GERMINATION PHYSIOLOGY OF AFRICAN BIRD'S EYE CHILI SEED

BY

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DECLARATION

Declaration by student

This thesis is my original work and has not been presented for award of any certificate or degree in any other institution or university. No part of this work should be reproduced without prior permission from the authors and/ or University of Eldoret.

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DEDICATION

To my academic mentors, Prof. Julius O. Ochuodho and Prof. Elmanda O. Auma; my dear mum Edith A. Okomba for their endless support, prayers and encouragement throughout these academic years of study.

ABSTRACT

Seed germination parameters may be determined by stage of seed maturity and the level of dormancy. These parameters subject the seed to varied responses on sensitivity to environmental factors that stimulate germination such as gibberellic acid, priming treatments, light and the range of temperature over which germination can occur. *Capsicum frutescens* seeds show erratic physiological maturity with low germination and vigour besides being physiologically dormant. The overall objective of the study was to determine the effects of fruit harvesting stage and seed treatments on physiological quality of seeds as well as the best conditions for seed germination. Seed samples were subjected to different germination regimes of constant temperatures (15^{o}C, 20^{o}C, 30^{o}C and 35^{o}C) and alternating temperatures (20^{o}C/30^{o}C and 25^{o}C/30^{o}C) under different photoperiodic regimes. Seed samples from differently matured fruits were subjected to priming using KNO_3 (concentrations of 0.2%, 0.5% and 0.8%), and polyethyl glycol (PEG_{6000}) (concentrations of -0.2Mpa, -0.6Mpa and 1Mpa); and gibberellic acid treatments (concentrations of 0.02%, 0.05% and 0.08%). Germination assays were done under light and temperature conditions. Fruits were tagged at flowering and seeds harvested at different fruit maturity dates from 20 days after flowering to 75 days after flowering with a step of five days. Percentage germination, mean germination time, first count, 1000 seed weight, seed moisture content, seed dry weight and electrical conductivity tests were performed. The experimental data obtained from different parameters tested was subjected to analysis of variance (ANOVA) and separation of means was done by Contrast Comparison at 95% level of significance. Temperature had significant effect on germination ($p \leq .001$), with the highest percentage germination of 57% being recorded at a temperature of 25^{o}C. Primed seeds and seeds treated with gibberellic acid had high percentage germination and germination vigour as compared to controls irrespective of the fruit colour and germination conditions ($p < .001$). The highest values were recorded from seeds that were harvested from red fruits and germinated under darkness ($p < .001$). Seeds that were harvested from fruits at 75 days after flowering when the fruits were red in colour had maximum germination and vigour values. For maximum seed vigour and germination, seeds should be harvested from red fruits and germinated under constant optimum temperatures of 25^{o}C. Where priming is a necessity, potassium nitrate is the most suitable at concentrations of 0.5%. Treatment with GA_3 should be done at a concentration of 0.08% and germination is more optimum if treatments are germinated in high temperatures in the presence or absence of light.

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ABBREVIATIONS

ABE	African Bird's Eye.
ACC	1-aminocyclopropane.
AVG	Amino Ethoxyvinylglycine.
DGB	Dark Green Boston.
E.C	Energy Charger.
eRH	Equilibrium Relative Humidity.
Fintrac HDC	Fintrac Horticulture Development Centre.
HCDA	Horticultural Crops Development Authority.
ISTA	International Seed Testing Association.
KHCP	Kenya Horticulture Competitiveness Project.
KHDP	Kenya Horticultural Development Programme.
LEA	Late Embryogenesis Accumulated.
PLI	Public Ledger Issue.
STS	Silver Thiosulphate.
βGlu1	Class 1β-1, 3-glucanase.

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To God the father, I am forever indebted to you for your love, Grace and Provision throughout my life and reminding me that I was born with a purpose.

CHAPTER ONE

INTRODUCTION

1.1 Origin and botany of chillies

The genius *Capsicum frutescens* (L) is in the large family solanaceae, subfamily solanoideae and tribe capsiceae (Knapp, 2002; Knapp *et al.*, 2004; Bosland and Votava, 2000). The original range of red pepper is unknown, but it is believed to have been domesticated in Central America, possibly Panama, thousands of years ago (Bosland and Votava, 2000). Center of diversity for *C. frutescens* believed to be Western Amazonia, Central America (Colombia to Peru) (Hernandez-Verdugo *et al.*, 1999).

Capsicum frutescens, also known as chili pepper, is a short-lived evergreen shrub usually 1 to 1.5m in height and 1 to 3cm in basal stem diameter. The shrub is supported by a short to long taproot, many spreading lateral roots, and moderately abundant fibrous roots. The stem and larger branches of mature plants are woody but moderately soft and weak. Bark of stem and older branches is light gray. The form is upright, the abundant branching is often dichotomous, and the branches and twigs are slender (de Swart *et al.*, 2004; Rodriguez-Rey *et al.*, 2000).

The larger of the leaves are 4 to 12cm long and 1 to 4.5cm broad. Greenish white to yellowish-white flowers with blue, violet, or yellow anthers occur in groups of two or more at the nodes. The berries are red or red-orange at maturity, elongated with pointed or rounded tip, 1.5 to 3.5cm long and 0.5 to 1.2cm thick (Bosland and Votava, 2000; Ben Chaim *et al.*, 2003; Thorup *et al.*, 2000; Huh *et al.*, 2001).

The fruits are somewhat dry and contain few to many creams to yellow lenticular seeds about 3mm in diameter (Knapp, 2002; Andrews, 1995), which develops from a compylotropous ovule. Within a pod, the many seeds are attached to the placenta walls in close rows, mainly near the calyx end. The seeds are disk-like with a deep chalazal depression. The embryo is surrounded by a well defined endosperm with protein and lipids as storage reserves (Chen and Lott, 1992). The fruits, especially the seeds and placenta, have a biting, pungent taste. The species has 2n=24 chromosomes (Bosland and Votava, 2000; Lioger, 1995).

1.2 *Capsicum frutescens* uses, production potential and constraints

Capsicum frutescens is among the hottest varieties of pepper in the world and comprises numerous chemicals including steam-volatile oils, vitamins, capsaicinoids, carotenoids, proteins, fibre and mineral elements. Many chilli pepper constituents have importance for nutritional value, flavour, aroma, texture and colour. The two chemicals of greatest interest are the capsaicinoids and the carotenoids. The capsaicinoids are alkaloids that give hot chilli pepper's nutritional value and colour of the chilli peppers (Bosland and Votava, 2000; Hornero-Mendez *et al.*, 2002; Britton and Hornero-Mendez, 1997).

Apart from its traditional and culinary uses, its therapeutic and pharmacological uses are well documented, as green chilli forms an excellent combination of healthy ingredients and essential nutrients, good source of vitamins (A,B,C,E, and P), mineral (Iron, Magnesium and Potassium), dietary fibres and macronutrients (Parle and Kaura, 2012). Capsaicin is recognised as a treatment for osteo-arthritic pain, topically applied capsaicin, either as a cream or plaster helps in reducing lower back pain (Kim *et al.*, 2006), managing headaches, including cluster and migraine headaches. The

high amounts of beta-carotene in green chilli, flavonoids, vitamin-A and vitamin-C, which are useful in preventing problems like lung infections, asthma, and emphysema (Caterina and Julius, 2001).

Green chili reduces blood cholesterol, triglyceride levels, and platelet aggregation, hence lowers risk of heart attack, stroke and pulmonary embolism. Green chili also prevents the deposition of fats along blood vessel walls caused by free radicals, which is the first step in the development of atherosclerosis (Ahuja and Ball, 2006), provides protection against developing stomach ulcers by killing ingested bacteria, increasing the secretion of mucus and protective buffer solution as capsaicin possesses antibacterial property particularly against the bacteria *Helicobacter pyroli* (which is one of the causative agent of stomach ulcers) (Parle and Kaura, 2012).

Malawi is the world leading supplier of African Birds Eye (ABE) chilies historically shipping about 400-500 metric tons per year to Western Europe and other markets (PLI, 2010). Other suppliers including Zimbabwe, South Africa, Nigeria and Uganda, produce much smaller volumes and typically on a more speculative basis (Fintrac HDC, 2004). Over the last decade, weather and other production problems have slowed down the ability of Malawi as a supplier to provide ABE chilies on a regular basis. Supply from Zimbabwe has also been inconsistent (KHCP, 2011). Supply fluctuations have additionally caused prices to vary widely from $2000 to $4000 per metric ton (Fintrac HDC, 2004; KHCP, 2011).

To take advantage of the current market openings, FINTRAC HDC, in conjunction with local commercial partners are leading in efforts to develop Kenya as a reliable source of African Birds Eye chillies (HCDA, 2008; Fintrac HDC, 2004; KHCP,

2011). Fintrac started working with a handful of farmers who started supplying ABE chilli to two export processors; Mace foods and Equator products (Fintrac HDC., 2004). As at that time (2004) more than ninety two thousand seedlings had been transplanted in some parts of South Nyanza with average farmer having 500 to 100 plants on plots ranging in size from one-eighth to one-quarter of an acre (Fintrac HDC, 2004;KHCP, 2011). At the end of the pilot year of production, it was estimated that about 1000 farmers would be growing the variety with projected increase to 20000 farmers by the end of the project (HCDA, 2008; KHCP, 2011). As at now, Mace foods averages about 40 metric tons per year but the market demand is estimated to be three or four times more.

The interest in ABE chillies is growing rapidly due to availability of market both locally and internationally (KHCP, 2011; Fintrac HDC, 2004; HCDA, 2008). ABE chillies require few if any inputs and it's minimal requirements for rainfall makes it very suitable for production in marginal areas with little rainfall and poor soils such as Homa Bay, Busia and Siaya in Western Kenya (Nyanza and Western counties), (KHDP, 2009; Fintrac HDC, 2004).

The problem however is the unavailability of high quality seeds of the species as farmers experience poor germination and emergence hence reducing the potential for higher output at the end of the cropping season. There is also the mixture of seeds and plant types which is being handled. The demand has necessitated looking at the ABE chilies seed production system as there will be a high demand of the same.

The department of Seed, Crop and Horticultural Sciences at the University of Eldoret has been carrying out an experiment to try and develop a protocol that will enable farmers to have access to the best seed available. After separating the two plant types, through selection, it was noticed that physiological seed quality was still a problem hence this research project. The procedure has involved the establishment of experimental plots in Bungoma and Busia, where two types of the chili were planted and harvesting of fruits at different maturity level using the fruit colour as a maturity marker was carried out. The collected samples were then processed and several seed quality assessment procedures were done.

1.3. Justification

Seed development and maturation study is essential because seeds need to be harvested timely to ensure good yield associated with germination, vigour and field performance (Tekrony and Hunter, 1995; Demir *et al.*, 2002). Physiological changes might set in; if the seeds are retained on the mother plant for longer duration after physiological maturity is reached. As such there is increased risk of developing hard seeds and by extension secondary seed dormancy (Oluoch and Welbaum, 1996b; Kumar *et al.*, 2002; and Dias *et al.*, 2006). Seed yield and quality largely depends on the stage of maturity (da Silva *et al.*, 2002; Vidigal *et al.*, 2011; de Souza *et al.*, 2011), and as such timely harvest at the best stage of maturity is critical since harvesting either at an early or later stage will results in poor quality seeds, hence necessitating the study of the same effect on African Birds Eye chilies.

1.4. Problem statement

Germination is a critical stage in the life of plants and often controls population dynamics, with major practical implications. To produce quality seeds, timely harvest is crucial to maximize seed vigour and germination. Visual indicators of physiological maturity have been used as an indicator of seed maturity.

Apart from seed maturity, germination dynamics such as percentage, rate and vigour are greatly influenced by the characteristics of the seed that determines the conditions required for germination. Any treatment or germination conditions that widen the environmental requirements for germination is thus considered as dormancy release factor. As such cardinal germination conditions are defined by the temperature range requirements, and light or a combination of both over a defined incubation time where seed dormancy is suspected.

Seed treatments such as application of gibberellic acid and priming have been used to improve germination positively by a complex interaction with environmental conditions. This research was set up to determine the appropriate stage of harvesting the chilies based on the fruit colour for the highest quality seed possible; establish the optimum germination conditions and overcome seed dormancy where dormancy is suspected through gibberellic acid application and priming procedures.

1.5. Objectives

1.5.1. Overall objectives

To determine the effects of fruit harvesting stage and seed treatments on the physiological quality of seeds as well as the best conditions for seed germination of ABE chilies.

1.5.2. Specific objectives

i. To establish optimum germination conditions for ABE chilies.
ii. To determine appropriate seed treatment using gibberellic acid and priming for improved seed germination of ABE chilies.
iii. To determine the optimum seed harvesting stage using fruit colour as maturity indicator.

1.6. Hypothesis

1.6.1. Objective 1 hypothesis

H_A: The germination percentage of ABE chili seeds is affected by different incubation conditions.

1.6.2. Objective 2 hypotheses

H_A: Seed treatment with gibberellic acid and seed priming improve the vigour and germination of ABE chili seeds.

1.6.3. Objective 3 hypotheses

H_A: Seeds in fruits that undergo different colour change show different seed germination percentage and vigour.

CHAPTER TWO

LITERATURE REVIEW

2.1. Seed development and maturation

Seeds start to develop after fertilization and undergo a number of morphological and physiological changes until they reach the point of dispersal (Bewley and Black, 1994). Therefore, as dry matter transfers from the maternal plant to the seed, moisture contents begins to fall, although seed equilibrium relative humidity (eRH) remains high (Hay and Probert, 2000). Formation of an abscission layer marks the end of reserve accumulation and attainment of mass maturity (Ellis and Pieto Filho, 1992).

Production of good quality seeds requires timely harvest at physiological maturity for maximal seed vigour. The identification of the time of physiological maturity is usually a subject of controversy as far as seed maturation is concerned (Ellis and Filho, 1992; Zanakis *et al.*, 1994).

Factors such as seed water content cannot be considered as a good indicator of physiological maturity (Demir *et al.*, 2002). This is because they can be affected by genotype and environmental conditions. Maximal germination in maize occurred at high moisture levels early in development before seeds attained physiological maturity (Tekrony and Hunter., 1995). In *Brassica* (Sinniah *et al.*, 1998), rape and cabbage (Still and Bradford, 1998), Sumauma (Lima *et al.*, 2000), maximum dry mass did not coincide with physiological maturity.

2.2 Indicators of seed maturity and harvest Time in relation to germination

markers of seed maturity such as fruit colour or a combination of a number of field markers can be used as excellent indicators of physiological maturity (still and

Bradford, 1998). Potential markers might vary according to fruit type i.e., fleshy or dry fruits hence change in fruit colour may therefore be unreliable indicator of seed maturity (Demir and Samit, 2001). In plant species that have indeterminate fruiting periods, easily identifiable fruit maturation characteristics that relate to seed quality are essential to determine the optimum harvest stage (Dias *et al.*, 2006b).

Seed maturation in fleshy fruits may precede fruit maturity. In overripe tomato fruits, high percentage of Seeds may undergo precocious germination while in muskmelon fruits; seeds are either dead or had very low vigour if harvest was delayed until fruits started decomposing (Oluoch and Welbaum, 1996b). In physic nut seeds (*Jatropha curcas* L.), seeds of high physiological quality were obtained from yellow and yellow-brown fruits while green fruits had the lowest physiological quality (da Silva *et al.*, 2012). Dry weight increased with the maturation process with the highest value from yellowish brown or brown fruits (Zanakis *et al.*, 1994). In sweet Pepper, Seeds with high germination and vigour were harvested when fruits were completely Red at 75 days after Anthesis D.A.A.), (de Souza *et al.*, 2011).

In the drupe of *Rhus aromatic* Ait and *R. glabra* L., fruit colour changes appear to be associated with embryo achieving Full size and developing the ability to germinate (Li *et al.*, 1999). Maximum dry matter correlates With changes In fruit colour, 1000 seed weight increased gradually with the progress of fruit maturity (Egli and Tekrony,1997), in tomato (Dias *et al*., 2006), and sweet pepper seeds (Vidigal *et al.*, 2011).

These morphological markers (fruit colour) are at best a coarse tool used to make preliminary assessments (Finch Savage *et al.*, 2002; Demir and Samit, 2001). On the positive side they indicate increasing seed maturity and with it increased seed longevity (Shantappa *et al.*, 2006; Alan and Eser, 2008), reducing the likelihood of harvesting seeds which may not have achieved the maximum dry weight, germinability or desiccation tolerance. On the negative side, the colour changes are often subjective (Muthoka *et a*l., 2003).

2. 3. Dormancy classification and germination in *Capsicum frutescens.*

Capsicum frutescens has seeds that are flattened discoid with curved embryos having no visible distinction between testa rapture and endosperm rapture (Bosland and Votava, 2000), often berries as fruits (Baskin and Baskin, 2007). *Solanaceae* family has been classified as having non-deep physiological dormancy (Baskin and Baskin, 2004). Mechanical resistance from combined testa and endosperm dormancy which is greater than the embryo growth potential opposing it appears to be the cause of non-deep physiological dormancy in seed model systems such as *Arabidopsis thaliana* and *Solanaceae* species (Koornneef *et al.*, 2002; Leubner-Metzger, 2003). Embryos excised from these seeds that exhibit non-deep physiological dormancy produce normal seedlings and gibberellic acid treatment can has been shown to break this type of dormancy (Baskin and Baskin, 2004).

Based on pattern of change in physiological responses to temperature, five types of non-deep physiological dormancy can be distinguished. This is based on temperature range at which seed germination can occur, which increases gradually during the progression of no-deep dormancy release from low to higher or from high to lower

temperature (types) (Baskin and Baskin., 2004). The sensitivity of the seeds to light and gibberellic acid increases as non-deep physiological dormancy is progressively released (Cadman *et al.*, 2006).

A combination of the dormancy breaking system not only shifts the seed from dormancy to a quiescence state, but it also induces germination (Finch-Savage and Leubner-Metzger, 2006; Ali-Rachedi *et al.*, 2004). Much work has been done to break dormancy and improve germination through the use of various temperature regimes, light, growth regulators and inorganic salts (Van Rooyen and Theron, 2002; Corbineau *et al.*, 2002; Veassey and Texeira de Freitas, 2002).

2.4. Gibberellic acid application and dormancy release

Gibberellic acid plays a vital role in seed development as far as fertilization, embryo growth, assimilate uptake, fruit growth and prevention of seed abortion in *Solanaceae* and *Brassicaceae* species are concerned (Hays *et al*, 2002; Koornneef *et al.*, 2002). Absisic acid hormone has been suggested to be responsible for induction and maintenance of seed dormancy and gibberellins as responsible for seed germination (Debeaujon and Koornneef, 2000). It has been suggested that gibberellins regulate dormancy release and germination positively by a complex interaction with ABA and environmental conditions (Kucera *et al.*, 2005; Koornneef *et al.*, 2002; Leubner-Metzger, 2003b). Gibberellins also overcome germination constraints imposed by both seed coat and ABA-related embryo dormancy (Debeaujon and Koornneef, 2000).

Experiments on gibberellic acid and testa deficient mutants of *Arabidopsis* have shown that gibberellic acid is required to generate sufficient embryo growth potential (Debeaujon and Koornneef, 2000). Gibberellic acid also raptures the endosperm and

testa in the final stages of germination, though the influence of gibberellic acid on dormancy itself is not readily apparent (Nonogaki, 2006). In GA- deficient lines of model species in dormancy studies such as *Arabidopsis* and tomato, it has been observed that without the external GA application, seeds do not germinate. Germination rates and final percentages increased with higher external GA concentrations under decreasing osmotic potential (Kucera *et al.*, 2005; Ali-Rashedi *et al.*, 2004).

Although GA can stimulate germination of dormant seeds in some species, there are some scenarios where GA alone is ineffective. It has been suggested that GA is necessary but not sufficient for dormancy release (Finkelstein *et al.*, 2008).

2.5. Priming on seed germination, vigour and dormancy release

Priming is a controlled hydration process that involves exposing seeds to low water potential that restricts germination but permits pre-germinative physiological and biochemical changes to occur (Varier *et al.*, 2010; Varier and Vari, 2010). Primed seeds may exhibit faster rates of germination, more uniform emergence and alleviation of phytochrome-induced dormancy in some crops (McDonald, 2000). In vegetable crops and flower species, enhancement of germination was achieved due to seed priming (Suzuki and Khan, 2000), in tomato (Corbineau *et al.*, 1999), pepper (Lee *et al.*, 1997), as well as carrot, lettuce and onion (Jeong *et al.*, 2000b).

In muskmelon and tomato seeds, optimal priming treatment is required to achieve the best criteria in seed vigour (Olouch and Welbaum, 1996 a, b). While imbibition of tomato seeds in PEG results in sharp increases in adenosine triphosphate (ATP), energy charge and ATP/ADP (Adenosine Diphosphate) ratio (Corbineau *et al.*,

1999). Priming has been shown to improve longevity of low vigour seeds, but reduces that of high vigour seeds (Pandita and Nagarajan, 2000). In some crop species, priming has been shown to overcome dormancy (McDonald, 2000). In thermo-sensitive varieties of lettuce, germination is reduced or completely inhibited at high temperatures such as 35^{o}C, a condition that is overcome by osmo-priming seeds with P.E.G (-1.2Mpa) at 15^{o}C with constant light (Nascimento, 2004; Cantiffe *et al.*, 2000).

In some freshly harvested seeds of certain wild capsicum species, dormancy was shown to be reduced by treatment with 0.2M potassium nitrate solution under white light (750-1250 lux) and alternating temperatures (30/20^{o}C or 30/15^{o}C), (Hernandez-Verdugo *et al.*, 2001).

Seeds at different stages of maturity have shown significant response to pre-sowing treatments (Hae and Finnah, 2011).

Immature seeds that are not physiologically mature tend to show a greater response to priming than seed lots comprised of older seeds. This has led to the suggestion that priming can effectively improve the quality of seed lots harvested prematurely or that have not reached full physiological maturity (Welbaum and Bradford, 1991). Response of seeds to priming has been found to depend on the osmotica, priming temperature, seed maturity and crop type (Nascimento, 2003; Jeong *et al.*, 2000 c, d).

2.6. Temperature and light effects on germination and dormancy release

Dormant seeds demonstrate varied response or sensitivity to environmental factors that stimulate germination such as light, nitrate and the range of temperature over which germination can occur. Non-dormant seeds are more responsive to these

factors and germinate over a wide range of temperature (Hilhorst, 1995, 1997; Hilhorst and Toorop, 1997). It is widely accepted that temperature regulates both dormancy and germination and that light regulates germination though the role of light as a dormancy regulator is debatable (Baskin and Baskin, 2004; Fenner and Thompson, 2005; Kucera *et al.*, 2005). Temperature affects the germination capacity, the germination rate and frequency alongside the incubation time (Kocabas *et al.*, 1999). Temperature range permissive for germination widens as dormancy is released and narrows as dormancy is induced (Benech-Arnold *et al.*, 2000; Cadman *et al*, 2006; and Baskin and Baskin, 2004). Dormancy that's controlled by an inhibitor-promoter balance could be corrected by exposing dry seeds to higher temperatures or imbibing at low temperatures (Copeland and McDonald, 1995).

Seed species respond to the environment with optimal growth and development according to the light they receive (Maloof *et al.*, 2000). While some seeds exhibit similar germination patterns in light and darkness, while others germinate more readily either under light or darkness (Colbach *et al.*, 2002). It has been demonstrated that some seeds species need constant light to germinate and others can germinate either under light or darkness conditions but need temperature fluctuations. In other species stratifications or high temperatures replace light requirements for germination (Baskin and Baskin, 2004; Fenner and Thompson, 2005; and Kucera *et al.*, 2005). In *Cleome gynandra,* germination increased with temperature in darkness but also the interaction between light and temperature had a negative influence on germination especially at high temperature (Ochuodho and Modi, 2005). While light reacts with temperatures to break dormancy, it induces secondary dormancy at low temperature in some species (Groot and Karssen, 1992).

Dormancy is not just associated with the absence of germination; rather it is a characteristic of the seed that determines the conditions required for germination hence any environmental cue that alters the conditions required for germination is by definition altering dormancy (Finch-Savage and Leubner-Metzger, 2006). These factors are integrated overtime to alter the depth of dormancy and the sensitivity to other factors (Ali-Rachedi *et al.*, 2004; Cadman *et al.*, 2006).

Occurrence of maximum seed quality during seed development and maturation, and its association with seed and fruit features are important factors to define the ideal harvest time. The objective of this research was to harvest the seeds at their prime quality based on the fruit maturation stage, determined by the external fruit colour; to determine the cardinal germination temperatures and light requirement for optimum germination of *Capsicum frutescens* and improvement of germination by gibberellic acid application and seed priming procedures.

CHAPTER THREE

METHODOLOGY

3.1 Experiment 1: Establishing cardinal germination conditions.

Seeds samples of African Birds Eye chili (ABE) species were used for this experiment. Prior to germination assays, all seeds were surface sterilized by immersion in 1% sodium hypochlorite solution for five minutes and washed afterwards with distilled water.

Seeds were then arranged in Petri dishes (10cm diameter) prepared with a double layer of Whiteman filter paper placed on each Petri dish (Four replicates; 100 seeds per dish), 10ml of distilled water was added before application of germination regimes and henceforth all germination assays followed a completely randomized design with four replicates of 100 seeds for each treatment.

Germination Assays were conducted in germination chambers having fluorescents tubes of white light and light/ darkness timer. Seeds were subjected to constant germination temperatures of 15^{o}C, 20^{o}C, 25^{o}C and 35^{o}C under darkness regime of 24 hours and alternating temperature of 20/30^{o}C and 25/30^{o}C under darkness and light regimes of 24 hours.

Petri dishes were sampled daily and germination count was done over a fourteen days germination period, seeds were considered to have germinated when the radical was observed to have emerged. Percentage germination was determined in each case as per ISTA rules (ISTA, 2004).

3.2 Experiment 2: Germination of freshly harvested seeds from differently matured fruits of ABE chili varieties under predetermined germination temperature

Chili fruits were harvested from two sites; namely from Busia and Bungoma experimental sites. Fruits were harvested from two African Bird's Eye chili varieties; the red variety (where fruits mature from an initial green colour to red colour), and yellow variety (where fruits mature from an initial green colour to an intermediate yellow fruit colour and final red maturity colour). These are temporal experiment names as these varieties are in DUS tests under KEPHIS.

In each site and variety, the fruits in their respective maturity stages based on fruit colour were harvested from each individual plant and the total collections then formed the sample collection for that particular desired colour after which the collected fruit samples were dried under room temperature until the fruits were dried enough to facilitate seeds to be extracted.

Seeds that were extracted based on fruit colour from the two varieties were subjected to normal germination test carried out according to ISTA (2004). Four hundred seeds were counted at random from a well mixed pure seeds; four replicates of 100 seeds were obtained at random from the initial sample of 400 seeds and germinated in germination trays.

Germination was carried under improvised germination growth box under 24 hours light regime, provided by white fluorescent tubes at a temperature of 27^{o}C +/- 1.5^{o}C and under total darkness regime in the germination chamber at a temperature of 25^{o}C +/- 1.5^{o}C (as per the results based on 3.1). Germination counts were recorded on the

7^{th}, 14^{th}, and 21^{st} days of incubation respectively. Normal germination recording were taken and percentage germination evaluated as per ISTA rules (2004).

3.3 Experiment 3: Treatment of ABE chili with gibberellic acid concentrations and germination under light and temperature conditions

Gibberellic acid was prepared into three concentrations of 0.02%, 0.05% and 0.08% respectively and hence the concentrated solutions were used to treat the seeds that were extracted as per experiment 2. The germination substrates were moistened with the GA_3 concentrations prior to germination procedures. The treated seeds were subjected to germination under pre-determined germination conditions (experiment 3.1). Non treated seeds were used as a control in this experiment. Germination assays were conducted in a completely randomized design.

First count, normal percentage germination at 7^{th}, 14^{th}, and 21^{st} days of incubation period and the mean germination time were taken as germination parameters at the end of the germination period as per ISTA rules (2004).

3.4 Experiment 4: Seed priming of ABE chili germination under light and darkness conditions

Three PEG_{6000} solutions of osmotic potential of -0.2MPa, -0.6MPa and 1MPa and KNO_3 solutions of 0.2%, 0.5% and 0.8% respectively, were used to moisten the germination substrates after which the primed seeds were transferred to incubation temperatures of $15^{o}C$ for five days after which the primed seeds where transferred to germination chamber for normal germination to continue under pre- determined (experiment 3.1) optimum germination conditions.

Seeds were hydro-primed for 12 hours at 25^{o}C and then the seeds were transferred to the normal germination conditions that had been pre-determined (experiment 3.1) and hence acted as control to the primed seed samples.

First count, normal percentage germination at 7th, 14th, and 21st days of incubation period and the mean germination time were taken as germination parameters at the end of the germination period as per ISTA rules (2004).

3.5: Experiment 5: Changes in seed quality during fruit maturation process of ABE chili

Fifty ABE chili seedlings (*Capsicum frutescens*) of each of the two varieties were transplanted in 100 potting bags and grown to maturation in the green house.

Flowers were tagged at anthesis, and the fruits were harvested from 20 to 75 days after flowering (DAF) with step of 5 days. Then, harvested fruits were classified according to the maturation stage based on fruit colour on the outside, e.g. completely green (20 to 40 DAF), yellow (45 to 50 DAF), red (55 to 65 DAF) and intense red (70 to 75 DAF) for the yellow variety and immature green (20 to 40 DAF), mature green (45 to 50 DAF), red (55 to 65 DAF) and intense red (70 to 75 DAF) for the red variety.

Fruits were harvested and dried under room temperature until the seeds could be extracted. A thousand seed weight tests; standard germination tests, electrical conductivity tests, seed water content determination tests, and seed dry matter content determination tests according to ISTA rules (2004).

3.6: Data analysis

The data were subjected to analysis of variance (ANOVA) using Genstat Statistical programme Version 12.3 and separation of means was done by Contrast Comparison at 95% level of significance.

CHAPTER FOUR

RESULTS

4.1 Optimum germination conditions and dormancy release in abe chili seeds

4.1.1 Effects of constant temperature regimes on percent germination of *C. frutescens.*

Seed germination of ABE chili was very low despite being subjected to different temperature regimes though response to those temperature regimes was significant ($p \leq .001$, Appendix 1.1). As the germination temperature increased, so did the percentage germination (Figure1). The highest percentage germination of 57% was achieved when seeds were germinated at temperature regime of 25^{o}C. At the highest germination temperature of 30^{o}C and 35^{o}C, the seeds did not germinate.

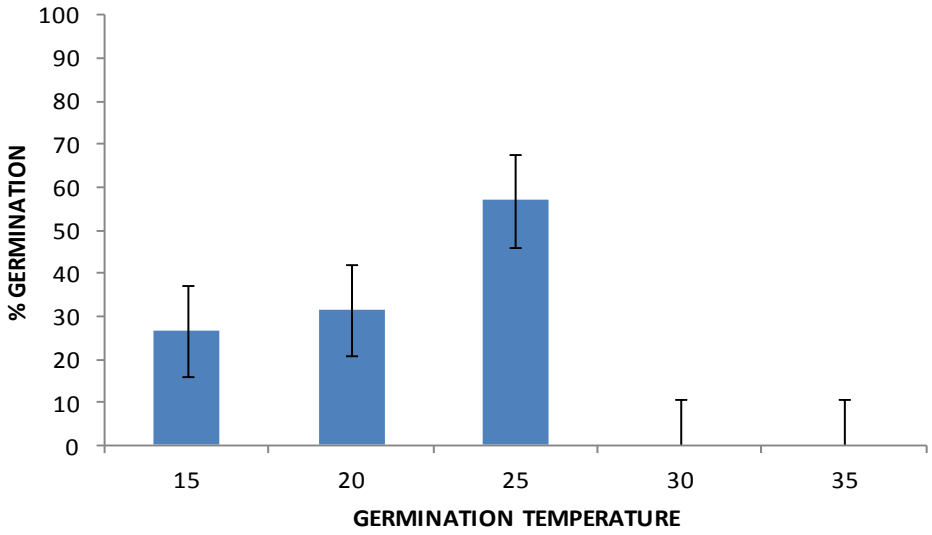

Figure 1: Effects of constant germination temperature regimes on percent germination of ABE chili in darkness. Error bars represent standard error.

4.1.2 Alternating temperature regimes and light conditions on percentage germination of ABE chili

Seeds that were germinated under alternating temperature regimes of 20/30^{o}C and 25/30^{o}C under continuous darkness and light regimes responded differently as far as germination percentage was concerned ($p \leq .001$, Appendix 1.1) (Figure2). Under alternating temperatures of 25/30^{o}C , the highest percentage germination of 50.9% was recorded when seeds were germinated in darkness as compared to those that were germinated in continuous light that had a germination percentage of 46.4%. Seeds that were germinated under alternating temperatures of 20/30^{o}C recorded high germination percentage of 50.6% under light conditions, compared to germination percentage of 41.6% under darkness. Germination percentages under alternating temperatures of 25/30^{o}C under continuous darkness was similar to 20^{o}C/30^{o}C under continuous light.

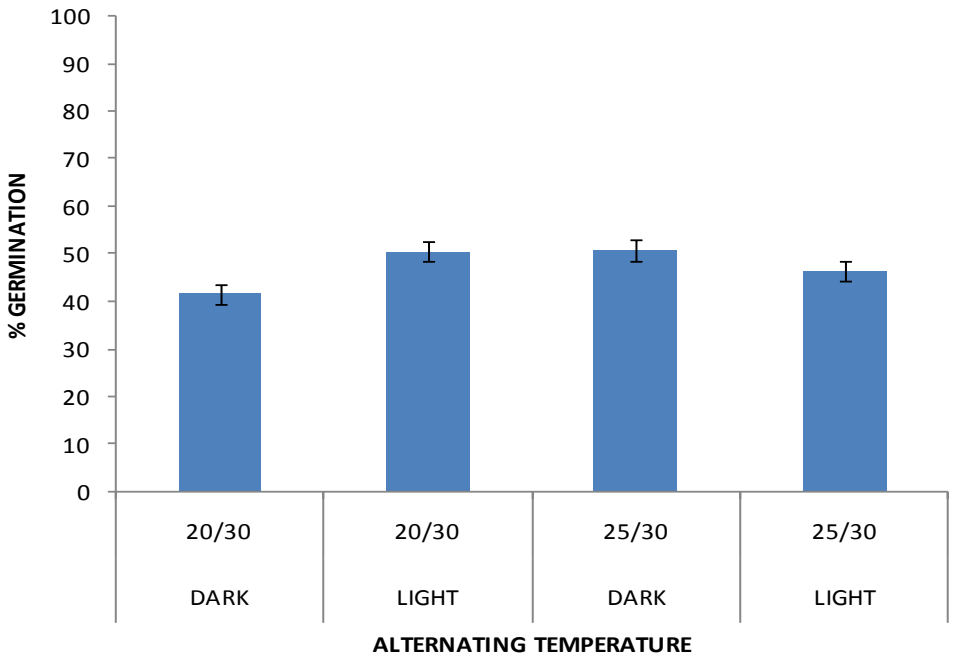

Figure 2: Effects of alternating temperature and light regimes on percent germination of ABE chili. Error bars represent standard error.

4.2.3 Harvesting stage and interaction with germination conditions; and incubation period on percentage germination and mean germination time of ABE chili

In both the yellow and red varieties of ABE chili, the stage of seed harvesting based on fruit colour significantly influenced germination ($p \leq .001$ (Appendix 1.2 and 1.3). Seeds from red fruits in both varieties had the highest mean percent germination as compared to seeds obtained from green and yellow fruits in case of the yellow variety; and green fruits in case of the red variety respectively (Table1.1 and 1.2). In the yellow cultivar, seeds from green and yellow fruits did not have significant difference as far as percentage germination was concerned (Contrast 3, Appendix 1.2), (Table1).

Table1: Fruit harvesting stage, germination conditions and incubation period on percent germination of yellow variety of ABE chili.

Germination conditions	Harvesting stage	Days			MEAN	GRAND MEAN
		7	14	21		
Light	Green	33.5	37.8	66.3	45.9	47.7
	Yellow	34.8	36.5	67	46.1	
	Red	33	44.5	75.8	51.1	
Dark	Green	44	60	75	53.7	59.9
	Yellow	42.8	61.8	74.8	59.8	
	Red	44.8	71	82.8	66.2	

	H. stage (HS)	Condition (C)	Days (D)	HS x C	HS x D	C x D	HS x D x C
F. probability	<.001	<.001	<.001	0.123	<.001	<.001	0.003
S.E	0.262	0.214	0.262	0.371	0.454	0.371	0.642
S.E.D	0.371	0.303	0.371	0.524	0.642	0.524	0.908
C.V (%)	2.3						

Table 2: Fruit harvesting stage, germination conditions and incubation period on percent germination of red variety of ABE chili.

Germination conditions	Harvesting stage	Days			MEAN	GRAND MEAN
		7	14	21		
Light	Green	32.8	42.8	66	47.2	50.5
	Red	35.8	49	76.3	53.7	
Dark	Green	44.8	67	74.8	62.2	65.9
	Red	45.5	72	91	69.5	

	H.Stage(HS)	Condition(C)	Days(D)	HSxC	HSxD	CxD	HSxDxC
F.probability	<.001	<.001	<.001	0.233	<.001	<.001	<.001
S.E	0.243	0.243	0.297	0.343	0.42	0.42	0.595
S.E.D	0.343	0.343	0.42	0.486	0.595	0.595	0.841
C.V (%)	2						

Germination conditions had significant effect ($p \leq .001$, Appendix 1.2 and $p \leq .001$, Appendix 1.3) on the overall percentage germination in both varieties, irrespective of fruit colour from which the seeds were obtained. Seeds that were germinated at $25^{o}C$ under darkness had overall higher germination percentage than seeds that were germinated under light for both varieties (Table 1 and 2). This observation was replicated as far as the interaction of seeds harvested at different fruit colour from both varieties and germination conditions was concerned. Seeds from red fruits in both varieties germinated under both light and darkness conditions had the highest percent germination.

In both varieties and irrespective of fruit colour, percent germination increased as germination days were extended. The mean germination percentage was highest on 21^{st} day as compared to the 7^{th} and 14^{th} day respectively ($p \leq .001$, Appendix 1.4) and ($p \leq .001$, Appendix 1.5). Though on day 7, seeds harvested at different fruit colours did not differ in percentage germination (Table 1and 2). In the yellow variety, seeds from red fruits had higher percentage germination in subsequent germination days, an

observation that was also noted in the red variety where seeds from red fruits exhibited higher germination percentages as germination days where extended up to the 21st day.

Seeds harvested from different fruit maturity stage from the yellow variety did not show significant difference in germination rate at the end of incubation period (p 0.003, Appendix 1.6 and Table 3). This outcome was also observed from seeds obtained from the red variety (p 0.002, Appendix 1.7 and Table 4). Germination conditions however, had a significant effect ($p \leq .001$, Appendix 1.6 and Table 3) and p 0.325, Appendix 1.7 and Table 4) on the rate of germination of both the yellow and red varieties. Seeds germinated under darkness had shorter germination time than those germinated under light.

Table 3: Fruit harvesting stage and germination conditions on mean germination time of yellow variety of ABE chili.

Harvesting Stage	Dark	Light	MEAN
Green	10.6	11.7	11.1
Red	10.4	11.5	11
Yellow	10.4	11.4	11
MEAN	10.5	11.6	11

	H.stage(HS)	Condition(C)	HS x C
F. probability	0.003	<.001	0.374
S.E	0.049	0.040	0.069
S.E.D	0.069	0.057	0.098
C.V (%)	2.2		

Table 4: Fruits harvesting stage and germination conditions effects on mean germination time of red variety of ABE chili.

Harvesting Stage	Dark	Light	MEAN
Green	11.1	11.4	11.2
Red	11.1	10.9	11.0
MEAN	11.1	11.2	11.1

	H.Stage(HS)	Condition (C)	HSxC
F.probability	0.002	0.325	0.003
S.E	0.04	0.04	0.06
S.E.D	0.06	0.06	0.08
C.V(%)	1.8		

4.2 Gibberellic acid, dormancy release and improved germination in ABE chili.

4.2.1: Seed maturity stages and interaction with GA_3 on percent germination of ABE chili varieties under different germination conditions

Gibberellic acid had significant effect ($p \leq .001$, Appendix 2.1 and $p \leq .001$, Appendix 2.2) on germination of ABE chili irrespective of the stage of seed maturity of both varieties. In the yellow variety, seeds obtained from red fruits had clear germination difference from seeds obtained from green and yellow fruits (contrast 1, contrast 2 and contrast 3, Appendix 2.1)). Seeds from green and yellow fruits did not differ significantly in germination behaviour (Table 5). This observation was also recorded for the red variety where germination of seeds from red fruits was higher than those obtained from green fruits (Table 6).In both varieties seeds treated by gibberellic acid concentrations had better germination percentages than control.

4.2.2: Germination conditions and interaction with GA_3 and seed maturity stages on percent germination of ABE chili.

Germination conditions and interaction with GA influenced significantly ($p \leq .001$, Appendix 2.3 and $p \leq .001$, Appendix 2.2) the percent germination of seeds from the differently matured fruits in both varieties. Temperature interaction with all levels of GA_3 concentrations had more profound effects on percentage germination irrespective of seed maturity stages than germination under light in both varieties (Table 5 and 6). This is also observed among the control treatments across the different stages of seed maturity from the different fruit maturity stages in both varieties.

Table 5: Effects of fruit harvesting stage, germination conditions, incubation period and interaction with GA_3 concentrations' effect on percent germination of yellow variety of ABE chili.

Harvesting Stage	GA Treatment	Condition	Day 7	Day 14	Day 21	Mean	General mean	Grand Mean
Green	Control	Dark	44.0	60.0	75.0	59.7	52.7	52.7
		Light	33.5	37.8	66.3	45.8		
	0.02	Dark	64.5	65.3	89.8	73.2	69.3	72.2
		Light	62.8	58.5	75.3	65.5		
	0.05	Dark	66.5	64.5	92.3	74.4	72.8	
		Light	64.8	64.8	84.0	71.2		
	0.08	Dark	67.0	67.0	93.0	75.7	74.5	
		Light	67.0	66.5	86.8	73.4		
Yellow	Control	Dark	42.8	61.8	74.8	59.8	52.9	52.9
		Light	34.8	36.5	67.0	46.1		
	0.02	Dark	63.3	64.3	90.0	72.5	68.3	71.7
		Light	58.3	60.5	73.8	64.2		
	0.05	Dark	67.0	63.8	91.8	74.2	72.6	
		Light	65.0	62.3	85.8	71.0		
	0.08	Dark	66.0	67.3	92.0	75.1	74.1	
		Light	66.3	65.8	87.3	73.1		
Red	Control	Dark	44.8	71.0	82.8	66.2	58.6	58.6
		Light	33.0	44.5	75.8	51.1		
	0.02	Dark	63.5	75.5	90.0	76.3	75.0	77.6
		Light	58.0	70.8	92.0	73.6		
	0.05	Dark	67.3	76.5	92.5	78.8	78.5	
		Light	66.0	76.0	93.0	78.3		
	0.08	Dark	66.3	77.5	96.3	80.0	79.3	
		Light	68.0	75.8	92.3	78.7		

	H.Stage(H.S)	Treatment (T)	Condition (C)	Days (D)	H.SxT	H.SxC	TxC	H.SxD
F.probability	<.001	<.001	<.001	<.001	0.342	<.001	<.001	<.001
S.E	0.172	0.199	0.141	0.172	0.344	0.244	0.281	0.298
S.E.D	0.244	0.281	0.199	0.244	0.487	0.344	0.398	0.422
C.V (%)	2.4							

	TxD	CxD	H.SxTxC	H.SxTxD	H.SxCxD	TxCxD	H.SxTxCxD
F.probability	<.001	<.001	<.001	<.001	<.001	<.001	<.001
S.E	0.344	0.244	0.487	0.597	0.422	0.487	0.844
S.E.D	0.487	0.344	0.689	0.844	0.597	0.689	1.193
C.V (%)							

Table 6: Fruit maturity stage, germination conditions, incubation period and interaction with GA_3 concentrations' effect on percent germination of red variety of ABE chili.

Harvesting Stage	GA Treatment	Condition	Day 7	Day 14	Day 21	Mean	General mean	Grand Mean
Green	Control	Dark	44.0	60.0	75.0	59.7	52.7	52.7
		Light	33.5	37.8	66.3	45.8		
	0.02	Dark	64.5	65.3	89.8	73.2	69.3	72.2
		Light	62.8	58.5	75.3	65.5		
	0.05	Dark	66.5	64.5	92.3	74.4	72.8	
		Light	64.8	64.8	84.0	71.2		
	0.08	Dark	67.0	67.0	93.0	75.7	74.5	
		Light	67.0	66.5	86.8	73.4		
Yellow	Control	Dark	42.8	61.8	74.8	59.8	52.9	52.9
		Light	34.8	36.5	67.0	46.1		
	0.02	Dark	63.3	64.3	90.0	72.5	68.3	71.7
		Light	58.3	60.5	73.8	64.2		
	0.05	Dark	67.0	63.8	91.8	74.2	72.6	
		Light	65.0	62.3	85.8	71.0		
	0.08	Dark	66.0	67.3	92.0	75.1	74.1	
		Light	66.3	65.8	87.3	73.1		
Red	Control	Dark	44.8	71.0	82.8	66.2	58.6	58.6
		Light	33.0	44.5	75.8	51.1		
	0.02	Dark	63.5	75.5	90.0	76.3	75.0	77.6
		Light	58.0	70.8	92.0	73.6		
	0.05	Dark	67.3	76.5	92.5	78.8	78.5	
		Light	66.0	76.0	93.0	78.3		
	0.08	Dark	66.3	77.5	96.3	80.0	79.3	
		Light	68.0	75.8	92.3	78.7		

	H.Stage(H.S)	Treatment (T)	Condition (C)	Days (D)	H.SxT	H.SxC	TxC	H.SxD
F.probability	<.001	<.001	<.001	<.001	0.342	<.001	<.001	<.001
S.E	0.172	0.199	0.141	0.172	0.344	0.244	0.281	0.298
S.E.D	0.244	0.281	0.199	0.244	0.487	0.344	0.398	0.422
C.V (%)	2.4							

	TxD	CxD	H.SxTxC	H.SxTxD	H.SxCxD	TxCxD	H.SxTxCxD
F.probability	<.001	<.001	<.001	<.001	<.001	<.001	<.001
S.E	0.344	0.244	0.487	0.597	0.422	0.487	0.844
S.E.D	0.487	0.344	0.689	0.844	0.597	0.689	1.193
C.V (%)							

4.2.3 GA_3 concentrations, interaction with germination conditions and incubation period on germination percentage of differently matured seeds of ABE chili varieties.

GA_3 concentrations had significant effect ($p \leq .001$, Appendix 2.3 and $p \leq .001$, Appendix 2.2) on the final germination percentage as compared to control. As the concentration of GA_3 was increased, so did the increase in percent germination of ABE chili. This was observed in both varieties and across all the seeds extracted from differently matured fruits for red variety respectively.

Interaction of different GA concentrations with differently matured seeds had no significant effect (p 0.325, Appendix2.3 and p 0.002, Appendix 2.2) on the percent germination especially within a particular concentration. Though the observation was the case, seeds from red fruits in both varieties still recorded the highest percent germination across the three levels of concentrations when compared with the control (Table 5 and 6).

In both varieties and irrespective of the stage of seed maturity, percent germination was highly responsive to the interaction of GA_3 concentrations applied and the germination conditions. Temperature and GA_3 interaction had more pronounced effect than GA_3 and light interaction within and without the GA_3 concentration levels and seed maturity levels (Appendix 2.4 and Appendix 2.5). Seeds extracted from red fruits in both varieties had the highest mean germination irrespective of the treatments interactions (Table 5 and 6).

Percentage germination was observed to increase as the germination days were increased with maximum germination percentage being recorded at the end of the germination period (21 days) (Appendix 2.3 and 2.2). The influence of the interactions of different treatment regimes as earlier mentioned remained the same in both varieties (Table 5 and 6).

4.2.4 Gibberellic acid application and interaction with seed maturity, germination conditions on mean germination time of ABE chili varieties.

In both capsicum varieties, external GA_3 application significantly affected the Mean Germination Time ($p \leq .001$, Appendix 2.4 and 2.5). The mean germination time decreased as the GA_3 concentration was increased irrespective of the stage of seed

maturity in both varieties (Table 7 and 8). In the yellow variety, the response to GA_3 application to differently matured seeds did not differ significantly as far as mean germination time was concerned (Table 7). In the red variety, seeds from red fruits differed significantly from seeds from green fruits as far as GA_3 concentrations was concerned and effects of interactions put into consideration (Table 8).

Germination conditions with different concentrations of GA_3 applied significantly contributed to seeds germinating faster, with effects of temperature interaction with GA_3 being greater than the interaction of GA and light (Table 7 and 8).

Table 7: Fruit maturity stage and interaction with gibberellic acid concentration and germination conditions on mean germination time of yellow variety of ABE chili.

Harvesting stage	GA treatment	Germination conditions		Mean	Grand Mean
		Dark	Light		
Green	Control	10.63	11.72	11.2	11.2
	0.02	9.48	10.38	9.9	9.6
	0.05	9.43	9.55	9.5	
	0.08	9.18	9.39	9.3	
Yellow	Control	9.57	11.39	10.5	10.5
	0.02	9.57	9.54	9.6	9.6
	0.05	9.55	9.72	9.6	
	0.08	9.64	9.67	9.7	
Red	Control	10.39	11.53	11	11
	0.02	9.68	10.55	10.1	9.7
	0.05	9.52	9.79	9.7	
	0.08	9.09	9.29	9.2	

	H.Stage(H.S)	Condition(C)	Treatment(T)	H.SxC	H.SxT	CxT
F.probability	0.002	<.001	<.001	0.842	1	0.014
S.E	1.30	1.06	1.51	1.84	2.61	2.13
S.E.D	1.84	1.51	2.13	2.61	3.69	3.01
C.V(%)	2.40					

Table 8: Fruit maturity stage and interaction with gibberellic acid concentration and germination conditions on mean germination time of red variety of ABE chili.

Harvesting stage	GA Treatment	Germination conditions		Mean	Grand Mean
		Dark	Light		
Green	Control	10.5	11.2	10.9	10.9
	0.02	10.6	10.0	10.3	9.8
	0.05	9.2	10.2	9.7	
	0.08	9.2	9.5	9.4	
Red	Control	9.3	11.2	10.3	10.3
	0.02	9.9	9.8	9.8	9.5
	0.05	9.5	9.7	9.6	
	0.08	8.8	9.6	9.2	

	H.Stage(H.S)	Condition(C)	H.SxT	H.SxC	TxC	H.SxTxC
F.probability	<.001	<.001	<.001	<.001	<.001	<.001
S.E	0.030	0.030	0.059	0.042	0.059	0.084
S.E.D	0.042	0.042	0.084	0.059	0.084	0.119
C.V(%)	2.900					

4.3: Priming treatment interaction with germination conditions and dormancy alleviation in ABE chili

4.3.1 Priming and interaction with seed maturity stages, germination conditions on percent germination of ABE chili varieties.

Seed maturity had significant effect ($p \leq .001$, Appendix 3.1 and 3.2) on germination of both ABE chili. In the yellow variety, seeds from red fruits showed clear germination percentage difference from seeds that were extracted from green and yellow fruits. Seeds from the green and yellow fruits did not show significant difference in terms of germination percentage (p 0.01, Appendix 3.1 and Table 9). The same observation was recorded in red variety where seeds from red fruit had the highest percentage germination than seeds extracted from green fruits ($p \leq .001$, Appendix 3.2 and Table 10). These results were observed irrespective of the priming treatment, incubation period or germination conditions.

In both varieties, germination conditions had a significant effect ($p \leq .001$, Appendix 3.1 and 3.2) on germination percentage at the end of germination period. Temperature had more influence on germination percentage than light. Though the observation was the case, the percentage germination difference margin was small within seeds sampled from the same fruit colour (Table 1.9 and 2). When germination conditions interacted with seed maturity based, the effects on percent germination was not significant (p 0.265, Appendix 3.1 and p 0.004, Appendix 3.2) in both varieties. This was more evidently shown in the yellow variety where seeds from green and yellow fruits recorded almost the same percentage germination (Table 9).

4.3.2 Priming concentrations and interactive influence on germination of differently matured ABE chili seeds.

Priming as compared to control had significant effect ($p \leq .001$, Appendix 3.2 and 3.3) on the percentage germination of both varieties. This was observed irrespective of the type of priming treatment and concentration. Seed priming with potassium nitrate had the highest percentage germination irrespective of the concentration, interaction with levels of seed maturity or the influence of germination conditions (Table 9 and 10). Priming with PEG_{6000} also performed better than hydro-primed seeds that were used as control for the experiment.

Within a particular priming treatment in both varieties, germination percentage was influenced by the concentration of priming chemical (Table 9 and 10). Seeds primed with potassium nitrate at 0.5% gave the overall mean highest percentage germination. This was irrespective of interaction with seed maturity stage and germination conditions. When the concentration was increased to 0.8%, the percentage germination was reduced as compared to the concentration at 0.5% with other factors

remaining constant for both varieties (Table 9 and 10). The same outcome is observed in seeds treated with PEG_{6000} whereby the highest germination was recorded in PEG_{6000} concentration of -0.6 MPa as compared to concentrations of -0.2 MPa and -1.0 MPa respectively, all other factors remaining the same. Though the different concentrations of potassium nitrate and poly ethyl glycol differed on seed germination percentage outcome, they performed better than control.

4.3.3 Germination response to priming treatment and germination days of differently matured ABE chili seed

In both varieties, germination increased as the germination period was extended (Table 9 and 10). Though in all treatment interactions, priming significantly influenced the final percentage germination as compared to the control. There was no difference in the first count in seed sampled within a particular priming concentration and type. Though this was the case, germination percentage was influenced by the type of priming, germination conditions, priming concentration within a priming treatment, seed maturity, and the interaction of the mentioned factors (Table 9 and 10).

Table 10: Seed maturity and interaction with priming treatments, concentrations, and germination conditions on percent germination of red variety of ABE chili.

Harvesting Stage	GerminationCondition	Priming treatment	Day 7	Day 14	Day 21	Mean	General Mean
Green	Dark	Control	34.8	51.3	82.0	56.0	56.0
		KNO3 (0.2)	53.5	83.3	97.5	78.1	79.7
		KNO3 (0.5)	58.3	87.5	98.0	81.3	
		KNO3 (0.8)	55.5	87.0	97.0	79.8	
		PEG (0.2)	51.3	54.5	80.3	62.0	66.2
		PEG (0.6)	58.5	65.0	90.8	71.4	
		PEG (1.0)	52.8	57.0	85.5	65.1	
	Light	Control	23.5	53.0	80.8	52.4	52.4
		KNO3 (0.2)	45.0	71.8	96.8	71.2	76.0
		KNO3(0.5)	61.5	76.5	97.3	78.4	
		KNO3(0.8)	51.8	86.5	97.3	78.5	
		PEG (0.2)	20.8	44.8	76.3	47.3	60.8
		PEG (0.6)	56.0	65.0	87.0	69.3	
		PEG (1.0)	52.5	57.3	87.5	65.8	
Red	Dark	Control	35.5	53.3	91.0	59.9	59.9
		KNO3(0.2)	61.5	88.3	98.0	82.6	83.7
		KNO3 (0.5)	69.8	88.5	97.8	85.3	
		KNO3(0.8)	63.0	88.5	98.3	83.3	
		PEG (0.2)	55.3	67.5	86.5	69.8	72.4
		PEG (0.6)	63.8	71.3	97.5	77.5	
		PEG (1.0)	57.8	63.0	89.5	70.1	
	Light	Control	22.8	56.8	90.3	56.6	56.6
		KNO3 (0.2)	56.3	77.5	97.0	76.9	80.6
		KNO3(0.5)	71.3	79.8	98.0	83.0	
		KNO3(0.8)	61.0	86.8	98.3	82.0	
		PEG (0.2)	26.8	58.5	89.8	58.3	68.5
		PEG (0.6)	64.8	73.0	92.5	76.8	
		PEG (1.0)	55.0	65.5	90.5	70.3	

	H.Stage(H.S)	Priming(P)	Days(D)	Condition(C)	H.SxP	H.SxD	PxD	H.SxC
F.probability	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.004
S.E	0.109	0.203	0.133	0.109	0.288	0.188	0.352	0.154
S.E.D	0.154	0.288	0.188	0.154	0.407	0.266	0.498	0.218
C.V (%)	2							

	PxC	DxC	H.SxPxD	H.SxPxC	H.SxDxC	PxDxC	H.SxPxDxC
F.probability	<.001	<.001	<0.001	0.034	<.001	<.001	<.001
S.E	0.288	0.188	0.498	0.705	0.266	0.498	0.705
S.E.D	0.407	0.266	0.705	0.575	0.377	0.705	0.997
C.V (%)							

3.4 Priming types, concentrations and interaction with seed maturity, and germination conditions on mean germination time of ABE chili

Priming had a significant influence on mean germination time in both varieties ($p \leq .001$, Appendix 3.4 and 3.5) with primed seeds having lower mean germination time than unprimed seeds (Table 11 and 12). The effects of priming on mean germination time was influenced by the stage of seed maturity based on the fruit harvesting stage in both varieties (Appendix 3.4 and 3.5). In yellow variety, seeds from red fruits had the lowest mean germination time than seeds from yellow and green fruits. The germination time difference between seeds from yellow and red was marginal (Table 11). In the red variety, there was observed clear difference in mean germination time between seeds from green fruits and seeds from red fruits (Table 12).

Table 11: Seed maturity stage and interaction with priming types, concentrations and germination condition on mean germination time of yellow variety of ABE chili.

Harvesting stage	Priming treatment	Germination conditions		Mean	Grand mean
		Dark	Light		
Green	control	11.6	11.7	11.7	11.7
	KNO3(0.2)	10.2	10	10.1	9.7
	KNO3(0.5)	9.1	9.1	9.1	
	KNO3(0.8)	9.7	10.1	9.9	
	PEG(0.2)	10.7	11.5	11.1	10.8
	PEG(0.6)	10	10	10	
	PEG(1.0)	11.2	11.4	11.3	
Yellow	control	11.6	11.7	11.7	11.7
	KNO3(0.2)	9.7	9.4	9.6	9.5
	KNO3(0.5)	8.8	9	8.9	
	KNO3(0.8)	9.5	10.3	9.9	
	PEG(0.2)	10.6	11.6	11.1	10.8
	PEG(0.6)	10.3	9.8	10.1	
	PEG(1.0)	10.9	11.6	11.3	
Red	control	11.4	11.5	11.5	11.5
	KNO3(0.2)	9.5	9.7	9.6	9.2
	KNO3(0.5)	8.4	8.7	8.6	
	KNO3(0.8)	9.3	9.5	9.4	
	PEG(0.2)	10.3	11.4	10.9	10.4
	PEG(0.6)	9.9	9.5	9.7	
	PEG(1.0)	10.5	10.8	10.7	

	H.Stage(H.S)	Condition(C)	Priming(P)	H.SxC	H.SxP	CxP	H.SxCxP
F.probability	<.001	<.001	<.001	0.279	<.001	<.001	<.001
S.E	0.018	0.014	0.027	0.025	0.047	0.038	0.066
S.E.D	0.025	0.020	0.038	0.035	0.066	0.054	0.093
C.V(%)	2.2						

Table 12: Seed maturity stage and interaction with priming types, concentrations and germination conditions mean germination time of red variety of ABE chili.

Harvesting Stage	Priming treatment	Germination conditions		Mean	Grand Mean
		Light	Dark		
Green	Control	11.4	11.1	11.2	11.2
	KNO3 (0.2)	10.5	9.8	10.2	
	KNO3 (0.5)	9.8	8.8	9.3	9.9
	KNO3 (0.8)	10.1	10.1	10.1	
	PEG (0.2)	13.1	10.7	11.9	
	PEG (0.6)	9.9	10.0	10.0	11.0
	PEG (1.0)	11.2	11.0	11.1	
Red	Control	10.9	11.1	11.0	11.0
	KNO3(0.2)	9.4	9.1	9.2	
	KNO3(0.5)	8.6	8.2	8.4	8.8
	KNO3(0.8)	9.2	8.3	8.8	
	PEG (0.2)	10.7	9.9	10.3	
	PEG (0.6)	9.5	9.9	9.7	10.2
	PEG (1.0)	10.6	10.5	10.5	

	H.Stage(H.S)	Priming(P)	Condition(C)	H.SxP	H.SxC	PxC	H.SxPxC
F.probability	<.001	<.001	<.001	<.001	<.001	<.001	<.001
S.E	0.017	0.032	0.017	0.045	0.024	0.045	0.064
S.E.D	0.024	0.045	0.024	0.064	0.034	0.064	0.091
C.V(%)	2.2						

Potassium nitrate was the best priming material in terms of mean germination time followed by poly ethyl glycol, both performing better than seeds that were hydro primed (Table 11 and 12). Where priming type was concerned, priming concentration also influenced the mean germination time irrespective of the seed maturity stage and germination conditions. In seeds that were primed using potassium nitrate, concentration of 0.5% gave the least mean germination time while those primed with poly ethyl glycol, the concentration of -0.6 MPa gave the least mean germination time. In both types of priming used, all the concentrations used performed better than those seeds that were hydro primed (Table 11 and 12).

It was evident that interaction of priming types and concentration when interacted with germination conditions had significant effect ($p \leq .001$, Appendix 3.4 and 3.5) on mean germination time with temperature performing better than light. Though germination conditions influenced mean germination time especially within seeds harvested from same fruit colour, the differences were noted between different

priming types as compared to the control. Within the same concentration in a particular priming type, the difference in mean germination time is marginal in both varieties (Table 11 and 12).

4.4 Seed quality changes during fruit maturation process of abe chili varieties.

4.4.1 Seed dry weight, moisture content and one thousand seed weight during the maturation process of ABE chili fruit maturation.

During the maturation process of ABE chili varieties, significant changes occurred in dry matter, one thousand seed weight and moisture content parameters were concerned. Dry matter content of the seeds for both ABE chili varieties increased in percentage per mega gram of seed as the fruit age increased from the day of flowering. From 20 DAF when the fruits in both varieties were immature green in colour, the dry matter content increased gradually reaching optimum weight at approximately 65 DAF, when the fruits were at least 90% red in colour (Figure 3).

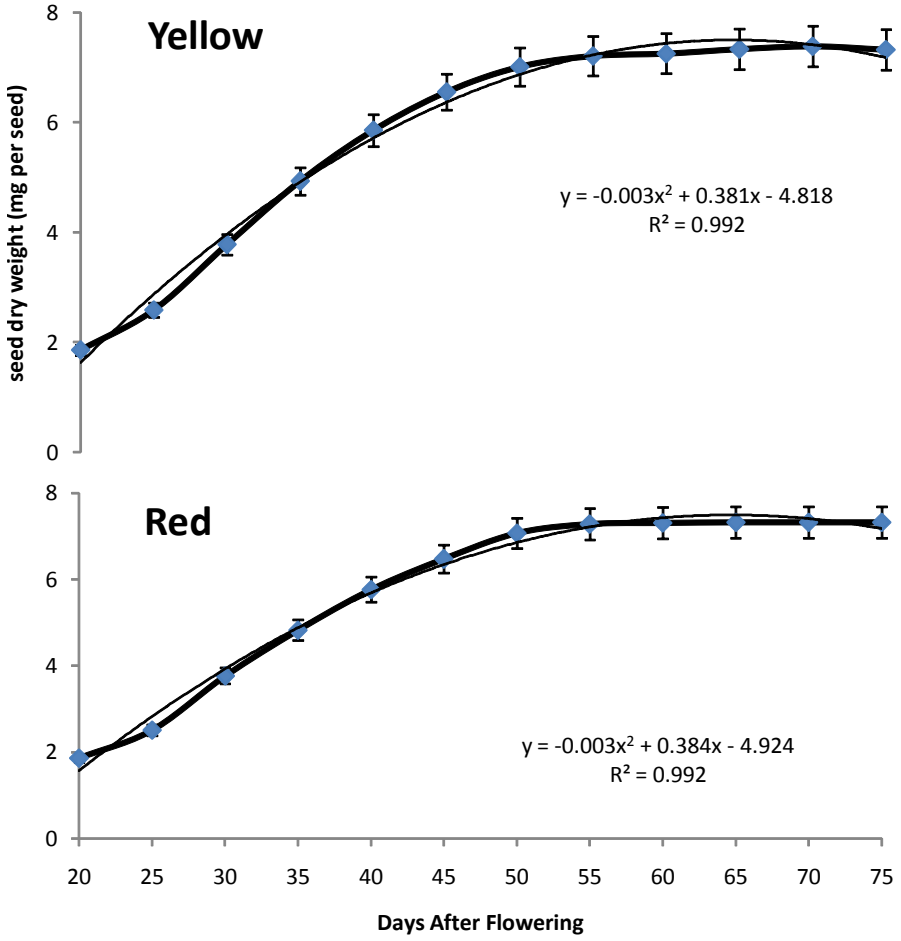

Figure 3: Changes in seed dry weight during fruit maturation process of yellow and red varieties of ABE chili.

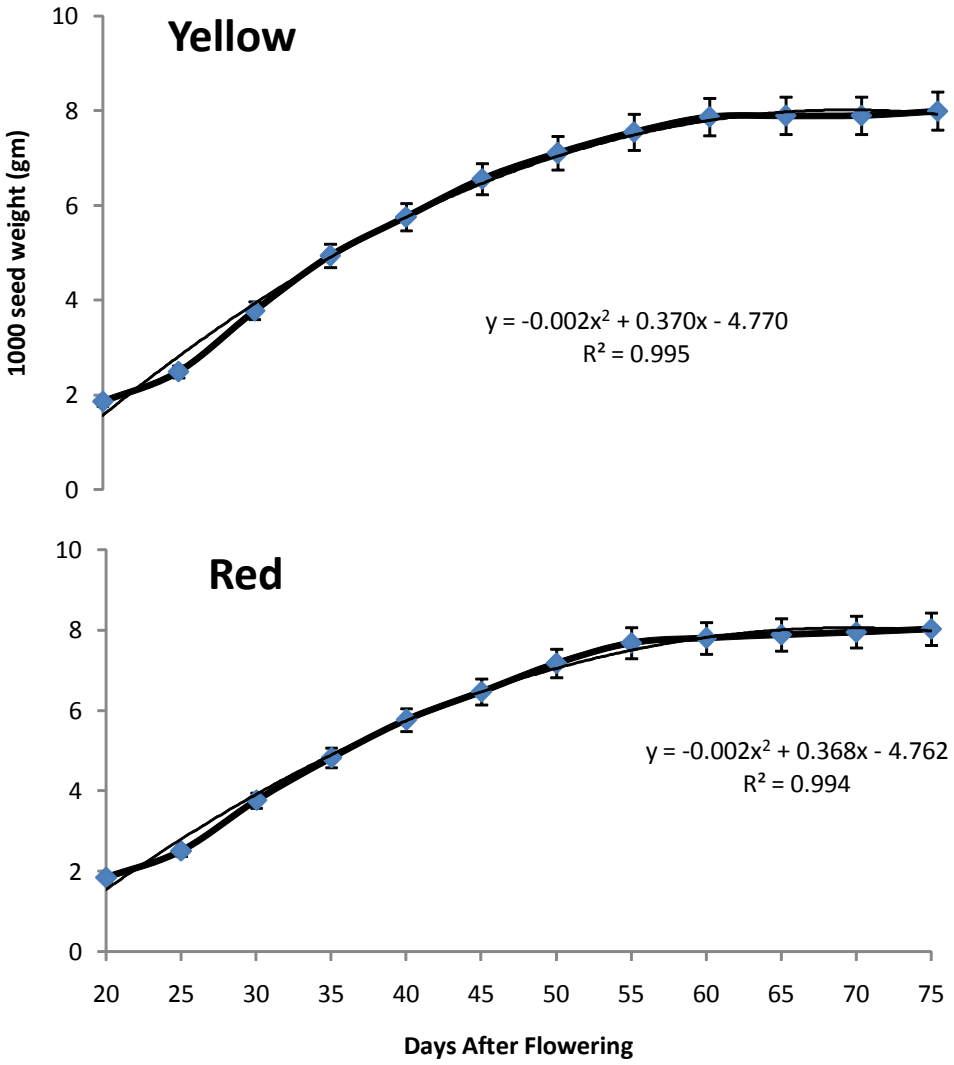

Figure 4: Changes in 1000 seed weight during fruit maturation process of yellow and red varieties of ABE chili.

From 50 DAF, the increase in dry matter content was gradual and reached the maximum at 65 DAF, when the fruits were approximately 90% red in colour. Thousand seed weight also increased with fruit maturity period, reaching maximum values at 65 DAF for both ABE chili varieties (Figure 4).

It is noted from the figures (Figure 3 and 4), that maximum dry matter content and one thousand seed weight for both varieties were attained simultaneously.

For both varieties, when the fruits were immature green in colour at approximately 20 DAF, the seed moisture content were 91.8% for the yellow variety and 92.4% for the red variety respectively (Figure 5).

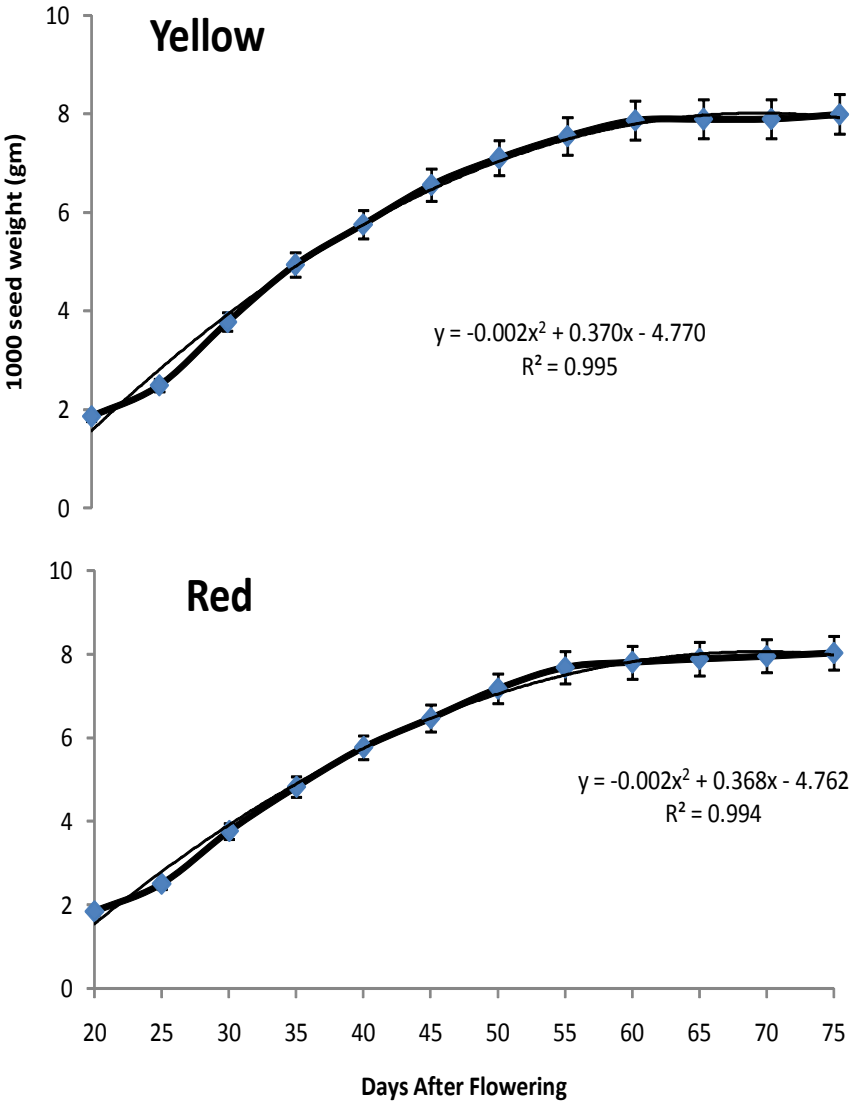

Figure 5: Changes in seed water content during fruit maturation process of Yellow and Red varieties of ABE chili.

Along the maturation process, the water content decreased to 49.1% for the yellow variety and 49.4% for the red variety, at approximately 55 DAF when the fruits were red attaining moisture content of 47.3% for the yellow variety and 47.34% for the red

variety respectively at 75 DAF. During the steady decline in water content, the dry matter continued to accumulate while moisture content was still high even after maximum dry matter had been attained.

4.4.2 Seed vigour changes during fruit maturation of ABE chili varieties.

Seeds harvested from immature green fruits in both the yellow and red varieties between 20 DAF to 40 DAF did not germinate. There was an increase in seed germination between 45 DAF to 65 DAF, which coincided with an increasing dry matter content of the seeds along maturation process, hence suggesting that the seeds had attained physiological maturity (Figure 6).

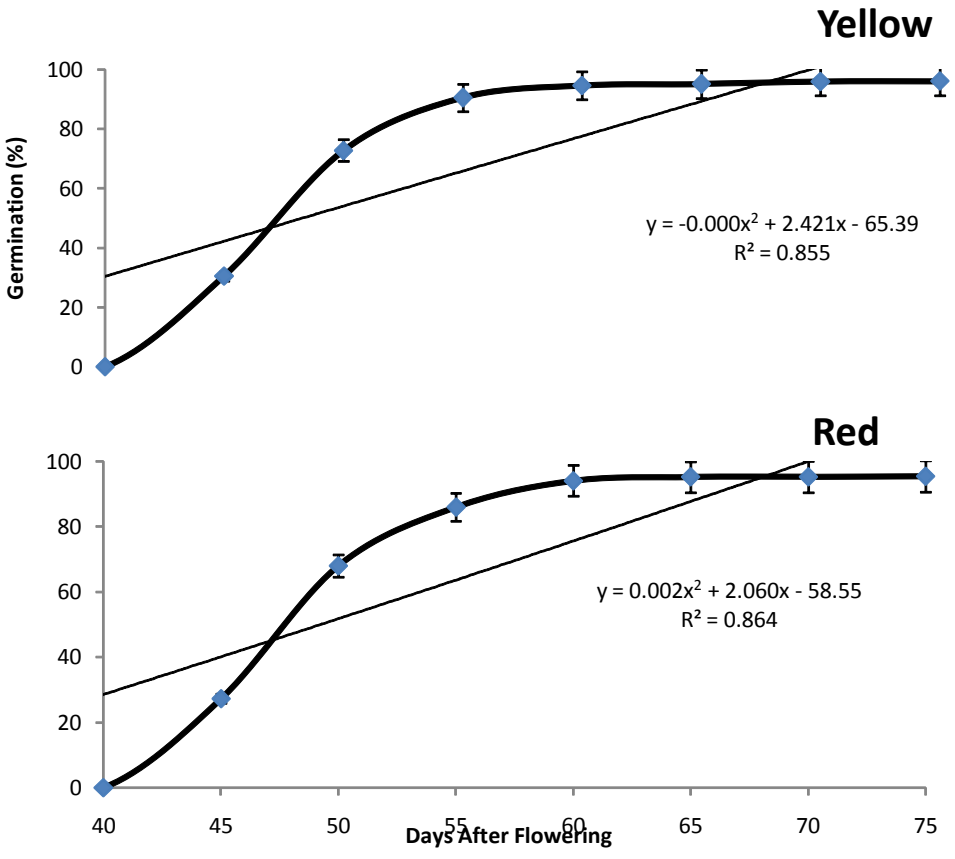

Figure 6: Changes in seed germination percentage during fruit maturation process of Yellow and Red varieties of ABE chili

Seed germination increased slightly up to the end of the maturation process at 75 DAF when the fruits where completely red in both varieties. The maximum dry matter content of the seeds in both varieties had been attained at between 60 to 65 DAF and leveling off towards 75DAF (Figure 3 and 6).

Seed vigour increased along fruit maturation process. The maximum seed vigour was recorded at 60-65 DAF for both varieties, with yellow variety recording a germination percentage of 95.1% and the red variety recording a germination percentage of 94.15% (Figure 6), when the fruits were red. High seed germination percentage was attained from 55 DAF increasing slowly until 60 to 65DAF where it levels off.

The highest electrical conductivity value was recorded at 20 DAF, when the fruits were immature green in both varieties. The electrical conductivity readings then started to decline gradually and stabilized at between 60 to 65 DAF (Figure 7).

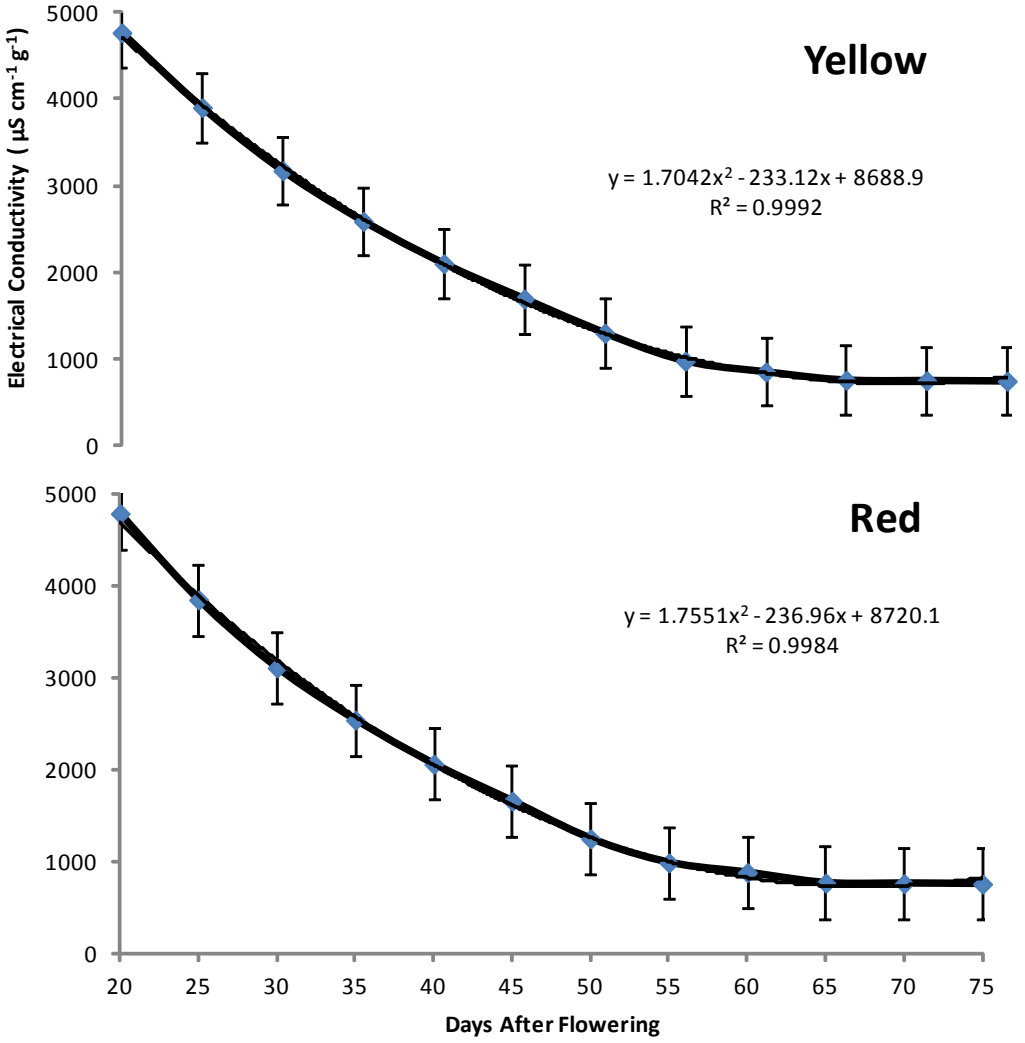

Figure 7: Changes in seed electrical conductivity during fruit maturation process of yellow and red varieties of ABE chili.

CHAPTER FIVE

DISCUSSIONS

5.1 Reaction of *Capsicum frutescens* to different temperature regimes.

ABE chili seeds exhibited increased percent germination as the temperature range for germination was increased during the germination period. The observed germination percentage differences might have been due to the dormancy depth of the seed species which prohibited germination at low temperature but germination increased as the temperature was increased (Baskin and Baskin, 2004; Cadman *et al.*, 2006). The applied temperature might have regulated the dormancy depth by affecting the hormonal balance of GA and ABA biosynthesis and catabolism which in turn determines the dominant hormone (Ali-Rachedi *et al.*, 2004; Cadman *et al.*, 2006). The lowest germination percentage was observed at 15 ^{o} C which was the lowest temperature at which the seeds were able to germinate while the highest germination percentage was observed at 25 ^{o} C, described as the optimum germination temperature for ABE chili (Alvarado and Bradford, 2002).

This phenomenon has also been observed in other crop species whereas seeds pass out of dormancy, three patterns of changes in temperatures requirements for germination can be distinguished (Baskin and Baskin, 2004). Seeds of some species germinate first at low temperatures, but with additional loss of dormancy the maximum temperature increases i.e. type1, e.g., *Arabidopsis thaliana* (Baskin and Baskin, 2004; Cadman *et al.*, 2006). Other species first gain the ability to germinate at high temperatures, but with additional loss of dormancy, the minimum temperatures for germination increase i.e. type2, e.g., *Helianthus annuus* (Finch-Savage and Leubner-Metzeger, 2006). In the third group of species, seeds start to germinate first at

intermediate temperatures, but with additional loss of dormancy they germinate at higher and lower temperatures e.g., *Aster ptarmacoides* (Baskin *et al.*, 2005).

Seeds that were subjected to alternating temperatures exhibited different germination behaviour. At high alternating temperatures of 25/30^{o}C, high germination percentage (50.9%) was observed in absence of light whereas alternating temperature of 20/30^{o}C, high percent germination (50.6%) was observed in presence of light. These differences of light influence on seed germination have been observed in other crop species which have shown greater germination in light than in darkness or vice versa (Milberg *et al.*, 2000). Red light has been shown to regulate the biosynthesis of bioactive GA in germinating seeds of *Arabidopsis* (Yamaguchi *et al.*, 2001), with the effectiveness of light in dormancy release being noted towards the end of the incubation period, when the degree of dormancy was sufficiently low (Derkx and Karssen, 1993). In photo dormant tobacco seeds, germination is blocked at a step before testa rupture. Neither testa nor endosperm rupture occur, even after several weeks of dark- imbibition (Leubner-Metzger *et al.*, 1996). Brief treatment of imbibed photo dormant seeds with red light activates the phytochrome signal induction pathway, resulting in the release of photo dormancy and the promotion of germination (Kretsh *et al.*, 1995; Emmler and Schafer, 1997), as light promotes the speed of endosperm rupture of non-photo dormant tobacco seeds (Leubner-Metzger, 2002).

Domesticated capsicum species have shown not to have light requirement for dormancy release and germination (Dell'Aquila, 2004), whereas seeds of wild *Capsicum annuum* var. *glabriusculum* do not germinate in constant darkness (Hernandez- Verdugo *et al.*, 2001b). Thus the role of light in dormancy release and

seed germination can effectively be termed as a depth sensing cue, preventing germination too deep in the soil or preventing seed germination on the soil surface, a strategy that is reasonable in situations where the soil dries rapidly (Bell, 1999; Bell *et al.*, 1995; Rokich and Bell, 1995).

In capsicum species, dormancy breaking has been shown to be responsive to either alternating temperatures (30/20^{o}C or 30/15^{o}C) (Hernandez-Verdugo *et al.*, 2001b) or a constant temperature range of 15^{o}C to 30^{o}C (Dell'Aquila, 2004). In this case, no special light requirements are necessary for germination of domesticated chilli pepper seeds. Seeds of wild *Capsicum annuum* var. *Glabriusculum* have been shown not to germinate in constant darkness (Hernandez-Verdugo *et al.*, 2001b). The same phenomenon has been observed in *Bunium persicum* where a narrow range of temperature (10^{o}C to 15^{o}C) has proven to be effective in breaking seed dormancy. Regimes with fluctuating temperatures (5^{o}C to 15^{o}C) during imbibition stage provided the best condition to stimulate germination. Therefore the choice of whether to use constant temperature or alternating temperature in seed germination where dormancy is suspected depends on the depth of dormancy exhibited by that seed lot irrespective of the seed type (Benech-Arnold *et al.*, 2000; Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006; Baskin *et al.*, 2005).

In as much as temperature plays a critical role in dormancy termination and promotion of germination, other seed factors also have a role to play in as far as the seeds response to temperature regimes is concerned. The level of seed maturity will determine the outcome of the germination percentage and by extension, germination rate. This has been shown in sweet pepper where seeds that were extracted from red fruits responded better to temperature application than seeds that were considered not

to have matured (de Souza *et al.*, 2011; Vidigal *et al.*, 2011). In tomato seeds, the highest percentage of germination were observed in seeds that were obtained from red fruits (Dias *et al.*, 2006a). Since the level of dormancy in an individual seed in a seed lot is most likely to vary, the effectiveness of temperature application can only be quantified over time such that as the germination period is extended, dormancy is progressively released as seeds become more responsive to the germination conditions, a phenomenon that has been well observed in *Arabidopsis thaliana* (Derkx and Karssen, 1993), and *Sisymbrium officinale* and *A. thaliana* (Hilhorst, 1990).

5.2 Role of gibberellic Acid in dormancy termination and promotion of germination.

From the results of gibberellic acid application on the germination of ABE chili seeds, germination improved tremendously on treated seeds than in control. The low percent germination exhibited by seeds under control treatment might be due to the fact that, in many species with coat-imposed dormancy, the seed envelope confers a physical constraint to radical emergence. In the solanaceous species, the micropylar endosperm and testa have this function being described as a thick β-1, 3-glucan layer. Endosperm weakening appears to be a prerequisite for germination. Class 1βGlu is transcriptionally induced in the micropylar endosperm of tobacco, tomato and other solanaceous seeds, capsicum species included just prior to radical emergence. βGlu induction and germination are tightly linked in response to plant hormones and environmental factors i.e., both are promoted by GA_3 and inhibited by ABA (Koornneef *et al.*, 2002; Leubner-Metzger, 2003a).

The high percent germination observed among the treated seeds might have been as a result of ABA and GA_3 interacting hence increased the ability of the embryo to rupture the endosperm and germinate. In such a situation, it has been shown that two antagonistic forces are in play; one is the embryo growth potential, and the other is the endosperm yield threshold or mechanical resistance of the endosperm to radical growth. When the embryo growth potential is greater than the endosperm yield threshold, germination occurs (Kucera *et al.*, 2005; da Silva *et al.*, 2004). GA_3 increased the growth potential of the embryo and is necessary to overcome mechanical restraint conferred by the seed covering layers. This is done through weakening of the tissues surrounding the radical (Koornneef, 2002; Leubner-Metzger, 2003b; Debeaujon and Koornneef, 2000), achieved by cell-wall hydrolysis through the collaborative or successive action of several GA_3-induced cell wall hydrolases (Bewley, 2003; da Silva *et al.*, 2005). ABA inhibits germination by decreasing embryo growth potential and possibly increasing endosperm yield threshold via the inhibition of cell wall hydrolases, inhibiting the expression of class 1 β-1, 3-glucanase (Kucera *et al.*, 2005; da Silva *et al.*, 2004). ABA inhibits the induction of βGlu 1genes during tobacco seed germination and specifically delays endosperm rapture (Leubner-Metzger and Meins, 2000).

Seed dormancy is controlled by the ABA:GA ratio rather than the absolute hormone content. Induction of seed dormancy would depend on ABA and GA_3 metabolism and sensitivity. Metabolism includes any process that changes the amount of the active form of the hormone such as synthesis, degradation, activation or deactivation. Processes that induce dormancy are ABA synthesis or activation, GA degradation or deactivation, increase of ABA sensitivity and increase of GA sensitivity. The opposite events will lead to dormancy alleviation and germination (Kucera *et al.*,

2005; Ali-Rachedi *et al.*, 2004). Millar *et al* (2006) and Gubler *et al* (2008) showed that dormancy maintenance in seeds of *Arabidopsis thaliana* and barley depends upon the balance of ABA synthesis and catabolism being shifted towards the former. Expression of the gene encoding ABA 8'- hydroxylase, a cytochrome P450 mono oxgenase that catalyses the hydroxylation of ABA to 8'- hydroxy ABA, was higher in imbibed non-dormant seeds compared with dormant seeds. This was inversely correlated with ABA concentration. The onset of dormancy in *Nicotiana tabacum* is correlated with a peak in ABA concentration which declines rapidly during further seed maturation (Jiang *et al.*, 1996; Phillips *et al.*, 1997). In *Arabidopsis Thaliana* ecotype Cvi seeds, alleviation of physiological dormancy occurs effectively by after-ripening, stratification or inhibition of ABA biosynthesis. Addition of GA_3 appears less effective as GA_3 treatment of dormant *A. Thaliana* Cvi seeds caused a transient increase in ABA concentration (Ali-Rashedi *et al.*, 2004). This suggests that in dormant seeds, a feedback mechanism exists that maintains a high ABA:GA ratio. In GA_3-deficient lines of *Arabidopsis* and tomato, germination under different external GA_3 concentrations has been present and shown that without the application of external GA_3, seeds do not germinate. Germination rates and final germination percentages increased with higher external GA_3 concentrations. At higher GA_3 concentrations, seeds germinated faster and at higher percentage under decreasing osmotic potential (Kucera *et al.*, 2005; Koornneef *et al.*, 2002; Ali-Rashedi *et al.*, 2004).

From the results, the interaction of GA_3 treated seeds with temperature resulted in better percent germination and mean germination rate than interaction with treatments and germination under light. These results correspond to others that have shown that GA_3 alone is not effective in stimulating germination (Finkelstein *et al.*, 2008).

Environmental conditions particularly alternating temperatures regulates seed germination and dormancy by affecting the plant hormone balance of GA_3 and ABA biosynthesis and catabolism which determines the dominant hormone (Ali-Rachedi *et al.*, 2004; Cadman *et al.*, 2006). This seems to be achieved through the environmental signal regulating the level of endogenous GA and it affects the ability of cells to respond to the hormone. More specifically, environmental cues like light and temperature can alter the tissue- specific localization of GA biosynthesis (Yamauchi *et al.*, 2004; Gonai *et al.*, 2004). In dormancy that's controlled by an inhibitor – promoter balance, dormancy can be corrected by exposing dry seeds to higher temperature or imbibing at low temperatures (Copeland and McDonald, 1995). In the regulation of thermo inhibition of lettuce seed germination, GA_3 application affected the temperature responsiveness of the seeds through ABA metabolism. GA_3 was substitute for the red – light trigger required to release photo dormancy and to induce testa rupture and subsequent endosperm rupture of tobacco seeds imbibed in the dark (Peng and Harberd, 2002).

Dormancy release also involves the light/gibberellin pathway which results in testa and endosperm rapture. Class 1 β-1, 3-glucanase (βGlu1) and other hydrolases are induced by light/GA_3 pathway in the micropylar endosperm and facilitate endosperm rapture and radical protrusion. Endosperm- specific βGlu1 expression and endosperm rapture are inhibited by abscisic acid (ABA). The light/GA_3 pathway also counteracts ABA effects by promoting ABA degradation (Kucera *et al.*, 2005). That being the case, in some instances light/GA_3 pathway has been shown not to promote dormancy release and germination. Dormancy release in imbibed annual ryegrass (*Lolium rigidum* Gaud) is promoted in the dark but inhibited in the light (Goggin *et al.*, 2009). Inhibitors of ABA metabolism had expected effects on seed germination

but did not influence ABA concentration, suggesting that they act upon other unknown factors regulating dormancy (Goggin *et al.*, 2009). Though GA_3 synthesis was required for germination, the influence of exogenous GA on both germination and dormancy release was minor or non-existent.

5.3 Reaction of ABE chili to Seed priming and priming techniques in enhancement of germination.

The improved seed germination percentage on the primed seeds compared to non-Primed seeds and control might be due to the fact that primed seeds have a lower water potential and a higher turgor potential than non-primed seeds (Bradford, 1995). This observation correlates with other findings such as in tomato, (Corbineau *et al.*, 1999), pepper (Lee *et al*, 1997), as well as carrot, lettuce and onions (Jeong *et al.*, 200b), where the rate of germination (inverse of time to germination) and improvement of seedling stands were observed to have been accelerated as a result of seed priming. The observed difference between primed and unprimed seeds is due to the fact that in primed seeds, the hydrostatic turgor pressure of the embryonic axes may increase. This is achieved through the accumulation of osmotic solutes allowing the radical to exceed the yield threshold of the tissues surrounding the radical (Bradford, 1995).

Primed seeds exhibit increased germination percentage, reduced mean germination time in different species such as grass (Hardegree and Van Vactor, 2000), lettuce seeds (Tarquis and Bradford, 1992), and sunflower seeds (Mahmood *et al.*, 2009). Better seedling vigour traits of primed seeds is a combined effect of better status of nutrient reserves in fleshly harvested seeds, increased seed nutrient level (Benech-Arnold *et al.*, 2000), enhanced permeability of loosened seed coat to water and

oxygen (Baskin *et al*, 2000), and earlier nutrient mobilization (Bewley, 1997). Improvement in germination is due to reserve mobilization of food material, activation and re-synthesis of some enzyme, DNA and RNA synthesis start during osmotic priming. Rapid embryo growth therefore results when obstacles to germination are removed (Khan, 1992). These changes include micro molecular synthesis, several enzymes activities, increase in germination power and vigour and overcoming of dormancy (Khan, 1992). Imbibition of tomato seeds in P.E.G has been shown to result in sharp increases in Adenosine Triphosphate (A.T.P), energy charge (E.C) and ATP/ADP (Adenosine Disphosphate) ratio (Corbineau *et al.*, 1999). These remain higher in primed seeds even after drying than in unprimed seed. During subsequent imbibition in water, the energy metabolism of the primed and dried seed is much more than that of the unprimed seed making the primed seed more vigorous.

The observed improved germination percentage might be due to the fact that during osmo priming, beneficial compounds that break dormancy or otherwise improve seed performance incorporated in the osmoticum were be taken up by the seed. This has been shown in other crop species such as lettuce seed where the weakening of endosperm layer (comprising galactomannan polysaccharides) is a pre-requisite to radical protrusion, particularly at high temperatures (Varier *et al.*, 2010). Endo-β-mannase is the key regulatory enzyme in endosperm weakening, which requires ethylene for activation. In lettuce, high temperature reduces germination primarily through their inhibitory effect on ethylene production by seeds. This in turn reduces the activity of endo-β-mannase (Nascimento *et al.*, 2004; Cantiffe *et al.*, 2000). Osmo priming of seeds with PEG (-1.2Mpa) at 15^{o}C with constant light could overcome the inhibitory effects of high temperature in thermo sensitive lettuce CV. Dark Boston (DGB) seeds in the absence of exogenous ethylene supply (Nascimento

et al., 2000). Imbibition of seeds of DGB in 1-aminocyclopropane-1-carboxylic acid (ACC, a precursor of ethylene) improved germination at 35^{o}C and also increased the activity of Endo-β-mannase. Osmo priming in the presence of amino ethoxyvinylglycine (AVG), an inhibitor of ethylene synthesis (it inhibits ACC synthesis) does not affect the enhancement of germination. Thus, osmo priming is able to overcome dormancy even when ethylene synthesis is interrupted. This occurrence can be attributed by the fact that osmo priming helps in releasing the ethylene within the embryonic tissues encased by the endosperm and seed coat and this would be sufficient to allow seed germination, and that priming actually lowers the need for a particular range of germination conditions by reducing or overcoming the dormancy in place (Varier *et al.*, 2010; Nascimento, 2004). Priming in the presence of Silver thiosulphate (STS) a putine specific inhibitor of ethylene inhibits germination, suggesting that ethylene activity is indispensable for the release of dormancy. This is supported by several studies that show an increased ability of primed seeds to produce ethylene, such as in germination of low viable cucumber seeds (Habdas *et al*, 2000), in lettuce seed germination (Cantiffe *et al.*, 2000), and in ageing tomato seeds (Siriwitayawan *et al.*, 2004).

Although priming improves the rate of germination, synchronous seedling emergence and growth, the effectiveness of priming depends on several factors. These includes concentrations of priming solutions, crop species, seed maturity, environmental conditions and type of osmotica used (Nascimento, 2003; Iqbal and Ashraf, 2005). Seeds at different stages of maturity responds differently to pre-sowing treatments (Hae and Finnah, 2011; Nascimento, 2003). The response to the treatments depends on apart from seed dormancy and hard-seed coats, maturity of fruits when harvested (Epkong, 2008; Samarah *et al.*, 2004). In muskmelon species, seed lots comprising of

immature seeds that are not physiologically mature showed a greater response to priming than seeds lots that comprised of older seeds. Therefore, priming effectively improved the quality of seed lots that were harvested prematurely or that had not reached full physiological maturity (Welbaum and Bradford, 1991).

Effectiveness of priming also depended on different priming agents and different concentrations of priming solutions (Iqbal and Ashraf, 2005; Jeong *et al.*, 2000c and d). Better scores have been reported when priming was done using potassium nitrate than poly ethyl glycol, results that have been also observed especially in freshly harvested tomato seeds compared with the untreated control (Liu *et al.*, 1996). Farooq *et al* (2005) concluded that though priming treatments (PEG -8000, KNO_3 and NaCl) resulted in improved germination and seedling vigour by dormancy breakdown compared with untreated seeds, the highest vigour was observed in seeds subjected to KNO_3, followed by NaCl. Hence salt priming is more effective. In hot pepper seeds, germination time was reduced by 50%, when seeds were primed with potassium nitrate.

5.4 Seed morphogenesis and maturation pathway in relations to germination parameters of ABE chili.

Seed germination in both varieties was observed to increase from 40 DAF onwards. This was because the seeds were undergoing a period of morphogenesis during which the embryo's body plan was being established through intensive cell divisions and the formation of embryonic organs (de Castro and Hilhorst, 2000; Meinke, 1995). This face was more likely followed by the second phase, considered a period of seed maturation. These includes the arrest of tissues and organ formation, the accumulation of nutrient reserves, changes in embryo size and in fresh and dry weights, suppression

of precocious germination, acquisition of desiccation tolerance, dehydration and quiescence, and in many species, the induction of dormancy (Kermode, 1995; Koornneef and Karssen, 1994).

This phenomenon has been noted in other vegetable species such as tomato seeds which attained maximum dry weight and its final curled shape between 35 and 50 days after pollination (DAP) (Berry and Bewley, 1991; Liu *et al.*, 1996). Several crucial events takes place simultaneously during the period of 35-50 DAP, i.e. seeds become fully germinable, desiccation tolerance is induced and water content decreases to approximately 50% (fresh weight basis). Primary dormancy may be induced in wild type seeds once full germinability is attained whereas Sit^{w} seeds do not become dormant. This correlates with the ABA content in seeds of both genotypes during development (de Castro and Hilhorst, 2000). Concurrently, the endosperm solidifies, abscission of the funiculus occurs and the testa turns brown (Liu *et al.*, 1996; de Castro, 1998). Some wild type and Sit^{w} seeds are able to germinate during embryo histo-differentiation, but are unable to produce viable seedling. At this stage, torpedo shaped embryos appear not to be yet fully differentiated and still undergo intense cell cycle activity, at low dry weight and high moisture content (de Castro and Hilhorst, 2000). Only after the completion of histo-differentiation and accumulation of reserves, i.e., from 35 D.A.P onwards, do germination results in normal seedlings (de Castro, 1998). The final stages of maturation process is marked by dehydration, in such a manner that during reserve deposition phase, there is also accumulation of potentially protective molecules especially LEA (Late Embryogenesis Accumulated) proteins and soluble sugars such as sucrose, raffinose and stachyose to prevent the membrane damage caused by water removal from the seed tissues (Bewley and Black, 1994). Apparently the switch from a developmental

mode to a germinative mode does not require the completion of histo-differentiation. Berry and Bewley (1991), concluded that the in ability of tomato seeds to provide viable seedlings at the early stages of development was due to lack of sufficient reserves in the still liquid endosperm as they observed that young embryos isolated from these seeds grew into viable seedlings when placed on a nutrient medium.

Seed maturity during development passes through the physiological maturity stage whereby the embryo has the capacity to germinate. At this stage, mass maturity is marked by attainment of maximum seed weight during the maturation process. In the development of sweet pepper seeds, seeds from fruits harvested between 20 and 40 DAA did not germinate. However, there was an increase in the germination percentage along maturation process, especially between 40 DAA and 50 DAA. Seed germination increased slightly up to the end of maturation when the maximum dry matter content of the seeds was achieved at around 75 D.A.A (Vidigal *et al.*, 2011). Dias *et al*, (2006a) showed that higher germination percentage in tomato was obtained before the maximum dry matter content of the seeds, at 75 DAA , when the fruits were 90% red. Contrasting results regarding maximum seed quality occurrence during seed development have been showed. In tomato, maximum dry matter content (Demir and Ellis, 1992a). Similar results were found in eggplant (Demir *et al.*, 2002) and pepper seeds (Oliveira *et al.*, 1999; Demir and Ellis, 1992b).

During seed maturity process, the attainment of seed vigour increases along maturation process. In sweet pepper, maximum seed vigour and maximum germination were recorded approximately at 60 DAA when the fruits were red. This coincided with mass maturity, represented by maximum dry matter content, which was attained from 55 DAA increasing slowly until 75 DAA (Vidigal *et al.*, 2001).

This hypothesis has also been supported by investigations in watermelon seeds (Demir *et al.,* 2004). Weakening of cell membrane in poor vigour seeds causes leakage of water soluble compounds like sugars, amino acids electrolytes when immersed in water. In pepper, the highest electrical conductivity value was obtained at 20 DAA, declining gradually till 65 DAA. This maximum electrical conductivity value coincided with the lowest germination percentage of the seeds, while lower conductivity values were recorded from 65 DAA until 75 DAA when seed vigour was high (Vidigal *et al*, 2011). This observation suggests that there was an increase of cell membrane integrity and subsequent reduction of electrolyte leakage along seed development. Similar phenomenon has been observed during development and maturation of tomato seeds (Demir and Ellis, 1992a and Dias *et al*, 2006b).

In developing seeds, the weight continues to increase after flowering. This increment is as a result of dry mass accumulation (Nedveda and Nikoleva, 1999). Increase in seed weight is linear and quadratic with the summit of the curve indicating 'mass maturity' of seeds. In pepper seeds maximum values of germination and dry matter was attained at 60 DAA and 50 D.A.A respectively (Oliveira *et al*, 1999). Demir and Ellis (1992b), found that maximum dry matter content in pepper seeds was obtained approximately 50 DAA, while fruits were 50% red. Vidigal *et al*, (2011) also found that the weight of one thousand seeds was maximum at the end of maturation process at 75 DAA, near the highest dry matter content value, when the fruits were red outside hence conceding with mass maturity (Ellis and Pieta Filho, 1992). Dias *et al* (2006a) obtained higher germination percentage before the maximum dry matter content of the tomato seeds at 75 DAA , when the fruits were 90% red. Physiological maturity may coincide with the end of seed filling period (reaching seed maximum weight), however, physiological maturity may occur after the end of seed filling period.

Seed moisture content decreases linearly during seed maturity and development. In early stages of seed formation, moisture content is high; seeds do not germinate which is related to inhibitory effects of ABA hormone. For most crops, physiological maturity occurs when seed moisture levels are high enough to prevent mechanical damage (Tekrony, 2003). In pepper seeds, at 20 DAA water content was 91.8% decreasing to 49.1% at 55 DAA (red fruits) attaining 47.3% at 75 DAA (Vidigal *et al*, 2011). This trend is one of the main characteristics of seed development in fleshy fruited vegetables in which seed water content remains at values of about 35-40% within the fruit (Tekrony and Egli, 1997). Whereas the seed water content declined steadily, the dry matter continued to accumulate and moisture content remained high even after maximum dry matter had been accumulated, a relationship noted in several species that produce seeds inside a fleshly fruit such as in tomatoes (Demir and Ellis, 1992a), aubergine (*Solanum melongena*) (Bewley and Black, 1994; Dias *et al.*, 2006a; Vidigal *et al.*, 2006). Seed water content however is not considered a good indicator of physiological maturity of seeds, since it can be affected by genotype and environmental conditions. Demir and Ellis, (1992a) obtained different moisture content values for tomato seeds at physiological maturity 53% and 72% in 1989 and 1990. Similar results were found by Demir *et al* (2002) in egg plant (*Solanum melongena* L.) seeds.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Temperature, priming, gibberellic acid seed treatment and germination

- The choice of temperature in germination of ABE chili depends on the depth of dormancy. When the dormancy is suspected to be deep, seeds will germinate very lowly at minimum temperatures increasing as the temperature is increased up to an optimum temperature where percent germination is at maximum and beyond which seeds will not germinate. In this case the optimum temperature for germination was 25 ^{o}C.
- Germination also responded well to alternating temperature regimes with or without light interaction. Contributory effect of light is dependent on alternating temperature ratio such that at lower alternating temperatures of 20/30^{o}C, light seems to be playing a supplementary role to temperature in seed germination. But at higher alternating temperatures of 25/30^{o}C, light seems to be having an inhibitory effect on germination of ABE chili.
- Though temperature is noted to significantly affect germination behavour on dormant seeds, maximum results are obtained when the interactive time between seeds and the temperature regime in which they are exposed to is extended beyond the standardized germination period.
- Seeds responded well to pretreatments such GA_3 and priming procedures. In both cases of seed treatments, even those that were considered to be "immature" from green fruits responded well in terms of percent germination and mean germination time compared to control experiments. In case of GA_3 treatment, the response to germination depended on the level of concentration, where the concentration of 0.08% was the most effective in overcoming seed

dormancy irrespective of the level of seed maturity or germination conditions. Interaction with temperature during germination period gave the best results as compared to interaction with light. Results not withstanding, seed maturity plays a critical role in having the best results desirable such that the highest level possible of matured seeds interacted with other factors will give the best results.

- The effectiveness of priming in overcoming dormancy in ABE chili depended on the type of osmotic used. In this case priming using potassium nitrate gave the best results in comparison to PEG. Potassium nitrate concentration of o.5% gave the best percent germination and increasing the concentration seemed to have a toxic effect on the seeds. This is also noted on seeds primed using PEG. Response to priming was depended on the level of seed maturity and the germination conditions.
- Wide range of factors therefore can be said to alter the physiological dormancy such as temperature range, priming concentrations and types, and gibberellic acid treatments. This factors need to be integrated overtime to alter the depth of dormancy and sensitivity to other factors, which indicate in a more immediate way that conditions are suitable to terminate dormancy and hence induce germination. Each of these factors therefore removes successive blocks to germination in a set order for germination process to be completed.

6.2 Physiological maturity and fruit colour

- The ABE chili seeds attained physiological maturity at between 60 to 65 days after flowering, when the fruits were red in colour coinciding with increasing dry matter content.

- At 65 days after maturity, the seeds are fully matured and harvesting them at this stage gives the best germination parameters desired in terms of thousand seed weight, dry matter content, seedling germination and vigour. At this stage, the fruits are 90% red in colour.

6.3 Recommendations.

- For maximum germination and vigour to be achieved in ABE chili, optimum germination temperature of 25 ^{o} C is recommended and where alternating temperature is used, supplementing with light is only desirable with lower alternating temperature. Experiments also need to be done to establish the response of ABE chili germination to alternating temperatures interaction with alternating time on light duration.
- Interaction of the temperature with either priming especially potassium nitrate (0.5%) or gibberellic treatment (0.08%) will shorten the germination time, though germination incubation period should be prolonged beyond the documented protocols to effectively overcome physiological dormancy in individual seeds in a germinating seed population. There is need to establish if high priming concentrations have toxic effects on ABE chili germination and if high levels of gibberellic acid have an effect in dormancy levels of ABE chili.
- For optimum seed germination attributes in ABE chili, it is commendable for seeds to be harvested when the fruits are matured red between 60 to 65DAF, when seeds are past physiological maturity and approaching harvest maturity. Post-harvest maturation experiments should be done to establish how it affects the seed quality of ABE chili that has been harvested both maturely and immaturely as an alternative to dormancy alleviation.

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APPENDICES

Appendix I: **ANOVA Table showing effects of cardinal temperatures and light regimes on percentage germination of ABE chili.**

Variate: %germination Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	5.51	1.84	0.08	
Temterature	4	2648.27	662.07	29.74	<.001
Contrast 1(15 vs 20)	1	48.51	48.51	2.18	0.153
Contrast 2(15 vs 20/30)	1	762.41	762.41	34.25	<.001
Contrast 3(15 vs 25)	1	1860.5	1860.5	83.58	<.001
Contrast 4(15 vs 25/30)	1	1296.54	1296.54	58.24	<.001
Contrast 5(20 vs 20/30)	1	364.1	364.1	16.36	<.001
Contrast 6(20 vs 25)	1	1308.16	1308.16	58.77	<.001
Contrast 7(20 vs 25/30)	1	782.04	782.04	35.13	<.001
Contrast 8(25 vs 25/30)	1	190.41	190.41	8.55	0.007
Contrast 9(25 vs 20/30)	1	635.84	635.84	28.56	<.001
Contrast 10(20/30 vs 25/30)	1	179.1	179.1	8.05	0.009
Residual	24	534.26	22.26		
Total	31	3188.04			

Appendix II: ANOVA Table showing effects of harvesting stage and interaction with germination conditions on germination percentage of yellow variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.35	0.35	0.21	
Harv_Stage	2	537.03	268.51	162.82	<.001
Contrast 1 (Red Vs Green)	1	414.19	414.19	251.16	<.001
Contrast 2 (Red Vs Yellow)	1	391.02	391.02	237.11	<.001
Contrast 3 (Green Vs Yellow)	1	0.33	0.33	0.20	0.655
condition	1	3626.68	3626.68	2199.18	<.001
Days	2	14817.36	7408.68	4492.54	<.001
Harv_Stage.condition	2	7.19	3.60	2.18	0.123
Contrast 1.condition	1	4.69	4.69	2.84	0.098
Contrast 2.condition	1	6.02	6.02	3.65	0.061
Contrast 3.condition	1	0.08	0.08	0.05	0.823
Harv_Stage.Days	4	257.22	64.31	38.99	<.001
Contrast 1.Days	2	198.50	99.25	60.18	<.001
Contrast 2.Days	2	187.17	93.58	56.75	<.001
Contrast 3.Days	2	0.17	0.08	0.05	0.951
condition.Days	2	1002.19	501.10	303.86	<.001
Harv_Stage.condition.Days	4	29.556	7.389	4.48	0.003
Contrast 1.condition.Days	2	18	9	5.46	0.007
Contrast 2.condition.Days	2	10.167	5.083	3.08	0.054
Contrast 3.condition.Days	2	16.167	8.083	4.9	0.011
Residual	53	87.403	1.649		
Total	71	20364.99			

Appendix III: ANOVA Table showing effects of harvesting stage and interaction with germination conditions on germination percentage of red variety of ABE chili.

Variate: %_Germ

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.35	0.35	0.21	
Harv_Stage	2	537.03	268.51	162.82	<.001
Contrast 1 (Red Vs Green)	1	414.19	414.19	251.16	<.001
Contrast 2 (Red Vs Yellow)	1	391.02	391.02	237.11	<.001
Contrast 3 (Green Vs Yellow)	1	0.33	0.33	0.20	0.655
condition	1	3626.68	3626.68	2199.18	<.001
Days	2	14817.36	7408.68	4492.54	<.001
Harv_Stage.condition	2	7.19	3.60	2.18	0.123
Contrast 1.condition	1	4.69	4.69	2.84	0.098
Contrast 2.condition	1	6.02	6.02	3.65	0.061
Contrast 3.condition	1	0.08	0.08	0.05	0.823
Harv_Stage.Days	4	257.22	64.31	38.99	<.001
Contrast 1.Days	2	198.50	99.25	60.18	<.001
Contrast 2.Days	2	187.17	93.58	56.75	<.001
Contrast 3.Days	2	0.17	0.08	0.05	0.951
condition.Days	2	1002.19	501.10	303.86	<.001
Harv_Stage.condition.Days	4	29.556	7.389	4.48	0.003
Contrast 1.condition.Days	2	18	9	5.46	0.007
Contrast 2.condition.Days	2	10.167	5.083	3.08	0.054
Contrast 3.condition.Days	2	16.167	8.083	4.9	0.011
Residual	53	87.403	1.649		
Total	71	20364.99			

Appendix IV: ANOVA Table showing effects of harvesting stage, incubation period and interaction with germination conditions on germination percentage of yellow variety of ABE chili.

Variate: % Germination Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.35	0.35	0.21	
Harv_Stage	2	537.03	268.51	162.82	<.001
Condition	1	3626.68	3626.68	2199.18	<.001
Days	2	14817.36	7408.68	4492.54	<.001
Contrast 1(7days Vs 14days)	1	2067.19	2067.19	1253.52	<.001
Contrast 2(7days Vs 21days)	1	14525.52	14525.52	8808.10	<.001
Contrast 3(14days Vs 21days)	1	5633.33	5633.33	3415.99	<.001
Harv_Stage.condition	2	7.19	3.60	2.18	0.123
Harv_Stage.Days	4	257.22	64.31	38.99	<.001
Harv_Stage.Contrast 1	2	198.50	99.25	60.18	<.001
Harv_Stage.Contrast 2	2	187.17	93.58	56.75	<.001
Harv_Stage.Contrast 3	2	0.17	0.08	0.05	0.951
Condition.Days	2	1002.19	501.10	303.86	<.001
condition.Contrast 1	1	638.02	638.02	386.89	<.001
condition.Contrast 2	1	15.19	15.19	9.21	0.004
condition.Contrast 3	1	850.08	850.08	515.48	<.001
Harv_Stage.condition.Days	4	29.56	7.39	4.48	0.003
Harv_Stage.condition.Contrast	2	15.17	7.58	4.60	0.014
Harv_Stage.condition.Contrast	2	10.50	5.25	3.18	0.049
Harv_Stage.condition.Contrast	2	18.67	9.33	5.66	0.006
Residual	53	87.40	1.65		
Total	71	20364.99			

Appendix V: ANOVA Table showing effects of harvesting stage, incubation period and interaction with germination conditions on germination percentage of red variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.00	0.00	0.00	
Harv_Stage	1	574.08	574.08	405.92	<.001
Condition	1	2852.08	2852.08	2016.62	<.001
Days	2	11142.38	5571.19	3939.22	<.001
Contrast 1 (7 Vs 14)	1	2592.00	2592.00	1832.73	<.001
Contrast 2 (7 Vs 21)	1	11137.78	11137.78	7875.20	<.001
Contrast 3 (14 Vs 21)	1	2983.78	2983.78	2109.74	<.001
Harv_Stage.condition	1	2.08	2.08	1.47	0.23
Harv_Stage.Days	2	268.79	134.40	95.03	<.001
Harv_Stage.Contrast 1	1	28.13	28.13	19.89	<.001
Harv_Stage.Contrast 2	1	258.78	258.78	182.98	<.001
Harv_Stage.Contrast 3	1	116.28	116.28	82.22	<.001
Condition.Days	2	405.79	202.90	143.46	<.001
condition.Contrast 1	1	325.13	325.13	229.89	<.001
condition.Contrast 2	1	1.53	1.53	1.08	0.31
condition.Contrast 3	1	282.03	282.03	199.42	<.001
Harv_Stage.condition.Days	2	40.54	20.27	14.33	<.001
Harv_Stage.condition.Contrast 1	1	0.50	0.50	0.35	0.56
Harv_Stage.condition.Contrast 2	1	34.03	34.03	24.06	<.001
Harv_Stage.condition.Contrast 3	1	26.28	26.28	18.58	<.001
Residual	35	49.50	1.41		
Total	47	15335.25			

Appendix VI: ANOVA Table showing effects of harvesting stage and interaction with germination conditions on mean germination time of yellow variety of ABE chili.

Variate: MGT Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.02	0.02	0.33	
Harv_Stage	2	0.95	0.47	8.26	0.003
Condition	1	20.23	20.23	352.04	<.001
Harv_Stage.condition	2	0.12	0.06	1.04	0.374
Residual	17	0.98	0.06		
Total	23	8.09			

Appendix VII: ANOVA table for effects of harvesting stage and interaction with germination conditions on mean germination time of red variety of ABE chili.

Variate: MGT Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.35	0.35	8.30	
Harv_Stage	1	0.71	0.71	16.90	0.002
Condition	1	0.04	0.04	1.06	0.325
Harv_Stage.condition	1	0.63	0.63	14.94	0.003
Residual	11	0.46	0.04		
Total	15	1.05			

Appendix VIII: ANOVA Table showing effects of harvesting stage and GA_3 interaction with germination conditions on germination percentage of yellow variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.09	0.09	0.03	
Harv_Stage	2	2084.59	1042.30	366.07	<.001
Contrast 1 (Red Vs Green)	1	1457.51	1457.51	511.90	<.001
Contrast 2 (Red Vs Yellow)	1	1662.63	1662.63	583.94	<.001
Contrast 3 (Green Vs Yellow)	1	6.75	6.75	2.37	0.125
Treatment	3	20647.29	6882.43	2417.20	<.001
Condition	1	2719.53	2719.53	955.14	<.001
Days	2	37860.42	18930.21	6648.55	<.001
Harv_Stage.treatment	6	19.41	3.24	1.14	0.342
Contrast 1.treatment	3	8.64	2.88	1.01	0.388
Contrast 2.treatment	3	11.85	3.95	1.39	0.248
Contrast 3.treatment	3	8.63	2.88	1.01	0.389
Harv_Stage.condition	2	56.27	28.14	9.88	<.001
Contrast 1.condition	1	41.26	41.26	14.49	<.001
Contrast 2.condition	1	43.13	43.13	15.15	<.001
Contrast 3.condition	1	0.02	0.02	0.01	0.932
Treatment.condition	3	1766.01	588.67	206.75	<.001
Harv_Stage.Days	4	1211.14	302.79	106.34	<.001
Contrast 1.Days	2	960.29	480.15	168.63	<.001
Contrast 2.Days	2	850.51	425.26	149.36	<.001
Contrast 3.Days	2	5.91	2.95	1.04	0.356
Treatment.Days	6	1377.49	229.58	80.63	<.001
Condition.Days	2	215.27	107.64	37.80	<.001
Harv_Stage.treatment.condition	6	96.40	16.07	5.64	<.001
Contrast 1.treatment.condition	3	62.56	20.85	7.32	<.001
Contrast 2.treatment.condition	3	80.43	26.81	9.42	<.001
Contrast 3.treatment.condition	3	1.60	0.54	0.19	0.905
Harv_Stage.treatment.Days	12	172.28	14.36	5.04	<.001
Contrast 1.treatment.Days	6	111.38	18.56	6.52	<.001
Contrast 2.treatment.Days	6	115.95	19.33	6.79	<.001
Contrast 3.treatment.Days	6	31.09	5.18	1.82	0.096
Harv_Stage.condition.Days	4	209.33	52.33	18.38	<.001
Contrast 1.condition.Days	2	179.04	89.52	31.44	<.001
Contrast 2.condition.Days	2	130.70	65.35	22.95	<.001
Contrast 3.condition.Days	2	4.26	2.13	0.75	0.474
Treatment.condition.Days	6	1040.98	173.50	60.93	<.001
Harv_Stage.treatment.condition.Days	12	203.08	16.92	5.94	<.001
Contrast 1.treatment.condition.Days	6	117.21	19.54	6.86	<.001
Contrast 2.treatment.condition.Days	6	142.93	23.82	8.37	<.001
Contrast 3.treatment.condition.Days	6	44.49	7.42	2.60	0.019
Residual	215	612.16	2.85		
Total	287	70291.75			

Appendix IX: ANOVA Table showing effects of harvesting stage, incubation period and interaction with GA_3 and germination conditions on germination percentage of red variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.88	0.88	0.30	
Harv_stage (Red Vs Green)	1	1856.30	1856.30	632.22	<.001
Condition(Light Vs Dark)	1	1215.05	1215.05	413.82	<.001
Treatment	3	10857.35	3619.12	1232.61	<.001
Days	2	27487.32	13743.66	4680.84	<.001
Contrast 1(7 Vs 14)	1	2056.01	2056.01	700.24	<.001
Contrast 2(7 Vs 21)	1	25849.70	25849.70	8803.94	<.001
Contrast 3(14 Vs 21)	1	13325.28	13325.28	4538.35	<.001
Harv_stage.condition	1	135.01	135.01	45.98	<.001
Harv_stage.treatment	3	46.39	15.46	5.27	0.002
Condition.treatment	3	1753.14	584.38	199.03	<.001
Harv_stage.Days	2	718.16	359.08	122.30	<.001
Harv_stage.Contrast 1	1	698.45	698.45	237.88	<.001
Harv_stage.Contrast 2	1	291.01	291.01	99.11	<.001
Harv_stage.Contrast 3	1	87.78	87.78	29.90	<.001
Condition.Days	2	117.28	58.64	19.97	<.001
condition.Contrast 1	1	106.95	106.95	36.42	<.001
condition.Contrast 2	1	5.70	5.70	1.94	0.166
condition.Contrast 3	1	63.28	63.28	21.55	<.001
Treatment.Days	6	1348.26	224.71	76.53	<.001
treatment.Contrast 1	3	1091.65	363.88	123.93	<.001
treatment.Contrast 2	3	909.21	303.07	103.22	<.001
treatment.Contrast 3	3	21.53	7.18	2.44	0.066
Harv_stage.condition.treatment	3	109.68	36.56	12.45	<.001
Harv_stage.condition.Days	2	64.82	32.41	11.04	<.001
Harv_stage.condition.Contrast 1	1	18.76	18.76	6.39	0.013
Harv_stage.condition.Contrast 2	1	64.70	64.70	22.03	<.001
Harv_stage.condition.Contrast 3	1	13.78	13.78	4.69	0.032
Harv_stage.treatment.Days	6	333.34	55.56	18.92	<.001
Harv_stage.treatment.Contrast 1	3	95.09	31.70	10.79	<.001
Harv_stage.treatment.Contrast 2	3	78.40	26.13	8.90	<.001
Harv_stage.treatment.Contrast 3	3	326.53	108.84	37.07	<.001
condition.treatment.Days	6	308.47	51.41	17.51	<.001
condition.treatment.Contrast 1	3	224.71	74.90	25.51	<.001
condition.treatment.Contrast 2	3	3.59	1.20	0.41	0.748
condition.treatment.Contrast 3	3	234.41	78.14	26.61	<.001
Harv_stage.condition.treatment.Days	6	169.68	28.28	9.63	<.001
Harv_stage.condition.treatment.Contrast 1	3	43.52	14.51	4.94	0.003
Harv_stage.condition.treatment.Contrast 2	3	137.09	45.70	15.56	<.001
Harv_stage.condition.treatment.Contrast 3	3	73.91	24.64	8.39	<.001
Residual	143	419.87	2.94		
Total	191	46941.00			

Appendix X: ANOVA Table showing effects of harvesting stage, incubation period and interaction with GA_3 and germination conditions on germination percentage of yellow variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.09	0.09	0.03	
Harv_Stage	2	2084.59	1042.30	366.07	<.001
Treatment	3	20647.29	6882.43	2417.20	<.001
Condition	1	2719.53	2719.53	955.14	<.001
Days	2	37860.42	18930.21	6648.55	<.001
Contrast 1 (7 Vs 14)	1	1490.76	1490.76	523.57	<.001
Contrast 2 (7 Vs 14)	1	34026.75	34026.75	11950.66	<.001
Contrast 3 (14 Vs 21)	1	21273.13	21273.13	7471.41	<.001
Harv_Stage.treatment	6	19.41	3.24	1.14	0.342
Harv_Stage.condition	2	56.27	28.14	9.88	<.001
treatment.condition	3	1766.01	588.67	206.75	<.001
Harv_Stage.Days	4	1211.14	302.79	106.34	<.001
Harv_Stage.Contrast 1	2	1185.54	592.77	208.19	<.001
Harv_Stage.Contrast 2	2	458.53	229.27	80.52	<.001
Harv_Stage.Contrast 3	2	172.64	86.32	30.32	<.001
Treatment.Days	6	1377.49	229.58	80.63	<.001
treatment.Contrast 1	3	942.22	314.08	110.31	<.001
treatment.Contrast 2	3	1074.38	358.13	125.78	<.001
treatment.Contrast 3	3	49.64	16.55	5.81	<.001
Condition.Days	2	215.27	107.64	37.80	<.001
condition.Contrast 1	1	202.13	202.13	70.99	<.001
condition.Contrast 2	1	105.02	105.02	36.88	<.001
condition.Contrast 3	1	15.76	15.76	5.53	0.02
Harv_Stage.treatment.condition	6	96.40	16.07	5.64	<.001
Harv_Stage.treatment.Days	12	172.28	14.36	5.04	<.001
Harv_Stage.treatment.Contrast 1	6	54.92	9.15	3.21	0.005
Harv_Stage.treatment.Contrast 2	6	93.84	15.64	5.49	<.001
Harv_Stage.treatment.Contrast 3	6	109.66	18.28	6.42	<.001
Harv_Stage.condition.Days	4	209.33	52.33	18.38	<.001
Harv_Stage.condition.Contrast 1	2	0.54	0.27	0.10	0.909
Harv_Stage.condition.Contrast 2	2	153.01	76.51	26.87	<.001
Harv_Stage.condition.Contrast 3	2	160.45	80.22	28.18	<.001
Treatment.condition.Days	6	1040.98	173.50	60.93	<.001
treatment.condition.Contrast 1	3	453.43	151.14	53.08	<.001
treatment.condition.Contrast 2	3	122.77	40.92	14.37	<.001
treatment.condition.Contrast 3	3	985.27	328.42	115.35	<.001
Harv_Stage.treatment.condition.Days	12	203.08	16.92	5.94	<.001
Harv_Stage.treatment.condition.Contrast 1	6	44.58	7.43	2.61	0.018
Harv_Stage.treatment.condition.Contrast 2	6	148.70	24.78	8.70	<.001
Harv_Stage.treatment.condition.Contrast 3	6	111.34	18.56	6.52	<.001
Residual	215	612.16	2.85		
Total	287	70291.75			

Appendix XI: ANOVA Table showing effects of GA_3 concentrations, germination conditions and interaction with differently matured seeds on mean germination time of yellow variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.10	0.10	0.00	
Harv_Stage	2	2084.60	1042.30	6.39	0.002
Condition	1	2719.50	2719.50	16.67	<.001
Treatment	3	20647.30	6882.40	42.19	<.001
Harv_Stage.condition	2	56.30	28.10	0.17	0.842
Harv_Stage.treatment	6	19.40	3.20	0.02	1
Condition.treatment	3	1766.00	588.70	3.61	0.014
Harv_Stage.condition.treatment	6	96.40	16.10	0.10	0.996
Residual	263	42902.20	163.10		
Total	287	70291.70			

Appendix XII: ANOVA Table showing effects of GA_3 concentrations, germination conditions and interaction with differently matured seeds on mean germination time of red variety of ABE chili.

Variate: MGT Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.00	0.00	0.04	
Harv_stage(Red Vs Green)	1	5.30	5.30	62.57	<.001
Treatment	3	45.25	15.08	178.00	<.001
Condition	1	13.84	13.84	163.36	<.001
Harv_stage.treatment	3	2.19	0.73	8.60	<.001
Harv_stage.condition	1	1.83	1.83	21.64	<.001
treatment.condition	3	16.03	5.34	63.04	<.001
Harv_stage.treatment.condition	3	6.38	2.13	25.11	<.001
Residual	47	3.98	0.08		
Total	63	34.30			

Appendix XIII: ANOVA Table showing effects of priming and germination conditions on germination percentage of differently matured seeds based on fruit colour of yellow variety of ABE chili.

Variate: MGT Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.00	0.00	0.04	
Harv_stage(Red Vs Green)	1	5.30	5.30	62.57	<.001
Treatment	3	45.25	15.08	178.00	<.001
Condition	1	13.84	13.84	163.36	<.001
Harv_stage.treatment	3	2.19	0.73	8.60	<.001
Harv_stage.condition	1	1.83	1.83	21.64	<.001
treatment.condition	3	16.03	5.34	63.04	<.001
Harv_stage.treatment.condition	3	6.38	2.13	25.11	<.001
Residual	47	3.98	0.08		
Total	63	34.30			

Appendix XIV: ANOVA Table showing effects of priming, priming concentrations and germination conditions on germination percentage of differently matured seeds based on fruit colour of red variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0	0	0	
Harv_Stage(Red vs Green)	1	2464.58	2464.58	1240.94	<.001
Condition	1	1312.19	1312.19	660.70	<.001
Priming	6	30202.45	5033.74	2534.54	<.001
Days	2	91205.90	45602.95	22961.57	<.001
Contrast 1(7 vs 14)	1	19203.02	19203.02	9668.92	<.001
Contrast 2(7 vs 21)	1	91005.47	91005.47	45822.21	<.001
Contrast 3(14 vs 21)	1	26600.36	26600.36	13393.56	<.001
Harv_Stage.condition	1	17.19	17.19	8.66	0.004
Harv_Stage.Priming	6	301.83	50.31	25.33	<.001
condition.Priming	6	1487.14	247.86	124.80	<.001
Harv_Stage.Days	2	55.79	27.90	14.05	<.001
Harv_Stage.Contrast 1	1	8.64	8.64	4.35	0.038
Harv_Stage.Contrast 2	1	55.00	55.00	27.70	<.001
Harv_Stage.Contrast 3	1	20.04	20.04	10.09	0.002
Condition.Days	2	602.22	301.11	151.61	<.001
condition.Contrast 1	1	171.50	171.50	86.35	<.001
condition.Contrast 2	1	601.29	601.29	302.76	<.001
condition.Contrast 3	1	130.54	130.54	65.73	<.001
Priming.Days	12	7155.98	596.33	300.26	<.001
Priming.Contrast 1	6	3826.73	637.79	321.13	<.001
Priming.Contrast 2	6	3909.38	651.56	328.07	<.001
Priming.Contrast 3	6	2997.86	499.64	251.58	<.001
Harv_Stage.condition.Priming	6	27.64	4.61	2.32	0.034
Harv_Stage.condition.Days	2	0.90	0.45	0.23	0.798
Harv_Stage.condition.Contrast 1	1	0.88	0.88	0.44	0.507
Harv_Stage.condition.Contrast 2	1	0.11	0.11	0.06	0.813
Harv_Stage.condition.Contrast 3	1	0.36	0.36	0.18	0.67
Harv_Stage.Priming.Days	12	861.42	71.79	36.14	<.001
Harv_Stage.Priming.Contrast 1	6	464.11	77.35	38.95	<.001
Harv_Stage.Priming.Contrast 2	6	656.84	109.47	55.12	<.001
Harv_Stage.Priming.Contrast 3	6	171.18	28.53	14.37	<.001
Condition.Priming.Days	12	2284.82	190.40	95.87	<.001
condition.Priming.Contrast 1	6	1430.50	238.42	120.05	<.001
condition.Priming.Contrast 2	6	1485.30	247.55	124.64	<.001
condition.Priming.Contrast 3	6	511.43	85.24	42.92	<.001
Harv_Stage.condition.Priming.Days	12	71.14	5.93	2.99	<.001
Harv_Stage.condition.Priming.Contrast 1	6	33.63	5.60	2.82	0.011
Harv_Stage.condition.Priming.Contrast 2	6	39.98	6.66	3.36	0.003
Harv_Stage.condition.Priming.Contrast 3	6	33.11	5.52	2.78	0.012
Residual	251	498.50	1.99		
Total	335	138549.70			

Appendix XV: ANOVA Table showing effects of priming concentrations, incubation days and germination conditions on germination percentage of differently matured seeds based on fruit colour of yellow variety of ABE chili.

Variate: %_Germ Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	9.45	9.45	5.31	
Harv_Stage	2	1145.50	572.75	321.65	<.001
Condition	1	510.02	510.02	286.42	<.001
Priming	6	47213.22	7868.87	4419.11	<.001
Days	2	165014.11	82507.06	46335.46	<.001
Contrast 1(7 vs 14)	1	40436.30	40436.30	22708.78	<.001
Contrast 2(7 vs 21)	1	165008.68	165008.68	92667.87	<.001
Contrast 3(14 vs 21)	1	42076.19	42076.19	23629.73	<.001
Harv_Stage.condition	2	4.75	2.38	1.33	0.265
Harv_Stage.Priming	12	598.30	49.86	28.00	<.001
condition.Priming	6	1201.91	200.32	112.50	<.001
Harv_Stage.Days	4	81.63	20.41	11.46	<.001
Harv_Stage.Contrast 1	2	60.90	30.45	17.10	<.001
Harv_Stage.Contrast 2	2	2.57	1.29	0.72	0.486
Harv_Stage.Contrast 3	2	58.97	29.49	16.56	<.001
Condition.Days	2	114.48	57.24	32.14	<.001
condition.Contrast 1	1	32.19	32.19	18.08	<.001
condition.Contrast 2	1	25.19	25.19	14.15	<.001
condition.Contrast 3	1	114.33	114.33	64.21	<.001
Priming.Days	12	18216.11	1518.01	852.50	<.001
Priming.Contrast 1	6	9580.12	1596.69	896.69	<.001
Priming.Contrast 2	6	10206.61	1701.10	955.33	<.001
Priming.Contrast 3	6	7537.44	1256.24	705.50	<.001
Harv_Stage.condition.Priming	12	55.28	4.61	2.59	0.003
Harv_Stage.condition.Days	4	4.52	1.13	0.64	0.638
Harv_Stage.condition.Contrast 1	2	0.11	0.06	0.03	0.969
Harv_Stage.condition.Contrast 2	2	3.02	1.51	0.85	0.429
Harv_Stage.condition.Contrast 3	2	3.65	1.82	1.02	0.36
Harv_Stage.Priming.Days	24	249.90	10.41	5.85	<.001
Harv_Stage.Priming.Contrast 1	12	174.44	14.54	8.16	<.001
Harv_Stage.Priming.Contrast 2	12	94.76	7.90	4.43	<.001
Harv_Stage.Priming.Contrast 3	12	105.66	8.81	4.94	<.001
Condition.Priming.Days	12	3783.64	315.30	177.07	<.001
condition.Priming.Contrast 1	6	3658.39	609.73	342.42	<.001
condition.Priming.Contrast 2	6	1288.85	214.81	120.64	<.001
condition.Priming.Contrast 3	6	728.21	121.37	68.16	<.001
Harv_Stage.condition.Priming.Days	24	167.78	6.99	3.93	<.001
Harv_Stage.condition.Priming.Contrast 1	12	81.80	6.82	3.83	<.001
Harv_Stage.condition.Priming.Contrast 2	12	109.56	9.13	5.13	<.001
Harv_Stage.condition.Priming.Contrast 3	12	60.31	5.03	2.82	0.001
Residual	377	671.30	1.78		
Total	503	239041.90			

Appendix XVI: ANOVA Table showing effects of priming, priming concentrations and germination conditions on germination percentage of differently matured seeds based on fruit colour of Yellow variety of ABE chili.

Variate: MGT Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.07	0.07	1.43	
Harv_Stage	1	58.93	58.93	1198.66	<.001
Priming	6	230.17	38.36	780.27	<.001
Condition	1	18.28	18.28	371.83	<.001
Harv_Stage.Priming	6	19.19	3.20	65.04	<.001
Harv_Stage.condition	1	3.22	3.22	65.55	<.001
Priming.condition	6	26.76	4.46	90.70	<.001
Harv_Stage.Priming.condition	6	9.57	1.60	32.45	<.001
Residual	83	4.08	0.05		
Total	111	126.22			

Appendix XVII: ANOVA for effects of priming, priming concentrations and germination conditions on germination percentage of differently matured seeds based on fruit colour of red variety of ABE chili.

Variate: MGT Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	0.07	0.07	1.43	
Harv_Stage	1	58.93	58.93	1198.66	<.001
Priming	6	230.17	38.36	780.27	<.001
Condition	1	18.28	18.28	371.83	<.001
Harv_Stage.Priming	6	19.19	3.20	65.04	<.001
Harv_Stage.condition	1	3.22	3.22	65.55	<.001
Priming.condition	6	26.76	4.46	90.70	<.001
Harv_Stage.Priming.condition	6	9.57	1.60	32.45	<.001
Residual	83	4.08	0.05		
Total	111	126.22			

Appendix XVIII: Table 9: Seed maturity based on fruit colour and interaction with priming treatments and concentrations, germination conditions and effects Son percentage germination of red variety of ABE chili.

Harvesting Stage	Priming	Days	Dark	Light	Mean (a)	Mean (b)	Mean (c)
Green	Control	Day 7	20.5	21.3	**20.9**	**50.6**	**50.6**
		Day 14	45.3	45.0	**45.1**		
		Day 21	85.0	86.8	**85.9**		
	KN (0.2)	Day 7	46.0	45.8	**45.9**	**73.7**	**77.0**
		Day 14	83.0	73.3	**78.1**		
		Day 21	96.8	97.3	**97.0**		
	KN (0.5)	Day 7	55.8	64.5	**60.1**	**80.3**	
		Day 14	88.8	77.8	**83.3**		
		Day 21	97.8	97.0	**97.4**		
	KN (0.8)	Day 7	47.0	47.8	**47.4**	**77.1**	
		Day 14	87.5	85.8	**86.6**		
		Day 21	97.3	97.3	**97.3**		
	P.E.G (0.2)	Day 7	51.0	28.3	**39.6**	**60.0**	**65.3**
		Day 14	55.8	58.8	**57.3**		
		Day 21	86.8	79.3	**83.0**		
	P.E.G (0.6)	Day 7	57.5	57.3	**57.4**	**70.4**	
		Day 14	66.3	66.8	**66.5**		
		Day 21	87.3	87.5	**87.4**		
	P.E.G (1.0)	Day 7	52.5	52.0	**52.3**	**65.7**	
		Day 14	58.5	56.3	**57.4**		
		Day 21	87.5	87.3	**87.4**		
Yellow	Control	Day 7	21.0	20.8	**20.9**	**50.5**	**50.5**
		Day 14	45.3	44.8	**45.0**		
		Day 21	84.5	87.0	**85.8**		
	KN (0.2)	Day 7	46.5	46.5	**46.5**	**74.0**	**77.4**
		Day 14	84.5	72.5	**78.5**		
		Day 21	97.3	97.0	**97.1**		
	KN (0.5)	Day 7	57.5	64.5	**61.0**	**80.7**	
		Day 14	88.3	78.3	**83.3**		
		Day 21	97.8	98.0	**97.9**		
	KN (0.8)	Day 7	47.5	48.5	**48.0**	**77.5**	
		Day 14	87.8	86.0	**86.9**		
		Day 21	97.5	97.5	**97.5**		
	P.E.G (0.2)	Day 7	53.0	27.3	**40.1**	**60.5**	**65.8**
		Day 14	57.5	59.3	**58.4**		
		Day 21	87.0	79.3	**83.1**		
	P.E.G (0.6)	Day 7	58.3	59.8	**59.0**	**71.3**	
		Day 14	66.5	66.8	**66.6**		
		Day 21	88.8	87.8	**88.3**		
	P.E.G (1.0)	Day 7	52.5	52.5	**52.5**	**65.7**	
		Day 14	59.3	55.8	**57.5**		
		Day 21	86.8	87.5	**87.1**		
Red	Control	Day 7	21.0	20.8	**20.9**	**50.8**	**50.8**
		Day 14	45.0	45.3	**45.1**		
		Day 21	87.0	86.0	**86.5**		
	KN (0.2)	Day 7	45.8	52.0	**48.9**	**76.5**	**78.9**
		Day 14	87.8	79.3	**83.5**		
		Day 21	97.8	96.8	**97.3**		
	KN (0.5)	Day 7	61.3	68.0	**64.6**	**82.5**	
		Day 14	89.0	79.5	**84.3**		
		Day 21	98.8	98.3	**98.5**		
	KN (0.8)	Day 7	47.5	49.8	**48.6**	**77.8**	
		Day 14	88.0	87.3	**87.6**		
		Day 21	97.8	96.8	**97.3**		
	P.E.G (0.2)	Day 7	55.5	30.0	**42.8**	**65.4**	**71.2**
		Day 14	62.5	65.3	**63.9**		
		Day 21	90.3	89.0	**89.6**		
	P.E.G (0.6)	Day 7	65.8	62.3	**64.0**	**77.7**	
		Day 14	73.8	76.0	**74.9**		
		Day 21	95.0	93.3	**94.1**		
	P.E.G (1.0)	Day 7	52.8	55.5	**54.1**	**70.5**	
		Day 14	69.8	63.3	**66.5**		
		Day 21	91.0	90.8	**90.9**		

	H.Stage(H.S)	Condition(C)	Priming(P)	Days(D)	H.SxC	H.SxP	H.SxD	PxD
F.probability	<.001	<.001	<.001	<.001	0.265	<.001	<.001	<.001
S.E	0.103	0.084	0.084	0.103	0.146	0.272	0.178	0.272
S.E.D	0.146	0.119	0.222	0.146	0.206	0.385	0.252	0.385
C.V (%)	1.9							

	PxC	DxC	H.SxPxD	H.SxPxC	H.SxDxC	PxDxC	H.SxPxDxC
F.probability	<.001	<.001	<.001	0.003	0.638	<.001	<.001
S.E	0.222	0.146	0.472	0.385	0.252	0.385	0.667
S.E.D	0.315	0.206	0.667	0.545	0.357	0.545	0.944
C.V (%)							