter content during post-storage for tomato.

Similarly, the maximum mean 1000 seed weight was 2.83 and 2.72 g when seeds were extracted from fruits harvested at 60 DAA and 70 DAA for *cv. Oforiwa* and *cv. Kpando* respectively without after-ripening. The observed difference could be due to cultivar. However, when fruits were stored for 10 - 15 days prior to seed extraction, mean 1000 seed weight increased significantly at earlier harvests (30 -50 DAA) than latter harvests

(60-70 DAA) by which stage seed filling was apparently complete (Demir *et al.*, 2002). Maximum increase in mean 1000 seed weight after-ripening occurred at 50 - 60 DAA in both cultivars when stored for 10 – 15 days under ambient conditions and either remained or declined marginally. Similar observations were made by Passam *et al.,* (2010a) in eggplants when maximum mean 1000 seed weight occurred at 55 DAAA and declined or remained after-ripening of fruits. The present study is consistent with earlier reports by Kortse and Oladiran (2013) who observed that after-ripening durations significantly influence 100 seed weight melon (*Citrullus lanatus* (Thumb) seeds.

6.4.3 Influence of harvest time and After-ripening on first germination count and percent germination of two cultivars of African eggplant

The results showed a significant difference for first germination count and final germination percentage for seeds harvested at different times, after-ripening and their interactive effects ($p \leq 0.001$) in both cultivars (Tables 14 & 15).

Table 14: The effect of after-ripening for 15 days on first germination count (%) and Germination (%) of cv. *Oforiwa* seeds extracted from fruits harvested at 30 -70 days after anthesis.

	After-ripening period (AF, days)							
	First germination Count (%)				Germination (%)			
Harvest time	0	5	10	15	0	5	10	15
30 DAA	0 d	0 d	0 d	7 d	0 c	0 e	6 d	46 c
40 DAA	0 d	6 cd	10 c	27 c	0 c	13 d	63 c	67 b
50 DAA	11 c	9 c	34 b	36 b	54 b	67 c	77 b	93 a
60 DAA	28 b	38 b	62 a	79 a	93 a	88 b	94 a	95 a
70 DAA	61 a	59 a	68 a	76 a	94 a	95 a	97 a	95 a

Means with the same letter within a column are not significantly different according to Tukey's HSD Test at 5% probability.

Table 15: The effect of after-ripening for 15 days on first germination count (%) and Germination (%) of cv. *Kpando* seeds extracted from fruits harvested at 30 -70 days after anthesis.

	After-ripening period (AF, days)							
	First germination Count (%)				Germination (%)			
Harvest time	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
30 DAA	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d
40 DAA	0 d	5 d	7 c	42 c	0 d	9 c	68 c	92 bc
50 DAA	8 c	11 c	29 b	53 b	43 c	32 b	78 b	91 c
60 DAA	37 b	60 b	75 a	87 a	78 b	92 a	94 a	98 ab
70 DAA	88 a	85 a	78 a	92 a	96 a	96 a	95 a	99 a

Means with the same letter within a column are not significantly different according to Tukey's HSD Test at 5% probability.

Results of the first germination count showed no or low germination (< 10 %) when fruits were harvested earlier (30 - 40 DAA) independent of after-ripening treatment for both cultivars (Table 14 and 15). However, when subjected to after-ripening period of 15 days under ambient condition, fruits harvested 40 or 50 DAA had a significant increase in germination of first count 27 -36 % and 42-53 % in *cv. Oforiwa* and *cv. Kpando* respectively (Tables 14 & 15). The maximum first germination count occurred in fruits harvested 70 DAA without after-ripening (61 - 88 %) in both cultivars which was not significantly different when fruits were harvested 10 days earlier and after-ripen for 15 days (79 – 87%) (Tables 14 & 15).

The performance of seeds from fruits harvested 60 or 70 DAA was much better in terms of seed vigour. This corroborates with earlier reports of Vidigal *et al.* (2009) for pepper

and Dias *et al.,* (2006b) for tomato where post-harvest fruit storage increased seed vigour as indicated by first count of germination and controlled deterioration test.

Results for the final germination percentage showed no germination of seeds for *cv. Kpando* when fruits were harvested 30 DAA irrespective of post fruit storage treatment. However, 15 days of after-ripening of fruit induced seed germination in *cv. Oforiwa* (46%). The present results is consistent with earlier observations in this report that when fruits were harvested 25-30 DAA, no or less than 10% germination. In pepper, Sanchez *et al.,* (1993) reported no germination of fruits harvested 30 DAA despite after-ripening for 28 days. Similarly, Barbedo *et al.,* (1999) found that cucumber seeds obtained from early harvested fruits (25 to 35 DAA) and after-ripened for 15 days had no or low seed germination.

For eggplant, Passam *et al*., (2010a) results corroborates with the present study in that germination did not occur in earlier harvests (25 – 35 DAA) until 45 DAA prior to after-ripening of fruits. In their case however, after-ripening for 20 days under 25 °C induced germination in these earlier harvests significantly between 44 to 100 percent.

After-ripening treatment had significant effect on seed germination particularly for fruits harvested earlier. The germination of seeds from 40 and 50 DAA started to increase with after-ripening treatment and was highest at 15 days of storage in both cultivars (Tables 14 & 15). In most cases, germination of seeds were above satisfactory levels (> 90%) except for cv. *Oforiwa* at 40 DAA (67%) (Table 14). Since after-ripening is considered as a main dormancy breaking treatment (Iglesias-Fernandes *et al.,* 2010), it is assumed that the no or low germination observed in the earlier harvests could be attributed to primary dormancy relating to physiological seed immaturity was released during the post-harvest

storage period. The observed positive effect of after-ripening on fruits harvested earlier (40 or 50 DAA) on seed germination could be attributed to the *in situ* priming principle reported by Welbaum and Bradford (1991a). This is achieved due to the fact that seeds at the maturity stage are held at moisture levels that is close to full imbibition, thus with sufficient oxygen supply, the seeds viability can be maintained for considerable period while preventing precocious germination (Bradford, 2004). This observation further concurs with the notion that important maturation event occurs within seeds after the vascular connections with the mother plant is lost (Leprince *et al*., 2017). The current results is consistent with other Solanaceous crops (Dias *et al*., 2006b; Passam *et al.,* 2010a, Kortse *et al.,* 2017, Ozden and Demir, 2018).

The results from the present study further showed that germination of seeds did not significantly improve by after-ripening fruits harvested 60 and 70 DAA. This is probably because, at this stage, the seeds had attained physiological maturity and had apparently completed its seed filling stage. These results are consistent with that of tomato seeds where post-harvest storage of fruits harvested 50 or 60 DAA did not significantly affect seed germination (Vidigal *et al*., 2006), in pepper (Vidigal *et al.*, 2009) and in eggplant (Passam *et al.,* 2010a). This suggests that when fruits of African eggplant are allowed to mature on the mother plant and harvested at 60 or 70 DAA, maximum seed vigour and germination quality can be obtained, without after-ripening treatment. This results agrees with an optimum harvest time of 62-76 DAA for this species studied under both tropical monsoon and temperate oceanic climates. However, when fruits are harvested earlier (40 or 50 DAA), after-ripen of fruits is necessary to obtain maximum seed vigour and germination.

6.4.4 Influence of harvest time and after-ripening on mean germination time (MGT), time of seed to acquire 50 percent germination (T_{50}) and Germination index (GI).

Among the quantitative germination parameters, mean germination time (MGT) and time to 50% germination have been recommended as efficient in measuring the germination time of a seed lot as these relate strongly to seed germinability (Ranal and Santana, 2006). In the present study, the mean germination time and time to 50% germination of both cultivars were significant ($p \leq 0.001$) in relation to fruit harvest time, after-ripening and their interaction (Figure 21).

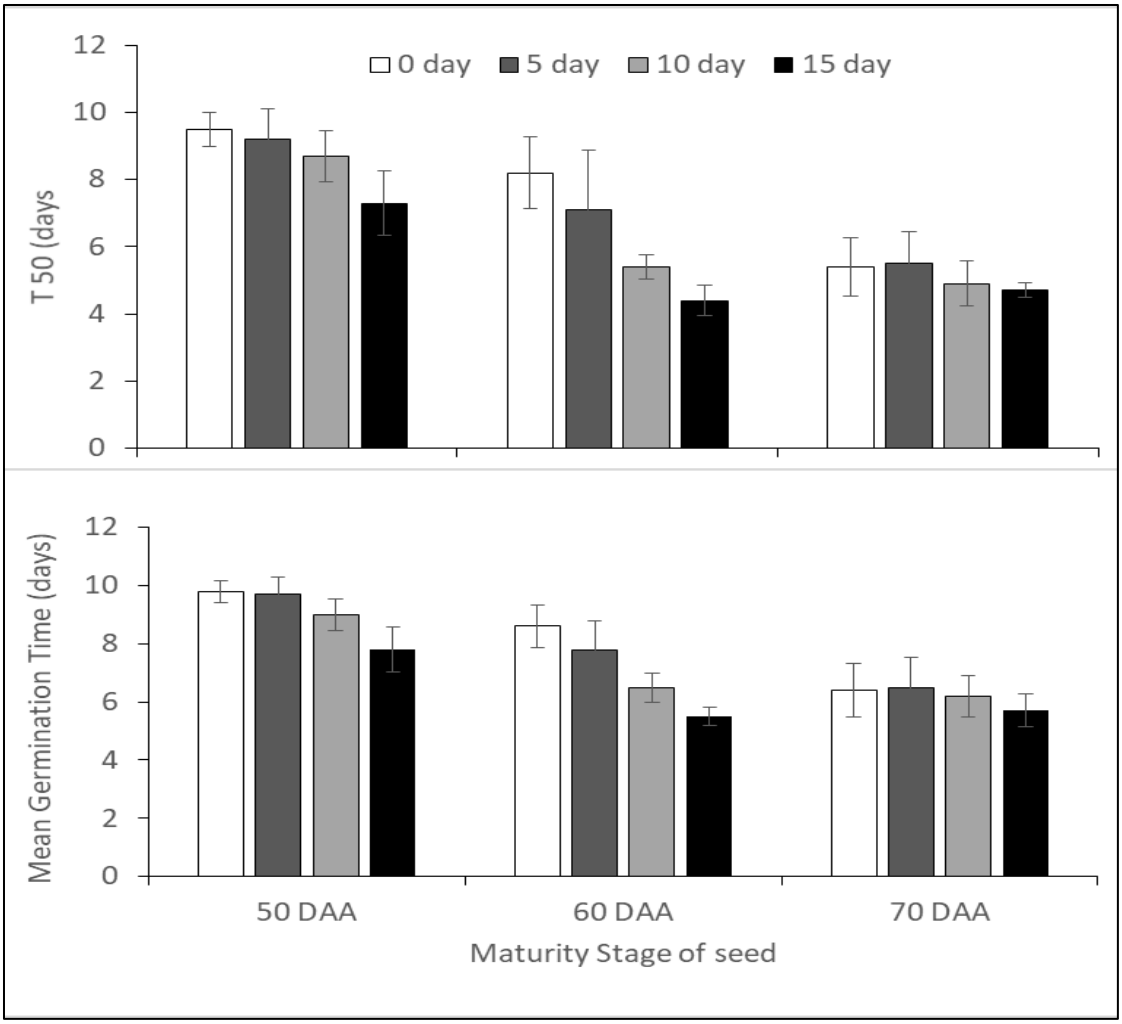


Figure 21: Mean values of the interactive effect of maturity stage and after-ripening on time to reach 50% germination (T_{50}) and mean germination time (MGT). Error bars represent standard deviation.

The time (days) required to complete the germination process in both cultivars decreased with seed maturation. In both cultivars, seeds extracted from fruits harvested 50 DAA took longer time (9 to 10 days) to complete germination but were faster (5 -7 days) when fruits were harvested at PM stage (70 DAA) (Fig. 21). When fruits were subjected to after-ripening, time to complete the germination process significantly reduced (Fig. 21). Similarly, time (days) to reach 50% germination (T_{50}) also significantly improved with seed maturation and after-ripening treatment in both cultivars (Fig. 21).

This results suggest that, harvesting fruits 60 or 70 DAA reduces the time (days) required to complete the germination process. This is because seeds harvested at this stage have maximum dry matter content and food reserve accumulation that gives the germinating seed enough energy. These quantitative traits in addition to the qualitative measurements of percentage seed vigour and germination thus give a better picture of the seed germination behaviour (Kader, 2005).

Table 16: Influence of Harvest maturity and After-ripening on Germination Index (GI) and Mean daily germination (MDG) of cv. *Oforiwa* and cv. *Kpando* seeds.

		After-ripening (post storage) period (days)							
		GI				MDG ($^{day-1}$)			
Cultivar	Harvest time	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
CV1	50 DAA	1.40 c	1.70 c	2.34 b	3.01 c	3.8 b	4.7 c	5.5 b	6.6 a
	60 DAA	2.84 b	2.96 b	4.04 a	4.68 a	6.6 a	6.2 a	6.7 a	6.7 a
	70 DAA	3.49 a	3.47 a	3.85 a	4.13 b	6.7 a	6.7 a	6.9 a	6.7 a
CV2	50 DAA	1.19 c	0.90 c	2.44 c	3.30 b	3.0 c	2.2 b	5.5 b	6.5 b
	60 DAA	2.56 b	3.52 b	3.99 b	4.79 a	5.5 b	6.3 a	6.7 a	7.0 a
	70 DAA	4.56 a	4.59 a	4.50 a	4.85 a	6.8 a	6.8 a	6.7 a	7.0 a

Means with the same letter within a column are not significantly different according to Tukey's HSD 5% probability. CV1: cv. Oforiwa, CV2: cv. Kpando

The results of the study showed a significant ($p \leq 0.001$) improvement of the germination index values (GI) values as a function of harvest time and after-ripening treatment independently and interactively (Table 17). Seeds obtained from fruits harvested at PM had higher GI values than those obtained from earlier harvests (50 DAA). (Table 17). This confirms the observation that seeds at PM possess higher percentage and rate of germination.

According to Bench-Arnold *et al*. (1991), germination index (GI) is a good measure of seed lot germination behaviour as it emphasizes both percentage of germination and its speed. Thus, GI further elucidate the seed vigour and germination capacity of a seed lot. Similarly, the quantitative measure of mean daily germination (MDG), measures the number of seeds germinating per day (Diavnshir and Pourbeik, 1976) also improved as seeds matures or when subjected to after-ripening treatments (Table 17). The number of seeds germinating per day increased from 3 to 6-7 seeds/day when seeds were at PM or fruits stored up to 15 days to after-ripen. It was further observed that these two measurements were strongly associated with the first count (seed vigour) and final germination percentage (Table 17).

Table 17: Correlation of qualitative and quantitative dynamics among seed germination variables of cv. *Oforiwa* in relation to harvest time (50, 60 and 70 DAA) and after-ripening for up to 15 days under ambient condition.

	FC	*GP*	*MGT*	T_{50}	*GI*	*MDG*
FC	1					
GP	0.999995	1				
MGT	-0.959299	-0.96018	1			
T_{50}	-0.975981	-0.97666	0.997778	1		
GI	0.901699	0.903044	-0.9871	-0.97423	1	
MDG	0.993036	0.993399	-0.98589	-0.99485	0.946356	1

FC: First Count (%); GP: Germination percentage (%); MGT: Mean germination time (days), T_{50}: Time to reach 50% of germination (days); GI: Germination index; MDG: Mean daily germination (number of seeds per day)

Table 18 shows the relationship of other seed quantitative parameters such as mean germination time, time to reach 50% germination, germination index and mean daily germination with final germination percentage. First germination count, germination index and mean daily germination were strongly and positively correlated (r = 0.99; 0.90; 0.99 respectively) with final germination percentage (Table 18). Time to reach 50% germination and mean germination time were strongly but negatively correlated (r = -0.97; -0.95 respectively) as expected (Table 18).

Germination is considered to be a qualitative developmental response of an individual seed that occurs at a point in time, although individual seeds within a seed lot respond differently at varied periods (Kader *et al.,* 1998). This implies that the final germination percentage alone is not sufficient enough to understand the seed germination behaviour of a seed lot. Thus, to get a better picture of the seed germination behaviour of a seed lot, it is recommended that in addition to the measure of seed vigour (first germination count)

and percentage germination as a qualitative measure, other quantitative measurements such as MGT, T_{50}, GI and MDG be determined as these variables have a strong correlation with the percentage germination as observed in this study.

6.5 Conclusion

Harvest time and after-ripening treatment improved seed physiological quality characteristics of African eggplant (*Solanum aethiopicum* L.), particularly when fruits are harvested 40 and 50 days after anthesis.

6.6 Recommendation

i. An after-ripening treatment up to 15 days is recommended if fruits are harvested (40-50 DAA) before attaining physiological maturity.

ii. For the purposes of seed production, African eggplant fruits can be harvested 70 DAA for maximum seed quality without after-ripening treatment.

6.7 Suggestion for further research

There is the need to investigate the biochemical changes during after-ripening treatment to elucidate the components associated with seed vigour and germination improvement in African eggplant and related species.

CHAPTER SEVEN

EVALUATION OF THE EFFECTS OF FERMENTATION AND DRYING METHODS ON SEED PHYSIOLOGICAL CHARACTERISTICS OF TWO CULTIVARS OF AFRICAN EGGPLANT (*Solanum aethiopicum* L.)

Abstract

Seed extraction and drying methods are important procedures employed after harvesting fruits of African eggplant as these methods affect the seed quality. Fermentation is one major technique employed prior to seed extraction. However, its effect on seed quality characteristics on seeds of Africn eggplant has not been reported. This study sought to evaluate the effects of fermentation and drying methods on seed physiological characteristcs of two cultivars of African eggplant (*Solanum aethiopicum* L.). Two independent experiments were carried out in this study. Fruits were harvested at 70 days after anthesis and subjected to various durations of fermentation in distilled water and different drying methods. In the first study, harvested fruits were immediately fermented in distilled water for 0, 6, 12, 24 and 48 hours before seed extraction. The experiment was laid out in a 2 x 5 completely randomized design in factorial arrangement. In the second experiment, seeds were extracted immediately from harvested fruits at physiological maturity and subjected to sun/24 hour; shade/24 hour; shade/48 hour; desiccant (silica gel)/24 hour; 30 °C/24 hour; 35 °C/24 hour; 45 °C/24 hour; and 50 °C/24 hour for drying. The experiment was laid out in a 2 x 8 CRD in factorial arrangement. Cultivar was one factor at two levels while fermentation and drying methods were second factors at 5 and 10 levels respectively. All data were taken in four replicates. The seed physiological characteristics measured were seed moisture content, seed dry weight, first count, seed germination and accelerated aging. The results suggest that seed germination of African eggplant seeds is not influenced by fermentation prior to extraction but can improve seed vigour. All drying methods were able to reduce seed moisture content to an ideal level for storage and maintained seed physiological quality. It is recommended to ferment fruits prior to extraction up to 12 hours if the purpose is to enhance seed vigour. Shade drying seeds for 48 hours or oven drying at 30 °C for 24 hours is ideal to maintain seed quality. The latent effect of fermentation and drying on seed physiological quality needs to be studied.

Introduction

The African eggplant (*Solanum aethiopicum* L.) is one of the most commonly consumed fruit vegetable in Ghana and West Africa. It is considered the third most cultivated and consumed after tomato, onion and before okra in Ghana (Horna *et al.,* 2007; Osei *et al.,* 2010).

Seeds that are borne in fleshy fruits are subjected to several postharvest handling practices which do affect the quality of seeds positively or negatively. Two of such important practices are seed extraction and drying methods.

Several seed extraction methods such as wet, dry, natural fermentation, chemical fermentation, and mechanical extraction have been used to extract seed from fruit vegetables such as tomato (Demir and Samit, 2001), egusi melon (Ogbonna and Odo, 2011), cucumber (Chethan *et al.,* 2013) and eggplant (Franca *et al*., 2013; Rahman *et al*., 2015). These various methods had varied effects on seed quality among different species. Silva *et al*., (1982) observed that natural fermentation gave a lower germination than acid treatment fermentation in tomato. Demir and Samit (2001a) also observed the same results. For eggplant, Franca *et al.,* (2013) observed no significance difference among seed extraction methods used on first germination count. A reduced percent seed germination was observed when fermentation duration was extended to 48 hours either with acid or without. In cucumber, seeds extracted with natural fermentation resulted in highest germination (94.5%) and seed vigour compared to alkali or acid extraction methods (Chethan *et al*., 2013). These studies suggest that each species has an ideal extraction method for maintaining maximum seed quality. There is few studies reported

on how natural fermentation, a common practice by smallholder farmers influence the seed quality of the African eggplant (*Solanum aethiopicum* L.).

After seed extraction from fleshy fruits, the next important step in maintaining the quality of seed is drying. Seed moisture content (MC) plays critical role in determining the longevity of seed in several vegetables (Wang *et al*., 2001). Generally, seeds are harvested at high moisture content and need to be dried before storage with a careful attention to the rate and extent of post-harvest drying (Babiker *et al.*, 2010). It has been reported that most vegetable seeds are harvested at moisture levels as high as and as high as 56% even at end of maturity (Marcos-Filho et al., 2018; Borges *et al*., 2019; Ramos *et al*., (2021). While most vegetable seeds can withstand drying to extend their storage life with low moisture content (Wang *et al*., 2001), this may differ with species.

This suggests that not all kinds of drying methods will suit equally well under given set of conditions in retaining viability and vigour of seeds, hence each crop should be evaluated accordingly. The extent to which these various drying methods reduce seed moisture and influence the physiological quality of African eggplant seeds is however not known. The present study sought to evaluate the physiological quality of African eggplant seeds subjected to different natural fermentation and drying methods.

7.1 Objective of the Study

To evaluate the effects of fermentation and drying methods on seed physiological characteristics of two cultivars of African eggplant (*Solanum aethiopicum* L.)

7.2 Research questions

i. To what extent does fermentation techniques prior to seed extraction affects seed quality?

ii. What drying methods are suitable for African eggplant seeds and to what extent does it maintain seed viability?

7.3 Materials and Methods

7.3.1 Plant Materials

Two cultivars cv. *Oforiwa* and *Kpando* used in previous studies were used for this study. Fruits of the two cultivars (cv. *Oforiwa* and *Kpando*) were harvested 70 days after anthesis (DAA) and processed same day or less than 48 hour to avoid possible after-ripening effect.

7.3.2 Evaluating the effects of fermentation on seed physiological characteristics.

7.3.2.1 Sample preparation

Fifty fruits of each cultivar was used for the fermentation process. For seed extraction, ten fruits were carefully cut individually with sharp knife and seeds were extracted immediately by hand from the pulp or put in a plastic container containing distilled water placed in an ambient condition for 6, 12, 24 and 48 hours for fermentation. Well-formed seeds after the fermentation periods separated from the pulp and remained at the bottom of the container while immature seeds which were floating were discarded. Seeds were thoroughly rinsed with running water.

7.3.3 Data collected

Seed moisture content determination: After each natural fermentation period, the seed moisture content was determined from sub-sample for each treatment using the oven method at 105 °C ± 3 for 24 hours on fresh wet basis (Brazil, 2009). The remaining seeds for each treatment were dried under shade for 48 hours. Seed quality tests were evaluated as follows:

Seed dry weight: Sub-samples of seeds extracted from each treatment were dried in oven at 30 °C for 24 hours after which seed weight by weighing four replicates of 100 seeds on an analytical balance to two decimal places and the mean results expressed in grams.

Germination test*:* Four replications of 50 seeds for each treatment were used. Seeds were evenly spread on a two layer Whatman paper moistened with distilled water and placed in a petri dish. The petri dishes were then incubated at 30/20 °C in an 8/16 hours alternating light/dark periods. Germination was conducted for 14 days. Daily germination was recorded until the 14^{th} day. Results were expressed as a mean percentage of normal seedlings for each lot.

First germination count: This was done together with the germination test by counting the number of normal seedlings identified on 7^{th} day after sowing as expressed as a percentage over total seeds sown.

Accelerated Ageing test: Accelerated aging was conducted in plastic boxes measuring 14 x 12 x 4.5 cm. A fiber mesh screen was suspended inside the plastic box on which seeds were evenly distributed to form a thin layer. Approximately 2.0 g of seeds from each treatment was used. To create the desired humidity condition for the test, 40 mL of water was added to each plastic box. The boxes were covered and maintained in an incubator at 42 ºC for 72 hours, after which seeds were submitted to the germination test as previously described. Evaluations were performed seven (7) days after sowing and the results expressed as mean percentage of normal seedlings for each lot.

7.3.4 Experimental Design and data analysis

The experiment was laid out in completely randomized design with a 2 x 5 factorial arrangement. Cultivar and fermentation durations constituted the factors. Cultivar had two (2) levels; cv. *Oforiwa* and cv. *Kpando*. Fermentation had five (5) levels. Treatments were replicated four times. The fermentation durations were 0, 6, 12, 24 and 48 hours. The data collected were subjecting to analysis of variance (ANOVA) and treatment means compared using the Tukey test at 5% significant level.

7.3.5 Evaluating the effect of drying methods seed physiological characteristics of two cultivars of African eggplant.

7.3.5.1 Sample preparation

Seeds were extracted from five (5) harvested fruits of each cultivar at the same maturity stage as in the first experiment. Extracted seed were then washed immediately under running water and subjected to the various drying methods of sun/24 hour; shade/24 hour; shade/48 hour; desiccant (silica gel)/24 hour; 30 °C/24 hour; 35 °C/24 hour; 45 °C/24 hour and 50 °C/24 hour.

7.3.6 Data collected

The seed moisture content was measured after the different treatments following the same methodology as previously described in the first experiment. The seed physiological characteristics measured were first germination count as an indication of seed vigour and percentage seed germination. First germination count was recorded after 7 days of sowing. Final seed germination percentage was calculated after day 14 as number of seeds germinated against total number of seeds sown and expressed as a percentage.

7.3.4 Experimental Design and data analysis

The experiment was laid out in a completely randomized design (CRD) with a two (2) cultivar x eight (8) factorial arrangement. Factor one was cultivar at tow (2) levels while drying methods constituted factor two (2) at eight levels. All data were taken in four replicates. Data were subjected to normality tests (Shapiro-Wilks) before analysis of variance (ANOVA) and means compared using Tukey's test at the 5% significant level. Data was analyzed using SAS software.

7.4 Results and Discussion

7.4.1 The effects of natural fermentation on seed physical and physiological quality.

The effects of fermentation duration on seed moisture content and seed dry weight of the cultivars of African eggplant is presented in figure 23. The analysis of variance of result data showed a significant ($p \leq 0.05$) for the individual effects of fermentation and cultivar on seed moisture, dry seed weight (Fig. 23), first count, and accelerated ageing except on germination percentage (Table 19). The interactive effects were significant for all studied variables hence their individual effects is not further discussed.

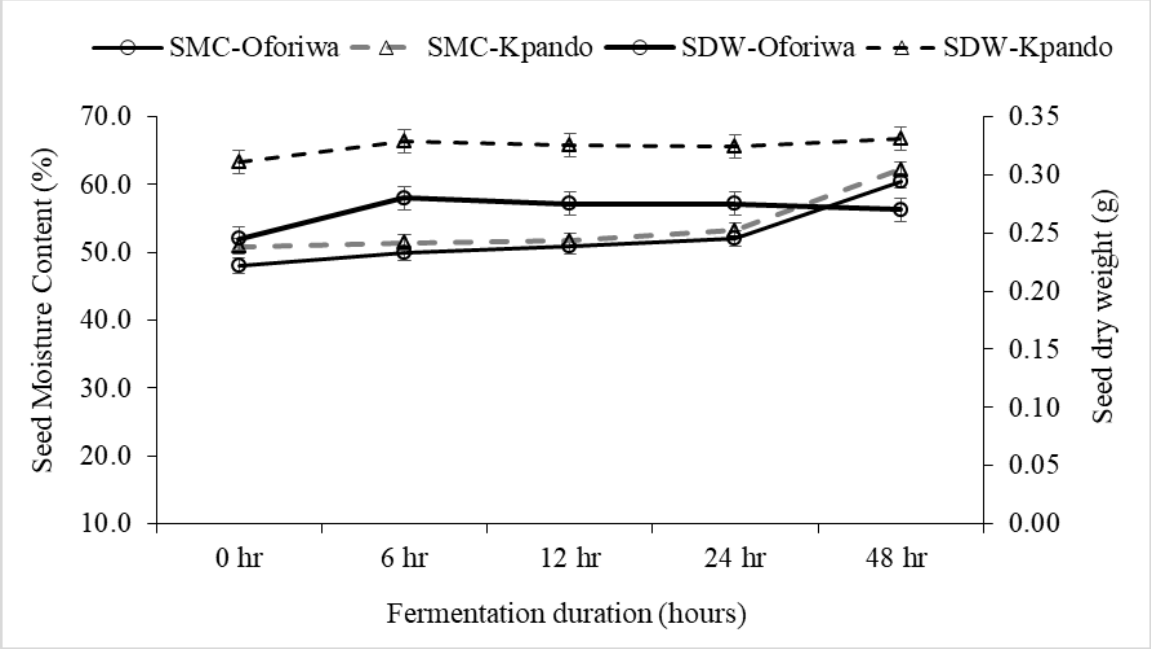


Figure 22: Seed moisture contents (SMC) and Seed dry weight (SDW) of cv. Oforiwa and cv. Kpando African eggplant seeds as influenced by various fermentation duration (means ± SEM bars).

The results showed that increasing the fermentation period of seeds increased seed moisture content in both cultivars. Seed moisture content increased from 47 % to 60.3% after 48 hours of fermentation (Fig. 23).

The cultivar *Kpando* showed a slightly higher absorption capacity compared to cv. *Oforiwa*. For normal eggplant (*Solanum melongena*), Franca *et al.,* (2013) also observed an increasing seed moisture content from 58 % to 74 % when seeds were fermented naturally or with acid. Similar trend of seed moisture content was reported by Lopes *et al.* (2001) for pomegranate seeds. Variation in moisture and water uptake among the cultivars is attributed to genetic differences as reported for tomatoes (Sabongari and Aliero, 2004). Cultivar *Kpando* which had a slightly higher water uptake (seed moisture) capacity is relatively heavier (dry seed weight (g) as observed in figure 23. This cultivar

is also larger in size with bigger surface area for absorption than cv. *Oforiwa* which has smaller seeds. This cultivar is also larger in size with bigger surface area for absorption than cv. *Oforiwa* which has smaller seeds. Cultivar *Kpando* had more dormant seeds compared to cv. *Oforiwa*, which suggests that cellular walls were not completely dry and had readily available openings to absorb more water (Sabongari and Alieno, 2004).

Table 18: Effect of fermentation on first germination count, percentage germination and accelerated ageing (mean ± standard deviation) for cv. *Ofoirwa* (CV1) and cv. *Kpando* (CV2) African eggplant seeds subjected to varied durations of natural fermentation.

	First germination Count (%)		[1]Germination (%)		Accelerate Ageing (%)	
Treatment	CV1	CV2	CV1	CV2	CV1	CV2
0 hr No fermentation	73 ± 5.03 ab	32 ± 9.79 e	99 ± 2.00 a	89 ± 5.03 ab	62 ± 1.70 c	52 ± 1.70 c
6 hr fermentation	76 ± 3.20 a	52 ± 7.30 c	99 ± 2.00 a	86 ± 4.00 c	61 ± 1.50 c	54 ± 1.50 c
12 hr fermentation	55 ± 3.82 c	39 ± 3.82 d	98 ± 2.30 a	86 ± 7.65 c	80 ± 0.95 a	82 ± 2.50 a
24 hr fermentation	67 ± 3.82 b	49 ± 6.83 c	98 ± 2.30 a	88 ± 8.00 c	71 ± 1.50 b	60 ± 1.70 b
48 hr fermentation	76 ± 4.61 a	47 ± 3.82 c	96 ± 3.26 ab	90 ± 6.92 bc	70± 1.50 b	83 ± 5.80 a

Means followed by the same letter in the column do not differ significantly based on Tukey test at 5% probability level. [1] data was transformed before statistical analysis.

The first germination count (seed vigour) was significantly ($p \leq 0.05$) influenced by the fermentation period and the cultivars (Table 19). The highest percentage first count was observed after 6 hours of fermentation in both cultivars (76% in cv. *Oforiwa* and 59% in *cv. Kpando*). These percentages were not significantly different when seeds were fermented up to 48 hours in both cultivars. However, when seeds were subjected to sub-optimal conditions through accelerated ageing (AA %), seeds fermented up to 12 hours proved resilient with 80 - 82 % seed germination in both cultivars (Table 19).

In normal eggplant (*Solanum melongena* L.), Chethan *et al.,* (2013) observed a higher seed vigour index I and II in cucumber seeds subjected to natural fermentation for 24 hours. This suggests that fermentation improves seed vigour in African eggplant although the period is less than those reported for eggplant. Rahman *et al*., (2015) also reported similar results of an improved seedling vigour when eggplant was extracted by the wet method which is similar to natural fermentation. The authors observed a higher seedling vigour index in wet seeds extraction than those extracted without fermentation (Rahman *et al.,* 2015).

Fermentation period did not improve seed germination percentage significantly ($p > 0.05$) (Table 19). However, germination capacity differed between the cultivars (Table 19). The difference was only observed among the cultivars, attributable to genetic difference. Results by Franca *et al*., (2013) for eggplant (*S. melongena*) on the contrary showed a significant decrease in percentage germination when seeds were fermented up to 48 hours. In the case of tomato, Sabongari and Aliero, (2004) reported an improvement in germination when seeds were soaked with maximum germination occurring after 24 hours but declined when this period was extended to 36 hours. The decreased observed at

longer fermentation periods could be due to reduced oxygen content (anoxia) in the water resulting in lower germinative capacity. Cardoso *et al.,* (2001) also reported an increase in germination percentage when yellow passion fruits were subjected to fermentation.

These varied results suggest that each crop species behave differently to fermentation. It has been reported that every species has a suitable fermentation period without causing anoxia (Franca *et al.,* 2013). Lopes *et al.,* (2001) reported 72 hours for pomegranate and Franca *et al*., (2013) recommended 24 hours for eggplant.

From this study, the African eggplant seeds can be extracted after harvesting at the optimum time without fermentation. For the purpose of improving seed vigour, up to 12 hours of fermentation would be ideal.

7.4.2 Effects of various drying methods on seed moisture content reduction of African eggplant

The various methods employed for drying seeds of African eggplant significantly ($p \leq 0.05$) reduced the seed moisture contents (Fig. 24). Oven drying of seeds at 35 °C, 45 °C and 50 °C for 24 hours reduced seed moisture content significantly from initial 47 % seed moisture content to 3.4 and 6.6 % compared to other types of drying (Figure 24). Oven drying temperatures applied in this study were able to remove 85.5 to 93 % moisture within 24 hours of drying. Shade drying removed the lowest moisture content (80.4%) followed by desiccant drying (81.7 %). The other drying methods reduced seed moisture down to 8.3 % and 9.2% except shade drying for 48 hours in *cv. Kpando* (6.1%) (Fig. 24).

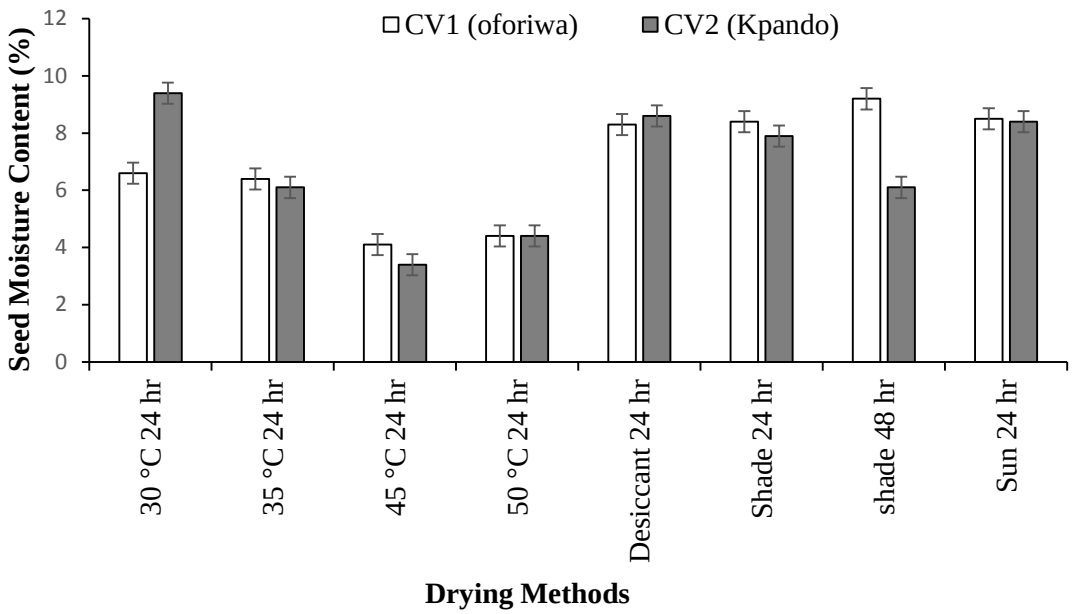


Figure 23: Seed Moisture content of cv. *Oforiwa* and *Kpando* African eggplant seeds as affected by different drying methods. Bars represent standard error of means (SEM).

The African eggplant seeds attain physiological maturity at high moisture contents ranging from 46 – 52 %. Therefore, the seeds should be extracted and dried early enough to obtain better quality seeds. The results from the present study is consistent with the observation made by Franca *et al.*, (2013) for eggplant. In their study, seed moisture content reduced ≤ 6 % when seeds were oven dried for 24 hours. Oven drying in the current study was able to dry seeds quickly at higher temperatures because the capacity of air to remove moisture depends on the surrounding temperature and humidity. The higher the temperature, the lower the humidity and the greater the moisture removal capacity of the surrounding air. Seeds dried in the open (sun or shade drying) have the tendency to reabsorb moisture from the surrounding air due to its hydroscopic nature. Ravi *et al*. (2007) also reported similar results for chilli seeds dried under sun (8.74 %) and shade

(9.58%). Chilli seeds dried using oven at 35 °C gave the lowest seed moisture content of 8.59% (Ravi *et al.,* 2007).

Taking into consideration the recommendation by George (2000) that 6% seed moisture content is suitable for eggplant seeds storage and a general observation that vegetable seeds stored longer at seed moisture content below 8% (Ells *et al*., 2020), it can be suggested from this study that oven drying at 35 – 50 °C for 24 hours can reduce African eggplant seeds to these required moisture levels. The other methods such as desiccant or shade drying period may be extended to obtain similar moisture levels.

7.4.3 Effects of various drying methods on first germination count (vigour) and percentage seed germination of African eggplant.

In the present study the cultivars showed significant difference ($p \leq 0.05$) for methods of drying. The interactive effect of drying methods and cultivar on first germination count was also significant ($p \leq 0.05$) (Fig. 25). First germination count was generally low for cv. *Kpando* (Fig. 25B) compared to cv. *Oforiwa* (Fig. 25 A), suggesting that seed vigour was affected by the drying methods more in cv. *Kpando* and this is attributable to genetic difference. Oven drying at 35 °C, shade drying for 48 hours and sun drying for 24 hours had less than 30 % first germination count in cv. *Oforiwa* (Fig. 25A).

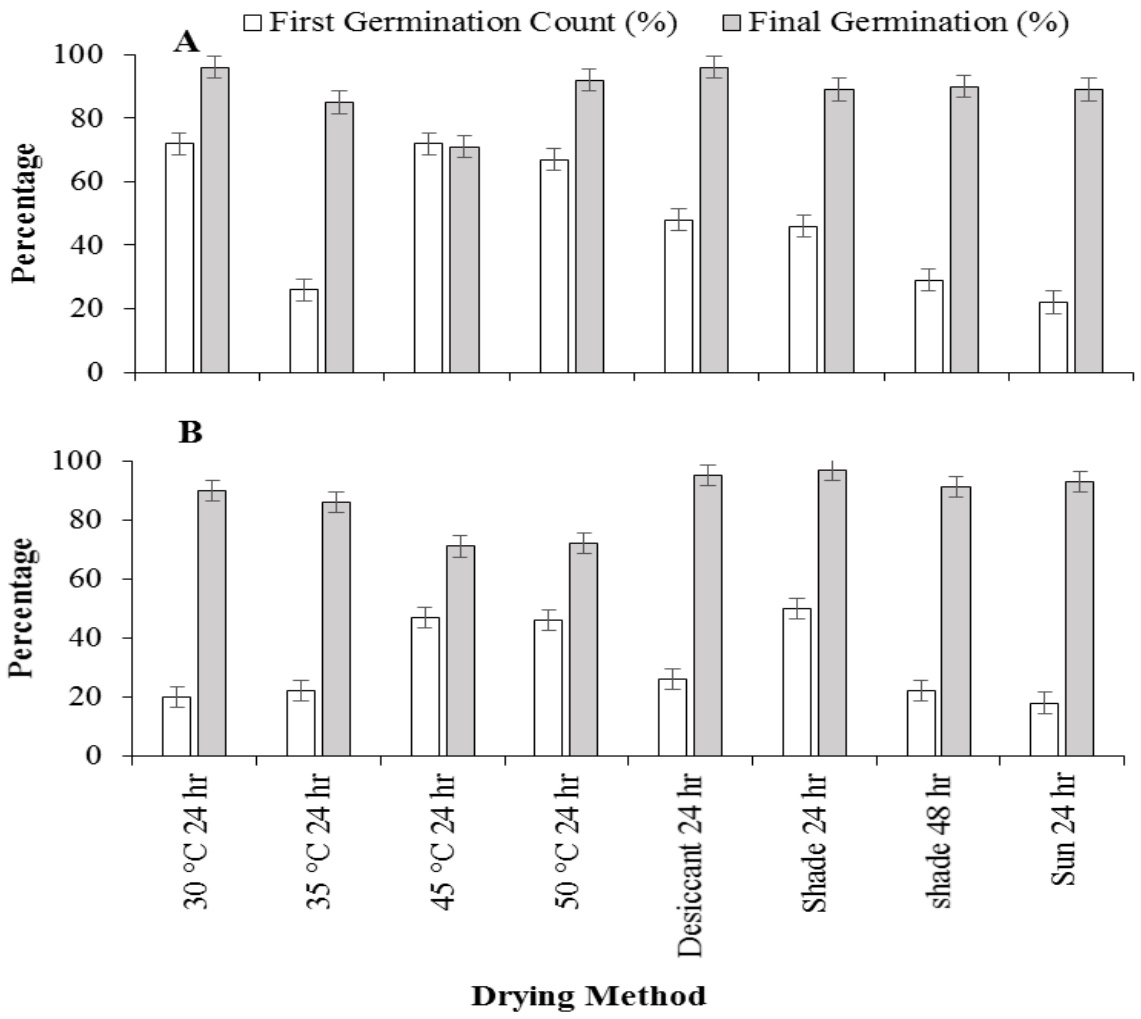


Figure 24: First germination count (%) and final germination (%) of cv. *Oforiwa* (A) and cv. *Kpando* (B) African eggplant seeds subjected to different methods of drying. Bars represent standard error of means (SEM).

Desiccant drying and longer hours of shade or sun drying generally had a significant effect on seed vigour as shown by the first germination count. This longer periods of exposure to such drying conditions could have caused some temporal effect on cell damage. However, since final germination was relatively high, this effect may not be conclusive.

Regarding final seed germination, the different drying methods caused minimal effect. There was however significant difference between the cultivars and their interactive

effect with drying ($p \leq 0.05$) (Fig. 25). Seed germination percentage for both cultivars germinated above 70%. Seeds dried in oven at low temperatures of 30 and 35 °C had comparable germination values ranging from 85 to 96 % as the other types of drying methods. Ravi *et al.* (2007) reported similar results (83 to 90 %) for chilli (*Capsicum annuum* L.) seeds when subjected to sun, shade and oven drying. Manish *et al.*, (2015) also reported above 70 % seed germination in three genotypes of sorghum seeds subjected to various drying methods with genotypes differing in responses as observed in the present study.

However, when oven temperatures were elevated to 45 and 50 °C, seed germination for cv. *Kpando* reduced by 16.2 to 21 %. Cultivar *Oforiwa* on the other hand reduced by 26 % when temperature was increased to 45 °C. This suggest that although oven drying was able to dry seeds quickly and efficiently, the high temperatures caused damage to seed viability. Increasing the temperature when drying seeds, especially at temperatures above 40 °C increases the drying rate, which can lead to damages to meristematic tissues and eventually caused injuries to the injured development of the embryonic axis (Afrakhteh *et al*., 2013). The present results corroborate with Siddique and Wright (2003) who reported a small decrease in germination percentage when peas (*Pisum sativum* L.) seeds were dried between 40 and 60 °C.

Similarly, seed physiological quality decreased at 60 °C and 70 °C drying temperatures for Adzuki beans (*Vigna angularis*) (Resende *et al.*, 2012). In evaluating sweet sweet sorghum seeds as affected by drying temperatures, Ullmann *et al*. (2015) reported a reduction in the germination when drying temperatures were above 40 °C. Similar observations was made for soybean, in which drying temperatures above 40 °C also

affected soybean seed germination and vigour (Hartmann Filho *et al.*, 2016). This observation is consistent with the view by Marcos-Filho (2005), that fast and excessive drying can affect seed viability.

According to Franca *et al.* (2013), the effect of drying on seed physiological quality may be observed immediately after drying or during storage as a latent effect. In the case of eggplant, drying did not affect seed germination immediately and after storing for 6 months (Franca *et al*. 2013). This could be as a result of the relatively low temperatures (38 °C) applied by the authors and the low moisture contents of seeds (4 - 6 %) prior to germination test. As reported by Harrignton (1972) and later affirmed by Almekinders and Louwaars (1999), the effect of higher drying temperatures, including thermal injuries to seeds is most damaging when seed moisture content is high. In the present study, the seed moisture contents prior to germination were low, hence the minor negative effect observed immediately after drying.

It is thus inferred that any possible negative effect could be latent during storage as observed by Araújo *et al.* (2000). In their study, the authors concluded that drying at temperatures of 50 °C and 60 °C and storing at higher relative humidity were harmful to seed quality of sweet corn (Araújo *et al.*, 2000) but with minor immediate effect. Similar results was observed by Hartmann Filho *et al.,* (2016) for soybeans, where increasing drying temperatures affected seed physiological quality of soybean, but more pronounced after storage for up to 150 days. This observations is consistent with the proposal by Roberts (1981) that physiological injuries caused by drying may reflect changes in subcellular systems including chromosomes, mitochondria, reduction of the number of starch granules at the embryo axis and increased in electrolyte and sugar leaching. This

suggests that such changes can only be observed at the latent stage and not immediate. Hartmann Filho *et al*., (2016) recommended drying temperatures not above 40 °C for soybean and for sorghum (Ullmann *et al*. (2015). Harrington (1972) also proposed temperature not exceeding 35 °C for drying of vegetable seed. In the present study, drying at 30 °C for 24 hours in oven or shade drying for 48 hours is recommended for African eggplant to maintain its seed physiological quality.

7.5 Conclusion

Fermentation of cultivars of African eggplant (*Solanum aethiopicum* L.) fruits prior to extraction does not influence overall seed percentage germination but can improve seed vigour while drying methods had immediate minimal effect on seed physiological characteristics.

7.6 Recommendation

i. For the purposes of improving seed vigour, fermentation of African eggplant seeds up to 12 hours is recommended.

ii. The suitable drying methods for African eggplant seeds with no immediate negative effect is drying at temperatures not exceeding 30 °C for 24 hours or shade drying for 48 hours.

7.7 Suggestions for further research

Further studies on the latent influence of drying and fermentation on the seed longevity of African eggplant (*Solanum aethiopicum* L.) is necessary.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General Discussion

This study broadly sought to establish the environmental, physiological and biochemical effects on the seed quality of African eggplant (*Solanum aethiopicum* L.). The present study constitutes the first or few detailed studies on the aforementioned factors and how they influence the unpredicatable seed germination characteristics of the *Solanum aethiopicum* L. This crop aside its immense nutritional importance, is the third most cultivated indigenous crop in Ghana and other West and Central African countries. It is however neglected in terms of research.

Two of the most important factors that influence seed germination include suitable temperature and light in some species. For the African eggplant, there has not been any such established conditions requirement to germinate the seed. Temperature responses by seeds can be quantified and fundamental to prescribing germination test conditions for the purposes of seed certification or conservation. Knowledge on these requirement informs national seed quality standards for crops for quality assurance purposes. The results of the present study showed that constant temperatures are not suitable for germinating *Solanum aethiopicum* seeds. At lower temperatures (15 °C) and higher constant temperatures above 30 °C, seed germination was significantly low or failed. This temperature regimes induce thermoinhibition in the seeds, hence preventing or reducing germination (Hill and van Staden, 2003). The study results established that for maximum seed germination in African eggplant, the suitable temperature to apply under control condition is 30/20 °C alternating temperatures under 8/16 hours alternating

ligh/dark periods. This condition mimics the tropical conditions that exist in where this crop is mainly cultivated. The proposed alternating temperature condition also reduces the time required for seeds to complete the germination process.

For seeds that are borne in fleshy fruits such as tomato, pepper, eggplant and African eggplant, the suitable maturity stage to harvest the fruits for maximum seed physiological quality is important. This is because some seeds within a fruit may attain physiological maturity when the fruit bearing the seeds has not and vice versa. Further, unlike other orthodox dry seeds such as cereals and legumes whose physiological maturity can easily be determined based on morphological characters, this stage of maturity is difficult for seeds borne in fleshy fruits such as African eggplant. Studies in this area have also given conflicting results vis-a-viz, whether maximum germination coincides with mass maturity or physiological maturity (Demir & Ellis, 1992b; Dias *et al.*, 2006; Vidigal *et al.*, 2011; Takac *et al*., 2015; Bortey & Dzomeku, 2016; Murrinie *et al*., 2019). In the present study, the results showed that the occurrence of physiological maturity or mass maturity differed among the cultivars used for the study. Cultivar *Oforiwa*, attained physiological maturity 60-62 days after anthesis ‘under both tropical and temperate oceanic climates. It however took 14 days more for cv. *Kpando* to reach its physiological maturity at 76 days anthesis under same seed production environments. The observed difference is attributed to genetic difference between the seeds including the seed sizes and weight. Cultivar *Kpando* had bigger and heavier seeds compared to cv. *Oforiwa* and thus require more time to accumulate food reserves to reach its maxium dry matter content.

Additionally, cv. *Kpando* seed maturation characteristics is consistent with the long held hypothesis that maximum seed germination conincides with physiological maturity or

mass maturity, (TeKrony & Egli, 1997) which in this study occurred at 76 days after anthesis. The results supports earlier observation for okra (Bortey & Dzomeku, 2016; Demir & Ermis, 2005). On the contrary, physiological maturity in cv. *Oforiwa* occurred 14 days before attaining maximum seed germination at 76 days after anthesis. Suggesting that for cv. *Oforiwa*, PM did not coincide with maximum germinability. Silva *et al*., (2015) reported similar results in a study of pepper seeds (*Capsicum annuum* L.), and (*Capscium baccatum* L.). The best seed quality was found for fruits harvested between 60 and 70 DAA respectively.

The seed maturation behaviour this cultivar is also consistent with the new hypothesis that some seeds that are borne in fleshy fruits that maximum seed germination occurs some time after seed filling stage or mass maturity stage. This is consistent with the reports by Demir & Ellis, (1992b) Dias *et al*., (2006) for tomato where dry matter accumulation did not coincide with maximum seed germination. Demir & Ellis, (1992c) and Demir *et al*., (2002) also observed similar results for pepper and eggplant respectively. It is however, been established in this study that for maximum seed germination, field emergence, thousand seed weight and reduced time to complete germination process, African eggplant seeds should be extracted from fruits harvested 70 -76 days after anthesis regardless of the seed production environment.

Several biochemical changes occur during the development and maturation of seeds with its associated influence on seed viability and vigour attributes (Henning *et al*., 2010; Han *et al.*, 2017). This association was studied to evaluate the biochemical component that has greater contributory role in maintenance of seed physiological quality. The results demonstrated that the mere high levels of protein and soluble sugars in themselves do

explain their direct link to the maintenance of seed germination and vigour quality. According to Santos *et al*. (2017) germination and seed vigour may rather be associated with the efficient metabolism of hydrolysis and mobilization of these reserves during the germination' process and 'not necessarily with the contents of these reserves present at maturity. This was evidenced by their negative correlations as reported in other studies for other crops. It is suggested that these seed reserves and their role in seed germination depends on the efficiency and mobilization capacity of the seed during germination process. Among the biochemical components, only tannin content and crude fat positively influenced physiological quality in terms of germination and vigour of African eggplant seeds. The roles of tannin in maintaining seed quality is linked to its ability to limit oxygen diffusion in the seed coat (Debeaujon *et al.,* 2007; Pourcel *et al*., 2007), enhancing seed coat solidity against environmental stresses and thereby improving viability (Debeaujon *et al.,* 2000; Chahtane *et al.,* 2017).

After fruits have been harvested precosiously (20 – 34 DAA), seeds extracted from such fruits failed to germinate or had < 10 % germination percentage. This is due to immature embryos at this stage of development. It has however, been reported in other fruits such as eggplant (*Solanum melongena*) (Passam *et al.,* 2010), Okra (Bortey & Dzomeku, 2016) and tomato (Demir and Samit, 2001) that seeds in such fruits continue to mature even when detached from the mother plant through a technique referred to as after-ripening or post-storage.

Studies on these crops (Passam *et al*., 2010a; Bortey & Dzomeku, 2016) have showed that the seeds therein continue to accumulate food reserves and dry matter thereby improving their quality. In this study, the seed quality of African eggplant harvested 30,

40, 50, 60 and 70 days after anthesis and after-ripened under ambient conditions up to 15 days was studied. The results showed that seeds continue to mature even when detached from the mother plant and stored for some time before extraction. This observation is consistent with other *Solanum* spp. such as eggplant or aubergine (Passam *et al.,* 2010a) and tomato (Vidigal *et al*, 2006). In addition to the accumulation of food reserves during this period of *in situ* storage of seeds, which improves seed quality, this technique can also relieve physiological dormancy resulting from GA/ABA hormonal imbalance (Iglesias-Fernandez *et al.,* 2010). The results further showed that after-ripening was not useful when fruits were extracted from precocious harvests and subjected to post-storage up to 15 days. This suggests that although the seeds continue to accumulate dry matter and mature after been detached from the mother plant, there is a critical stage at which such detachment is useful. Seeds extracted from fruits harvested too early (30 DAA) may not have well developed embryos and accumulated enough food reserves required for germination. The after-ripening technique was beneficial and significantly improved seed germination and vigour when fruits were harvested 40 or 50 DAA. This is attributable to increased food reserve and dry matter accumulation *in situ* as well as in situ priming effect. For fruits harvested at 60 or 70 DAA, after-ripening for 15 days was not significantly beneficial. This is because, at this stage, seeds had already attained physiological maturity as established in the earlier reports.

Lastly, two important methods employed after harvesting are seed extraction and drying. Farmers employ several methods for extracting seeds from fruits borne in fleshy fruits such as tomatoes, pepper, eggplant (Demir and Samit, 2001; Ravi *et al*., 2007; Chethan *et al.,* 2013; Franca *et al.,* 2013). For African eggplant, some farmers extract seeds directly

(dry extraction method) while others use wet method through natural fermentation. The duration of the fermentation process and how it affects the seed quality is however not known. Additionally, seeds are harvested at high moisture content and need to be dried before storage with a careful attention to the rate and extent of post-harvest drying. For the African eggplant, seed moisture content at the recommended harvest time ranges between 42 % and 47 %, hence require suitable drying method to maintain its quality.

The results from the present study showed that fermentation durations did not significantly differ when seeds were fermented up to 48 hours in both cultivars. Seeds extracted without fermentation had similar first germination count as those fermented up to 48 hours. However, when seeds were subjected to sub-optimal conditions through accelerated ageing (AA %), seeds fermented up to 12 hours proved resilient with 80 - 82 % seed germination in both cultivars. This observation is consistent with other studies in normal eggplant (*Solanum melongena* L.) and cucumber. This suggests that fermentation improves seed vigour in African eggplant' The fermentation process act as priming technique by improving imbition and intiating the metabolic processes preceeding radible emergence. The final seed germination percentage was however, not significantly affected by fermentation prior to extraction. This means, fermentation is required for African eggplant when seed vigour of seed lot requires a boost but not necessarily to enhance of seed germinability.

In terms of the suitable drying methods, all the drying methods employed in this experiment ranging from oven, sun, shade and desiccant drying were able to reduce the seed moisture content drastically to suitable moisture content levels between 3.4 % and 9.6 %. Drying methods affected seed vigour than germination. It was further observed

that oven drying methods at increasing temperatures above 30 °C was detrimental to seed germination. It is proposed that the drying effect on seed physiological quality of the African eggplant may be latent and not immediate as reported in other similar studies. This is because physiological injuries caused by drying often reflect changes at the subcellular levels and may manifest later in the storage life of the seed.

8.2 Conclusions

i. The interactive effect of temperature and light exposure improved seed germination of two cultivars of African eggplant (*Solanum aethiopicum* L.) particularly under alternating temperatures of 30/20 °C with limited light periods (8/16 hours).

ii. Fruit harvesting maturity stage influenced both seed physical and physiological quality with a strong relationship between fruit morphological traits (size, weight, and colour) and seed physiological characteristics of African eggplant (*Solanum aethiopicum* L.)

iii. Seed maturity stage influenced the biochemical characteristics including crude protein, sugars, antioxidant activity and tannin content of African eggplant (*Solanum aethiopicum* L.), with tannin content positively associated with seed quality maintenance.

iv. Harvest time and after-ripening treatment improved seed physiological quality characteristics of African eggplant (*Solanum aethiopicum* L.), particularly when fruits are harvested 40 and 50 days after anthesis.

v. Fermentation of the two cultivars of African eggplant (*Solanum aethiopicum* L.) fruits prior to extraction does not influence overall seed percentage germination but can improve seed vigour while drying methods had immediate minimal effect on seed physiological characteristics.

8.3 Recommendations

i. Seeds of African eggplant (*Solanum aethiopicum* L.) should be germinated at alternating temperatures of 30/20 °C under alternating light/dark 8/16 hours for maximum seed germination percentage and mean germination time.

ii. The suitable harvest maturity stage for maximum seed quality (germination, emergence, thousand seed weight and mean germination time) is 76 days after anthesis (DAA) for both cultivars irrespective of the seed production environment.

i. For maximum seed quality, bigger and heavier fruits with strong orange and vivid reddish-orange colours corresponding to N25A-C and N30A-C of the RHS colour chart should be harvested.

ii. Tannin content can serve as a suitable biochemical marker for seed quality traits selection in African eggplant improvement programmes.

iii. An after-ripening treatment up to 15 days is recommended if fruits are harvested (40-50 DAA) before attaining physiological maturity.

iv. For the purposes of seed production, African eggplant fruits can be harvested 70 days after anthesis for maximum seed quality without after-ripening treatment.

v. For the purposes of improving seed vigour, fermentation of African eggplant seeds up to 12 hours is recommended.

vi. The suitable drying methods for African eggplant seeds with no immediate negative effect is drying at temperatures method not exceeding 30 °C for 24 hours or shade drying for 48 hours.

8.4 Suggestions for further Research

i. To investigate further the temperature gradient for other cultivars of African eggplant to verify if the differences observed in their responses to temperature and light exposures is solely due to genetic.

ii. To evaluate the levels of tannin content during seed development and maturation and its role in seed viability maintenance.

iii. To establish whether the tannin content decrease with time during seed storage and its effect on seed longevity.

iv. To evaluate and establish the suitable harvest maturity stage for other *Solanum aethiopicum* L. crops for the purposes of quality seed production.

v. To investigate the biochemical changes during after-ripening treatment to elucidate the components associated with seed vigour and germination improvement in African eggplant and related species.

vi. Further studies on the latent influence of drying and fermentation on ‘the seed longevity of the African eggplant’ (*Solanum aethiopicum* L.) is necessary.

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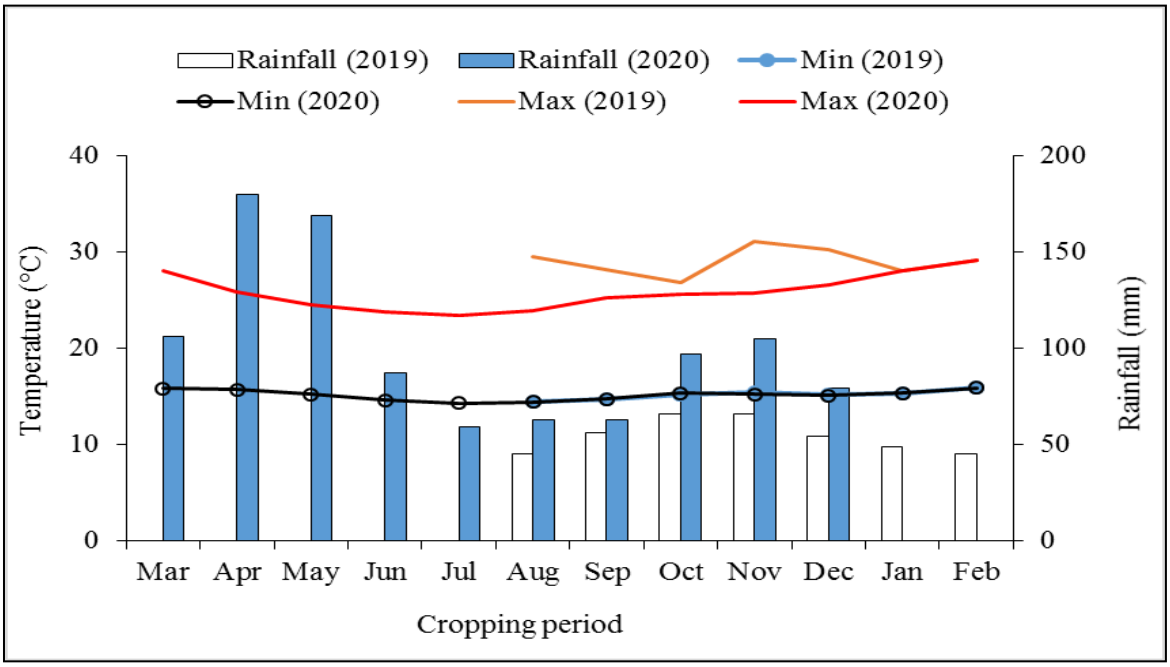
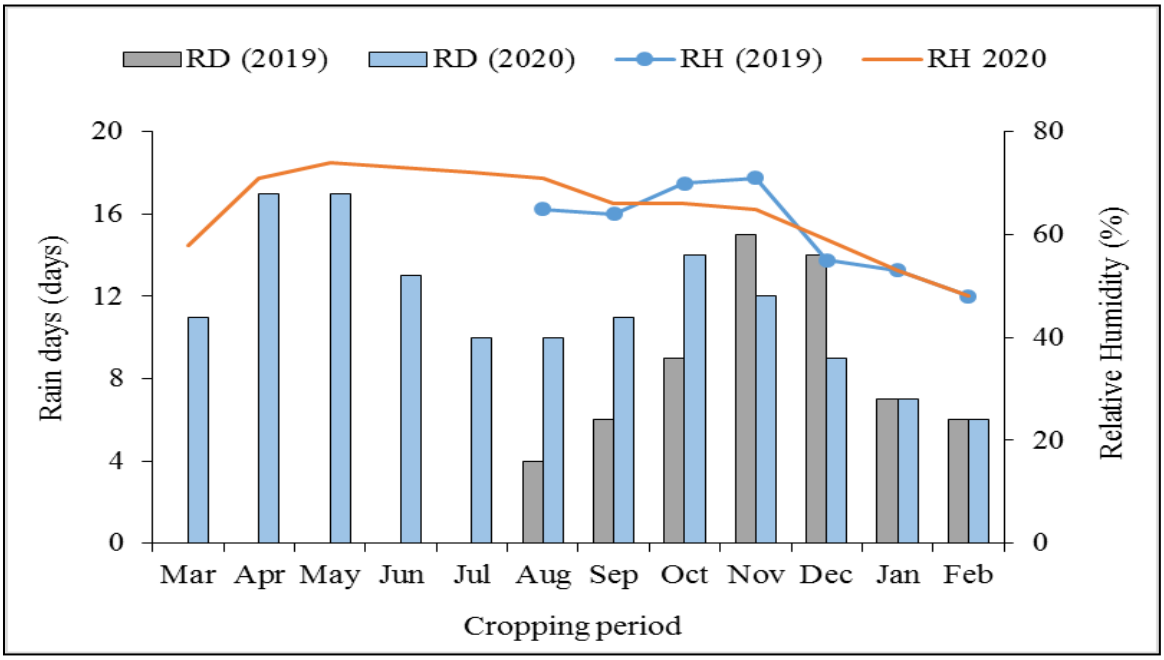
APPENDICES

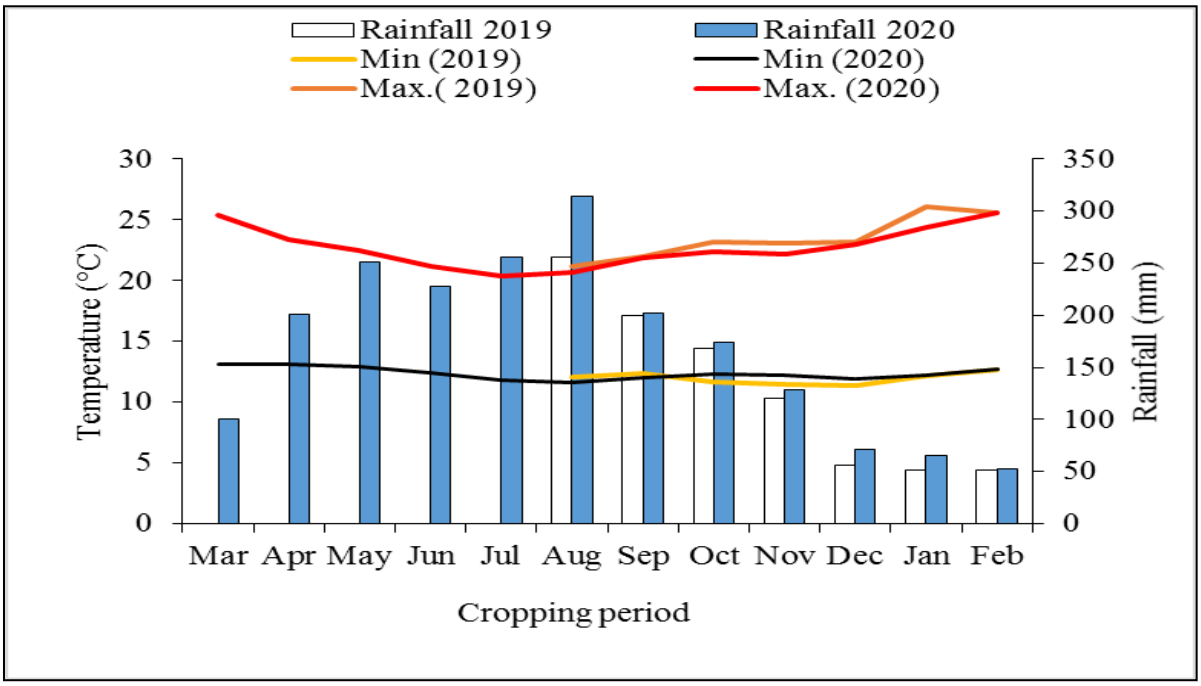
Appendix I: Rainfall distribution and Temperature during the cropping season in Bungoma (Tropical Monsoon climate).



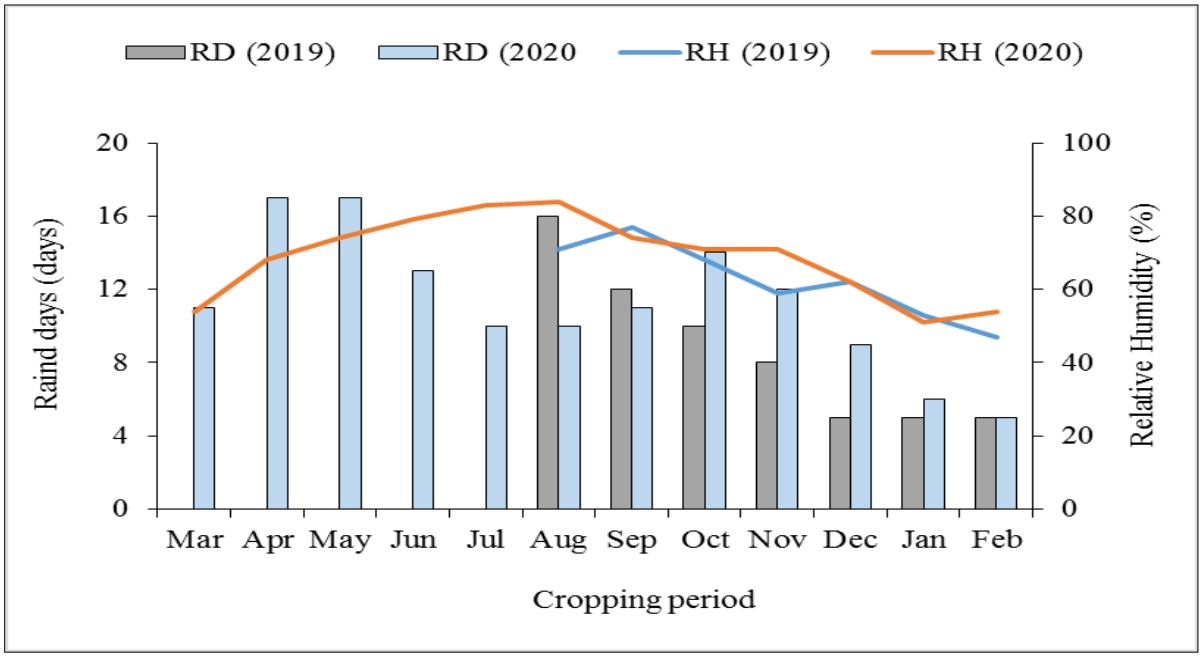
Appendix II: Relative Humidity and Rain days during the cropping season in Bungoma (Tropical Monsoon climate).



Appendix III: Rainfall distribution and Temperature during the cropping season in Eldoret (Temperate Oceanic climate)



Appendix IV: Relative Humidity and Rain days during the cropping season Eldoret (Temperate Oceanic climate).



APPENDIX V: Anova Summary showing effect of Constant and Alternating Temperature on percentage Seed Germination of two cultivars of African eggplant

Source	DF	SS	MS	F Value	Pr (> F)
Cultivar (CV)	1	7154.0833	7154.0833	112.96	0.0000
Temp.	7	38178.0000	5454.0000	86.12	0.0000
CV*Temp	7	8876.9167	1268.1310	20.02	0.0000
Error	32	2026.6667	63.3333		
Total	47	56235.6667			

CV (%) 27.05 Germ Mean 29.42

APPENDIX VI: Anova summary of effect of temperature and light on mean germination time (MGT)

Source	DF	SS	MS	F Value	Pr(> F)
Cultivar	1	4.8581	4.8581	2.88	0.0947
Light.Duration	2	179.7125	89.8562	53.34	0.0000
Temp	4	43.1120	10.7780	6.40	0.0002
Cultivar:Light.Duration	2	111.2586	55.6293	33.02	0.0000
Cultivar:Temp	4	26.1489	6.5372	3.88	0.0072
Light.Duration:Temp	8	182.5766	22.8221	13.55	0.0000
Cultivar:Light.Duration:Temp	8	105.2279	13.1535	7.81	0.0000
Error	60	101.0806	1.6847		
Total	89	753.9752			

CV(%) 18.28 MGT Mean 7.10

APPENDIX VII: Anova summary of effect of temperature and light on time to reach 50% germination: T_{50}

Source	DF	SS	MS	F Value	Pr(> F)
Cultivar	1	11.1232	11.1232	6.69	0.0121
Light.Duration	2	171.2762	85.6381	51.51	0.0000
Temp	4	53.8387	13.4597	8.10	0.0000
Cultivar:Light.Duration	2	109.3082	54.6541	32.87	0.0000
Cultivar:Temp	4	30.6256	7.6564	4.61	0.0026
Light.Duration:Temp	8	185.9239	23.2405	13.98	0.0000
Cultivar:Light.Duration:Temp	8	98.0291	12.2536	7.37	0.0000
Error	60	99.7564	1.6626		
Total	89	759.8814			

CV(%) 19.77 T50 Mean 6.52

APPENDIX VIII: Anova summary of effect of temperature and light on percentage seed germination (G %)

Source	DF	SS	MS	F Value	Pr (> F)
Cultivar	1	14187.7778	14187.7778	207.29	0.0000
Light.Duration	2	203.4667	101.7333	1.49	0.2344
Temp	4	48144.5111	12036.1278	175.85	0.0000
Cultivar:Light.Duration	2	2645.4222	1322.7111	19.33	0.0000
Cultivar:Temp	4	5444.7778	1361.1944	19.89	0.0000
Light.Duration:Temp	8	6900.7556	862.5944	12.60	0.0000
Cultivar:Light.Duration:Temp	8	2129.0222	266.1278	3.89	0.0010
Error	60	4106.6667	68.4444		
Total	89	83762.4000			

CV (%) 18.89 G.age Mean 43.80

APPENDIX IX: Anova summary of seed moisture content as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate

Source of variation	df.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	3.73	1.24	0.08	
Cultivar	1	539.71	539.71	35.69	<.001
Harv_Stage	5	6852.14	1370.43	90.62	<.001
Cultivar.Harv_Stage	5	131.42	26.28	1.74	0.153
Residual	33	499.08	15.12		
Total	47	8026.08			

Grand mean: 52.6; CV (%) 7.4

APPENDIX X: Anova summary of dry seed weight (g) as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0036837	0.0012279	2.15	
Cultivar	1	0.0018875	0.0018875	3.30	0.078
Harv_Stage	5	0.2534207	0.0506841	88.61	<.001
Cultivar.Harv_Stage	5	0.0098539	0.0019708	3.45	0.013
Residual	33	0.0188765	0.0005720		
Total	47	0.2877223			

Grand mean 0.25; CV (%) 9.7

APPENDIX XI: Analysis of variance summary of fresh seed weight (g) as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.014383	0.004794	3.75	
Cultivar	1	0.081923	0.081923	64.15	<.001
Harv_Stage	5	0.171510	0.034302	26.86	<.001
Cultivar.Harv_Stage	5	0.023110	0.004622	3.62	0.010
Residual	33	0.042142	0.001277		
Total	47	0.333067			

Grand mean 0.52; CV (%) 6.9

APPENDIX XII: Analysis of variance summary of thousand seed weight (TSW, g) as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.026758	0.008919	2.40	
Cultivar	1	0.795675	0.795675	213.92	<.001
Harv_Stage	5	22.485242	4.497048	1209.06	<.001
Cultivar.Harv_Stage	5	0.683975	0.136795	36.78	<.001
Residual	33	0.122742	0.003719		
Total	47	24.114392			

Grand mean 2.66; CV (%) 2.3

APPENDIX XIII: Analysis of variance summary of seed size (seed length, mm) as influenced by harvest fruit maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0008917	0.0002972	0.83	
Rep.*Units* stratum					
Cultivar	1	0.0546750	0.0546750	152.80	<.001
Harv_Stage	5	0.0903750	0.0180750	50.51	<.001
Cultivar.Harv_Stage	5	0.2177750	0.0435550	121.72	<.001
Residual	33	0.0118083	0.0003578		
Total	47	0.3755250			

Grand mean 3.30; CV (%) 0.6

APPENDIX XIV: Analysis of variance summary of percentage seed germination as influenced by fruit harvest maturity stage and cultivar under tropical climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Harv_S	5	18.76056	3.75211	184.28	<.001
Cultivar	1	0.13312	0.13312	6.54	0.015
Harv_S.Cultivar	5	0.15414	0.03083	1.51	0.210
Residual	36	0.73299	0.02036		
Total	47	19.78082			

Grand mean 1.00 (transformed); CV (%) 14.3

APPENDIX XV: Analysis of variance summary of percentage emergence as influenced by fruit harvest stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	386.67	128.89	1.67	
Cultivar	1	1045.33	1045.33	13.53	<.001
Harv_Stage	5	48638.67	9727.73	125.92	<.001
Cultivar.Harv_Stage	5	2878.67	575.73	7.45	<.001
Residual	33	2549.33	77.25		
Total	47	55498.67			

Grand mean 42.7; CV (%) 20.6

APPENDIX XVI: Analysis of variance summary of time to reach 50% field emergence (T_{50}) as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Harv. Stage	4	116.372	29.093	26.57	<.001
Cultivar	1	0.032	0.032	0.03	0.866
HS.Cultivar	4	1.433	0.358	0.33	0.857
Residual	30	32.848	1.095		
Total	39	150.685			

Grand mean 6.44; CV (%) 16.3

APPENDIX XVII: Analysis of variance summary of mean germination time (MGT, days) to emergence as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep. stratum	3	32.88	10.96	0.66	
Cultivar	1	1.22	1.22	0.07	0.789
Harv_Stage	5	1617.92	323.58	19.35	<.001
Cultivar.Harv_Stage	5	2.54	0.51	0.03	1.000
Residual	33	551.91	16.72		
Total	47	2206.46			

Grand mean 10.30; CV (%) 9.3

APPENDIX XVIII: Analysis of variance summary for fruit length (mm) as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	18.282	6.094	1.26	
Cultivar	1	5009.845	5009.845	1032.62	<.001
Harv_Stage	5	1568.685	313.737	64.67	<.001
Cultivar.Harv_Stage	5	198.947	39.789	8.20	<.001
Residual	33	160.103	4.852		
Total	47	6955.862			

Grand mean 67.01; CV (%) 3.3

APPENDIX XIX: Analysis of variance summary of fruit width (mm) as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.062	0.687	0.23	
Cultivar	1	1949.475	1949.475	661.03	<.001
Harv_Stage	5	1167.095	233.419	79.15	<.001
Cultivar.Harv_Stage	5	40.722	8.144	2.76	0.034
Residual	33	97.322	2.949		
Total	47	3256.677			

APPENDIX XX: Analysis of variance summary of fruit weight (g) as influenced by fruit harvest stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	159.79	53.26	2.68	
Cultivar	1	5725.57	5725.57	287.67	<.001
Harv_Stage	5	14912.51	2982.50	149.85	<.001
Cultivar.Harv_Stage	5	225.97	45.19	2.27	0.070
Residual	33	656.80	19.90		
Total	47	21680.64			

Grand mean 69.6; CV (%) 6.4

APPENDIX XXI: Analysis of variance summary of number of seeds per fruit as influenced by fruit harvest maturity stage and cultivar under tropical monsoon climate.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	7487.4	2495.8	4.13	
Cultivar	1	194259.9	194259.9	321.49	<.001
Harv_Stage	5	193304.9	38661.0	63.98	<.001
Cultivar.Harv_Stage	5	13684.8	2737.0	4.53	0.003
Residual	33	19940.0	604.2		
Total	47	428677.0			

Grand mean 414; CV (%) 5.9

APPENDIX XXII: Analysis of variance summary of seed moisture content (%) as influenced by fruit harvest maturity stage and cultivar under temperate oceanic climate (TOC).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	18.345	6.115	1.33	
Cultivar	1	80.992	80.992	17.56	<.001
Harv_Stage	5	14758.609	2951.722	640.08	<.001
Cultivar.Harv_Stage	5	136.963	27.393	5.94	<.001
Residual	33	152.178	4.611		
Total	47	15147.086			

Grand mean 62.2; CV (%) 3.5

APPENDIX XXIII: Analysis of variance summary of dry seed weight (g) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0002415	0.0000805	0.34	
Cultivar	1	0.0015870	0.0015870	6.68	0.014
Harv_Stage	5	0.5482534	0.1096507	461.54	<.001
Cultivar.Harv_Stage	5	0.0014828	0.0002966	1.25	0.309
Residual	33	0.0078400	0.0002376		
Total	47	0.5594047			

Grand mean 0.20; CV (%) 7.8

APPENDIX XXIV: Analysis of variance summary of fresh seed weight (g) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0018178	0.0006059	0.65	
Cultivar	1	0.0015413	0.0015413	1.64	0.209
Harv_Stage	5	0.3242459	0.0648492	69.16	<.001
Cultivar.Harv_Stage	5	0.0322409	0.0064482	6.88	<.001
Residual	33	0.0309437	0.0009377		
Total	47	0.3907897			

Grand mean 0.49; CV (%) 6.3

APPENDIX XXV: Analysis of variance summary of thousand seed weight (g) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.002033	0.000678	0.08	
Cultivar	1	1.153200	1.153200	139.01	<.001
Harv_Stage	5	46.570967	9.314193	1122.74	<.001
Cultivar.Harv_Stage	5	1.129600	0.225920	27.23	<.001
Residual	33	0.273767	0.008296		
Total	47	49.129567			

Grand mean 2.40; CV (%) 3.8

APPENDIX XXVI: Analysis of variance summary of Seed Size (Seed length, mm) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.0018250	0.0006083	2.31	
Cultivar	1	0.0200083	0.0200083	76.11	<.001
Harv_Stage	5	6.1655417	1.2331083	4690.79	<.001
Cultivar.Harv_Stage	5	0.0547417	0.0109483	41.65	<.001
Residual	33	0.0086750	0.0002629		
Total	47	6.2507917			

APPENDIX XXVII: Analysis of variance summary of percentage seed germination as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Harv_Stag	5	91831.00	18366.20	538.42	<.001
Cultvar	1	616.33	616.33	18.07	<.001
Harv_Stag.Cultvar	5	2041.67	408.33	11.97	<.001
Residual	36	1228.00	34.11		
Total	47	95717.00			

Grand mean 55; CV (%) 10.7

APPENDIX XXVIII: Analysis of variance summary of percentage field emergence as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Harv_Stage	5	35648.00	7129.60	108.39	<.001
Cultivar	1	1541.33	1541.33	23.43	<.001
Harv_Stage.Cultivar	5	4878.67	975.73	14.83	<.001
Residual	36	2368.00	65.78		
Total	47	44436.00			

Grand mean 32; CV (%) 25.7

APPENDIX XXIX: Analysis of variance summary of mean germination time (days) for seedling emergence as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Harv_Stage	5	2464.2980	492.8596	657.10	<.001
Cultivar	1	53.9381	53.9381	71.91	<.001
Harv_Stage.Cultivar	5	415.8079	83.1616	110.87	<.001
Residual	36	27.0019	0.7501		
Total	47	2961.0458			

Grand mean 9.23; CV (%) 9.4

APPENDIX XXX: Analysis of variance summary of time to reach 50% field emergence as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Harv_Stage	5	2274.343	454.869	423.46	<.001
Cultivar	1	43.637	43.637	40.62	<.001
Harv_Stage.Cultivar	5	370.261	74.052	68.94	<.001
Residual	36	38.670	1.074		
Total	47	2726.911			

APPENDIX XXXI: Analysis of variance summary of number of seeds per fruit as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2521.4	840.5	1.70	
Cultivar	1	164806.6	164806.6	333.62	<.001
Harv_Stage	5	120529.8	24106.0	48.80	<.001
Cultivar.Harv_Stage	5	53293.0	10658.6	21.58	<.001
Residual	33	16301.8	494.0		
Total	47	357452.6			

Grand mean 238; CV (%) 9.3

APPENDIX XXXII: Analysis of variance summary of fruit length (mm) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	32.581	10.860	1.57	
Cultivar	1	1715.184	1715.184	248.07	<.001
Harv_Stage	5	4524.302	904.860	130.87	<.001
Cultivar.Harv_Stage	5	805.562	161.112	23.30	<.001
Residual	33	228.169	6.914		
Total	47	7305.799			

Grand mean 54.66; CV (%) 4.8

APPENDIX XXXIII: Analysis of variance summary of fruit width (mm) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	6.225	2.075	0.33	
Cultivar	1	2334.812	2334.812	368.21	<.001
Harv_Stage	5	2181.365	436.273	68.80	<.001
Cultivar.Harv_Stage	5	612.069	122.414	19.30	<.001
Residual	33	209.255	6.341		
Total	47	5343.726			

Grand mean 33.62; CV (%) 7.5

APPENDIX XXXIV: Analysis of variance summary of fruit weight (g) as influenced by fruit harvest maturity stage and cultivar under TOC.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	85.19	28.40	2.12	
Cultivar	1	6177.58	6177.58	462.05	<.001
Harv_Stage	5	9328.19	1865.64	139.54	<.001
Cultivar.Harv_Stage	5	633.87	126.77	9.48	<.001
Residual	33	441.21	13.37		
Total	47	16666.04			

Grand mean 38.9; CV (%) 9.4

Appendix XXXV: Summary of Multivariate Analysis of variance (MANOVA) for Germination percentage and Field emergence for two cultivars under two climates

Source		df	Mean Square	F	F ≤ 0.05
Climate	%FE	1	3750.000	58.391	.000
	%Germ	1	4648.167	129.315	.000
Cultivar	%FE	1	2562.667	39.903	.000
	%Germ	1	888.167	24.709	.000
HarvStage	%FE	5	14967.200	233.053	.000
	%Germ	5	32071.767	892.259	.000
Climate * Cultivar	%FE	1	24.000	.374	.543
	%Germ	1	28.167	.784	.379
Climate * HarvStage	%FE	5	927.200	14.437	.000
	%Germ	5	1426.167	39.677	.000
Cultivar * HarvStage	%FE	5	1476.667	22.993	.000
	%Germ	5	369.367	10.276	.000
Climate * Cultivar *	%FE	5	128.400	1.999	.089
HarvStage	%Germ	5	129.367	3.599	.006
Error	%FE	72	64.222		
	%Germ	72	35.944		
Total	%FE	96			
	%Germ	96			

% FE: percent field emergence, %Germ: percent germination

APPENDIX XXXVI: Anova summary of dry seed weight (mg) of cv. *Oforiwa* as affected by fruit harvest time and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	184405.1750	46101.2938	5732.80	0.0000
Error (a)	15	120.6250	8.0417		
After-ripen	3	2625.1000	875.0333	156.64	0.0000
Harv. * After-ripen	12	15245.5250	1270.4604	227.43	0.0000
Error (b)	45	251.3750	5.5861		
Total	79	202647.8000			

CV (a)% 1.39; CV(b)% 1.16; Mean 204.45

APPENDIX XXXVII: Anova summary of dry seed weight (mg) of cv. *Kpando* as affected by fruit maturity and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	231559.3000	57889.8250	13475.81	0.0000
Error(a)	15	64.4375	4.2958		
After-ripen	3	24581.2375	8193.7458	816.99	0.0000
Harv. *After-ripen	12	47928.2000	3994.0167	398.24	0.0000
Error(b)	45	451.3125	10.0292		
Total	79	304584.4875			

CV(a)% 0.8987; CV(b)% 1.37; Mean (mg) 230.64

APPENDIX XXXVIII: Anova summary of Thousand Seed weight (g) of cv. *Oforiwa* as affected by fruit harvest time and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	19.0817	4.7704	1415.90	0.0000
Error (a)	15	0.0505	0.0034		
After-ripen	3	0.3258	0.1086	119.59	0.0000
Harvest*After-ripen	12	1.4556	0.1213	133.58	0.0000
Error (b)	45	0.0409	0.0009		
Total	79	20.9544			

CV (a)% 2.82; CV (b)% 1.46; Mean (g) 2.06

APPENDIX XXXIX: Anova summary of Thousand Seed weight (g) of cv. *Kpando* as affected by fruit harvest time and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	23.6311	5.9078	5239.72	0.0000
Error (a)	15	0.0169	0.0011		
After-ripen	3	2.8572	0.9524	904.43	0.0000
Harv.*After-ripen	12	4.9015	0.4085	387.88	0.0000
Error (b)	45	0.0474	0.0011		
Total	79	31.4542			

CV(a)% 1.45; CV(b)% 1.40; Mean TSW (g). Mean

APPENDIX XL: Anova summary of seed moisture content (%) of cv. *Oforiwa* as affected by fruit harvest time and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	6983.4792	1745.8698	1098.12	0.0000
Error (a)	15	23.8481	1.5899		
After-ripen	3	1171.0474	390.3491	576.69	0.0000
Harv. * After-ripen	12	1088.8357	90.7363	134.05	0.0000
Error (b)	45	30.4594	0.6769		
Total	79	9297.6699			

APPENDIX XLI: Anova summary of seed moisture content (%) of cv. *Kpando* as affected by fruit harvest time and after-ripening period.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	3	3.984	1.328	1.58	
Harvest time	4	7660.930	1915.233	2283.69	<.001
Residual (a)	12	10.064	0.839	0.68	
After-ripen	3	1166.690	388.897	315.48	<.001
Harv.* After-ripen	12	1017.521	84.793	68.79	<.001
Residual (b)	45	55.472	1.233		
Total	79	9914.662			

APPENDIX XLII: Anova summary of first count (%) of cv. *Oforiwa* as affected by fruit harvest time and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	48602.8000	12150.7000	944.35	0.0000
Error (a)	15	193.0000	12.8667		
After-ripen	3	7811.8000	2603.9333	102.88	0.0000
Harv. *After-ripen	12	2957.2000	246.4333	9.74	0.0000
Error (b)	45	1139.0000	25.3111		
Total	79	60703.8000			

CV (a)% 11.94; CV(b)% 16.74; Mean First Count (%), 30.05

APPENDIX XLIII: Anova summary of first count (%) of cv. *Kpando* as affected by fruit harvest time and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	83237.2000	20809.3000	613.24	0.0000
Error (a)	15	509.0000	33.9333		
After-ripen	3	8915.8000	2971.9333	335.18	0.0000
Harv. *After-ripen	12	6649.2000	554.1000	62.49	0.0000
Error (b)	45	399.0000	8.8667		
Total	79	99710.2000			

CV(a)% 15.39; CV(b)% 7.87; Mean First.Count (%) 37.85

APPENDIX XLIV: Anova summary of percentage seed germination (%) of cv. *Oforiwa* as affected by fruit harvest time and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	83861.2000	20965.3000	944.38	0.0000
Error (a)	15	333.0000	22.2000		
After-ripen	3	12074.2000	4024.7333	310.66	0.0000
Harv. *After-ripen	12	11274.8000	939.5667	72.52	0.0000
Error (b)	45	583.0000	12.9556		
Total	79	108126.2000			

CV (a) % 7.62; CV(b)% 5.82; Mean of SG (%) 61.85

APPENDIX XLV: Anova summary of percentage seed germination (%) of cv. *Kpando* as affected by fruit maturity: and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	4	98552.8000	24638.2000	685.66	0.0000
Error (a)	15	539.0000	35.9333		
After-ripen	3	15339.8000	5113.2667	341.90	0.0000
Harv.*After-ripen	12	19135.2000	1594.6000	106.62	0.0000
Error (b)	45	673.0000	14.9556		
Total	79	134239.8000			

CV (a)% 10.33; CV(b)% 6.66; Mean SG (%) 58.05

APPENDIX XLVI: Anova summary of mean germination time (MGT, days) of cv. *Oforiwa* as influenced by fruit harvest time and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	56.6365	28.3183	194.26	0.0000
Error (a)	9	1.3120	0.1458		
After-ripen	3	33.3444	11.1148	39.64	0.0000
Harv.*After-ripen	6	7.6240	1.2707	4.53	0.0027
Error (b)	27	7.5711	0.2804		
Total	47	106.4880			

CV (a)% 4.78; CV(b)% 6.63; Mean MGT (days) 7.99

APPENDIX XLVII: Anova summary of mean daily germination (MDG, seeds/day) of cv. *Oforiwa* as influenced by fruit maturity stage and after-ripening (post-storage) period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	24.6373	12.3186	72.23	0.0000
Error (a)	9	1.5350	0.1706		
After-ripen	3	7.1632	2.3877	35.02	0.0000
Harv. *After-ripen	6	10.1224	1.6871	24.74	0.0000
Error (b)	27	1.8411	0.0682		
Total	47	45.2990			

CV (a)% 6.66; CV(b)% 4.21; Mean MDG (day^{-1}) 6.20

APPENDIX XLVIII: Anova summary of germination index (GI) of cv. *Oforiwa* as influenced by fruit harvest time and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	25.9064	12.9532	232.66	0.0000
Error (a)	9	0.5011	0.0557		
After-ripen	3	14.9263	4.9754	68.45	0.0000
Harv.*After-ripen	6	2.1845	0.3641	5.01	0.0014
Error (b)	27	1.9626	0.0727		
Total	47	45.4809			

CV(a)% 7.45; CV(b)% 8.51; Mean GI 3.17

APPENDIX XLIX: Anova summary of time to reach 50% germination (T_{50}, day) of cv. *Oforiwa* as influenced by fruit harvest time and after-ripening period.

Source'	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	99.5461	49.7730	189.14	0.0000
Error (a)	9	2.3685	0.2632		
After-ripen	3	57.6259	19.2086	41.09	0.0000
Harv.*After-ripen	6	19.4353	3.2392	6.93	0.0002
Error(b)	27	12.6225	0.4675		
Total	47	191.5981			

CV(a)% 7.08; CV(b)% 9.44; Mean T_{50} (day) 7.25

APPENDIX L: Anova summary of mean germination time (MGT, day) of cv. Kpando as influenced by fruit harvest time and after-ripening period.

Source	df	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	84.8431	42.4216	131.66	0.0000
Error (a)	9	2.8998	0.3222		
After-ripen	3	21.2044	7.0681	46.34	0.0000
Harv.*After-ripen	6	9.6189	1.6031	10.51	0.0000
Error (b)	27	4.1185	0.1525		
Total	47	122.6847			

APPENDIX LI: Anova summary of mean daily germination time (MDG, seed/day) of cv. *Kpando* as influenced by fruit harvest time and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	57.9829	28.9914	115.06	0.0000
Error (a)	9	2.2677	0.2520		
After-ripen	3	26.3876	8.7959	98.26	0.0000
Harv.*After-ripen	6	26.4008	4.4001	49.15	0.0000
Error (b)	27	2.4170	0.0895		
Total	47	115.4559			

CV(a)% 8.53; CV(b)% 5.08; Mean (MDG) 5.89

APPENDIX LII: Anova summary of germination index (GI) of cv. *Kpando* as influenced by fruit maturity stage and after-ripening period.

Source	DF	Sum of Square	'Mean Square	F Value	Pr(> F)
Harvest time	2	59.7661	29.8830	1294.96	0.0000
Error (a)	9	0.2077	0.0231		
After-ripen	3	17.6991	5.8997	94.64	0.0000
Harv.*After-ripen	6	8.2504	1.3751	22.06	0.0000
Error (b)	27	1.6832	0.0623		
Total	47	87.6065			

CV(a)% 4.42; CV(b)% 7.27; Mean (GI) 3.44

APPENDIX LIII: Anova summary of time to reach 50% germination (T_{50}, day) of cv. *Kpando* as influenced by fruit maturity stage and after-ripening period.

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
Harvest time	2	110.9148	55.4574	68.54	0.0000
Error (a)	9	7.2823	0.8091		
After-ripen	3	21.6387	7.2129	31.13	0.0000
Harv.*After-ripen	6	10.1794	1.6966	7.32	0.0001
Error (b)	27	6.2566	0.2317		
Total	47	156.2718			

CV(a)% 14.40; CV(b)% 7.71; Mean (T_{50}) 6.25

APPENDIX LIV: Anova summary of interactive effects of drying methods and cultivar on seed moisture content of African eggplant.

Effect	Num DF	Den DF	F Value	Pr > F
Drying Methods (DM)	7	50	51.49	<.0001
Cultivar (CV)	1	3	1.17	0.3581
DM*CV	7	50	8.87	<.0001

APPENDIX LV: Anova summary of interactive effects of drying methods and cultivar on first germination count of African eggplant.

Effect	Num DF	Den DF	F Value	Pr > F
Drying methods (DM)	7	50	15.58	<.0001
Cultivar (CV)	1	3	37.26	0.0088
DM*CV	7	50	5.99	<.0001

APPENDIX LVI: Anova summary of interactive effects of drying methods cultivar on percentage seed germination of African eggplant.

Effect	Num DF	Den DF	F Value	Pr > F
Drying Methods (DM)	7	50	13.66	<.0001
Cultivar (CV)	1	3	0.76	0.4480
DM*CV	7	50	4.40	0.0007

APPENDIX LVII: Anova summary of interactive effect of fermentation duration and cultivar on seed moisture content of African eggplant.

Effect	Num DF	Den DF	F Value	Pr > F
Fermentation duration (FM)	4	24	2234.18	<.0001
Cultivar (CV)	1	3	115.61	0.0017
FM*CV	4	24	12.94	<.0001

APPENDIX LVIII: Anova summary of interactive 'effect of fermentation duration and cultivars on first germination count' (%)

Effect	Num DF	Den DF	F Value	Pr > F
Fermentation (FM)	4	24	18.36	<.0001
Cultivar (CV)	1	3	89.40	0.0025
FM*CV	4	24	9.71	<.0001

APPENDIX LIX: Anova summary of interactive effect of fermentation duration and cultivars on seed germination percentage.

Effect	Num DF	Den DF	F Value	Pr > F
Fermentation (FM)	4	24	0.25	0.9096
Cultivar (CV)	1	3	21.50	0.0189
FM*CV	4	24	0.80	0.5353

APPENDIX LX: Anova summary of interactive effect of fermentation and cultivar on Accelerated Ageing (AA).

Effect	Num DF	Den DF	F Value	Pr > F
Fermentation (FM)	4	24	157.88	<.0001
Cultivar (CV)	1	3	11.99	0.0406
FM*CV	4	24	32.62	<.0001

APPENDIX LXI: Mean squares of analysis of variance for sucrose, glucose and fructose (sugars) of African eggplant cv. *Oforiwa* at six harvest maturity stages.

		Mean square		
Source of Variation	**Degrees of freedom**	**Surose**	**Glucose**	**Fructose**
Maturity Stage (between groups)	5	77.65**	35.57**	42.617**
Within groups	12			
Total	17			

**Significant at 1 % probability level

APPENDIX LXII: Mean squares of analysis of variance for crude protein, crude fat, antioxidant activity (AOA) and tannin content of African eggplant cv. *Oforiwa* at six harvest maturity stages.

		Mean square			
Source of Variation	**Degrees of freedom**	**Crude Protein**	**Crude Fat**	**AOA**	**Tannin**
Maturity Stage (between groups)	5	1.637 ns	23.46**	14623.24**	10630.34**
Within groups	12				
Total	17				

**Significant at 1% probability level, ns: not significant.

APPENDIX LXIII: Mean squares of analysis of variance for germination (G), vigour by germination index (GI), mean daily germination (MDG) and mean germination time (MGT) of African eggplant cv. *Oforiwa* at six harvest maturity stages.

		Mean square			
Source of Variation	**Degrees of freedom**	**% G**	**MGT**	**GI**	**MDG**
Maturity Stage (between groups)	5	7661.600**	21.067**	17.572**	16.616**
Within groups	18				
Total	23				

**Significant at 1% probability level.

Appendix LXIV: Similarity Report

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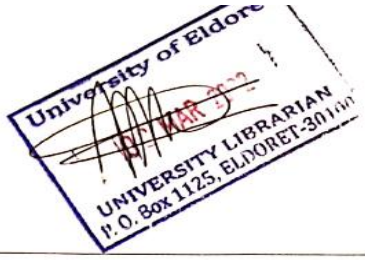

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