THE EFFECT OF WATER STRESS AND STORAGE CONDITIONS ON SEED QUALITY OF CHICKPEA GENOTYPES CHARACTERIZED BY DIFFERENCES IN SEED SIZE AND COAT COLOUR

BY

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DECLARATION

I, Busisiwe Vilakazi (student number: 16023588), hereby declare that this dissertation for Master of Science (MSc.) degree in Agriculture (Plant Production) submitted by me at the University of Venda is my own work and has not been submitted previously for any degree at this or any other University. It is original in design and execution, and all references have been duly acknowledged.

Student: Miss B. Vilakazi  18 April 2018
Signature Date

As the supervisors/co-supervisor of the candidate, we agree to the submission of this dissertation.

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Signature Date

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Signature Date 18 April 2018

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DEDICATION

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an excellent utilizer of residual soil moisture in agricultural ecosystems. However, its seed quality and hence reproduction is constrained by water stress, seed size and storage conditions. This study was carried out at the University of KwaZulu- Natal (UKZN), Pietermaritzburg Campus. It was conducted to evaluate the performance of chickpea genotypes (Desi-K, Saina-K and ICCV-K) with different seed sizes on seedling emergence (i), seed ageing effect on seed quality and imbibition of genotypes produced under water stressed and non-stressed conditions (ii), and (iii) the effect of water stress during seed development on sugars and protein accumulation, germination and seed vigour. Pot experiments were conducted under glasshouse / tunnel conditions at the Controlled Environment Facilities (CEF). The experiment for objective 1 was laid out as a single factor in completely randomized design (CRD). Data on emergence rate, final hypocotyl and complete emergence was collected. The small seeded Desi-K showed higher and faster emergence compared to medium sized Saina-K and large seeded ICCV-K. In the experiment of the second objective, seeds of the three genotypes were first obtained by production under water stressed and non-stressed growing conditions. They were then aged for 0, 1, 3, 5, or 7 days at 41 ^{o}C and 100% relative humidity to form a 2 x 3 x 5 (water levels x genotypes x ageing) factorial design. Data was collected on germination percentage (GP), mean germination time (MGT), electrical conductivity (EC), tetrazolium chloride test (TZ) and imbibition weight. Seed ageing caused progressive loss of seed viability and vigour in all genotypes, which resulted in lower GP, delayed MGT, reduced TZ staining, cell death and high solute leakage from the seeds produced under the two water regimes. However, the effect was more severe under water stressed conditions. In the experiment for objective 3, seeds of all three genotypes were larger when grown under non-stressed condition compared to those under water stressed condition. These larger seeds had higher seed viability and germination percentage but lower electrical conductivity and mean germination time. Stressed seeds had higher soluble sugars than non-stressed seeds. It was deduced that irrigation during seed development reduces the final sugars and protein content but increases the seed size and physiological quality parameters allied to production of chickpea. Therefore, water provision to chickpea crop is critical during seed development.

Key words: chickpea, seed quality, water stress, storage conditions, seed size, sugars

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LIST OF ABBREVIATIONS

AA	Accelerated Ageing
EC	Electrical Conductivity
GP	Germination Percentage
MGT	Mean Germination Time
SS	Seed Size
SV	Seed Viability
TZ	Tetrazolium Chloride
CEF	Controlled Environment Facility
CRD	Completely Randomized Design
RH	Relative Humidity
PE	Production Environment
RFOs	Raffinose Family of Oligosaccharides
PCA	Principal Component Analysis
LSD	Least Significant Difference
SED	Standard Error Difference

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Chickpea (*Cicer arietinum* L.), a member of the Fabaceae family, is an important grain legume that could add to crop diversification and contribute to food security in smallholder farming systems. It is generally grouped into two types; the desi type with small angular, dark coloured and rough seeds, and the kabuli type with large, light coloured smooth seeds. This legume has an enormous potential for improving the economic status of resource poor smallholder farmers as a rotation crop because of its nitrogen fixation capacity (Gowda et al., 2013) and high protein content (Gaur et al., 2010). Chickpea may also act as an insurance crop in poor seasons when the main crop fails due to drought since it is a fairly drought tolerant crop that is able to extract water from deep layers in the soil profile with its deep rooting system (Ogola and Thangwana, 2013).

The crop is cultivated in over 50 countries throughout the tropical, subtropical and temperate regions in south, west and east Asia, Australia, east and north Africa, north and south America and southern Europe (FAOSTAT, 2016). Asia accounts for 89.2% of total area under chickpea production and 84.5% of global yield (FAOSTAT, 2016). In Africa, Ethiopia is the leading producer accounting for 2.5% of world and more than 55% of Africa's chickpea production (Korbu et al., 2016). However, there is limited chickpea production in south African countries including South Africa while there is high and ever increasing demand for food (Kassie et al., 2009; Munirathnam and Sangita, 2009).

Chickpea productivity is constrained by several factors including water stress, high temperature, low soil fertility and poor seed quality (Gowda et al., 2013) that results to significant yield losses (Basu et al., 2009). Although chickpea is considered as a fairly drought tolerant crop due to its deep rooting system, water stress can reduce chickpea seed yield by 42 to 70% (Shrestha et al., 2006). These loses mainly emanate from pod abortion (Behboudian et al., 2001; Leport et al., 2006), reduced pod production (Behboudian et al., 2001) and reduced grain-filling duration (Ghassemi-Golezani and Ghassemi, 2013). Furthermore, high temperatures after flower opening can also reduce chickpea yield by decreasing the number of pods, seeds per plant and weight per seed (Wang et al., 2006). Moreover, poor seed quality is a major factor that limits chickpea production. Important aspects of seed quality include genetic purity, the presence of inert matter, germination percentage, vigour, appearance and freedom from diseases (Yadav et al., 2005). Seed quality of most crops is affected by abiotic stresses like water stress during seed

development (Turner et al., 2001). However, there is a dearth of information in literature on the response of chickpea seed quality development to water stress. In most crops, water stress occurring at, or soon after pollination induces embryo abortion and limits the total number of seeds produced (De Souza et al., 1996; Leport et al., 1999; Fang et al., 2009). At dry matter accumulation stages of later seed development, water stress may lead to reduced seed fill process (Brevedan and Egli, 2003; Egli, 2006). Therefore, water stress during seed development may affect seed vigour (Bewley et al., 2012). Moreover, high temperatures during seed development can cause early pod ripening and rapid seed maturation in legumes and result in small seeds with poor quality (Thomas et al., 2009). However, there is no evidence in literature on the response of chickpea seed quality to temperature stress.

Furthermore, it has been reported that in grain legumes, the imbibition damage and seed ageing during storage, and interaction of the two are the major factors affecting seed quality (Matthews and Powell, 2006). During seed ageing, seeds lose their vigour and viability for germination (Maity et al., 2000) and the losses are increased if seeds are stored at high temperatures and/or high relative humidity. These storage conditions hasten the rate of biochemical processes causing rapid deterioration that results in rapid losses in seeds with high moisture content (Shelar et al., 2008). Therefore, storage humidity and high temperature increases the deterioration speed of seeds (Pukacka et al., 2009). Imbibition damage also adversely influences the seed quality (viability and vigour) in grain legumes (Matthews and Powell, 2006). It results from the rapid uptake of water into the cotyledons, which kills some of the surface cells and so increases the solute leakage. This is more severe on the white seeded lots with loose adherence of testa that allows rapid movement of water through the seed in the gap between the testa and cotyledons (Chachalis and Smith, 2000; Peksen et al., 2004) resulting in low vigour and poor field emergence. In contrast, high vigour seeds imbibe slowly due to the brown and black testa that adheres closely to the cotyledons and restrict water movement into the seed which limits imbibition damage.

Chickpea exhibits a wide range of seed size due to genotypic differences. Seed size is one of the major components of seed quality that affects the growth and development of the crop (Adebisi et al., 2011). According to Jerlin and Vadivelu (2004), seed size is a widely accepted measure of seed quality, and large seeds have higher seedling growth and establishment. Emenky and Khalaf (2010) reported that the large seeds of chickpea recorded superiority in growth traits as compared to small seeds. Similar results were also obtained by Roozrokh et al. (2005).

In the last decade, observations from the field and pot experiments in Limpopo and Mpumalanga Provinces showed that chickpea seeds exhibit poor germination and seedling establishment after

planting, thus resulting in poor plant population and consequently low yields. The poor germination and seedling establishment has been observed to be more severe in the kabuli types compared to the desi types in both summer and winter sowings (both in the glasshouse and in the field) irrespective of storage conditions. This could probably be due to poor storage conditions characterized by high relative humidity and temperature which can lead to a rapid deterioration of seed viability and vigour. However, there is dearth of information in literature on the likely causes of poor seed quality in chickpea and factors that may explain the differences in quality between the kabuli and desi types. Presently, studies on chickpea seed physiology are limited. Although there is evidence of chickpea being a drought tolerant crop, there is no satisfactory evidence to associate plant drought tolerance with seed quality in response to water stress. Moreover, the increase of chickpea production to most African countries such as South Africa which produce insignificant amounts can drastically increase food security, since chickpea has a strong potential to be an export crop. Therefore, this study assessed the effect of water stress and storage conditions, particularly seed ageing influence on seed quality of different chickpea genotypes varying in seed size.

1. 2 Hypothesis

- Water stress causes alterations in seed chemical composition with regard to the final content of sugars and proteins, and affects germination and seed vigour of chickpea genotypes.
- Chickpea genotypes with different seed sizes have an effect on seedling emergence.
- Seed ageing during storage affects seed quality and imbibition of different chickpea genotypes produced under water stress or non-stress conditions.

1. 3 Research Aim and Objectives

The main objective of this study was to determine the effect of water stress and seed storage conditions on seed quality of chickpea genotypes characterized by differences in seed size and coat colour.

The specific objectives of the study were to:

- Determine the effect of water stress during seed development on sugars and proteins content, germination and seed vigour of chickpea genotypes.
- Investigate the performance of chickpea genotypes with different seed sizes on seedling emergence.

- Investigate the effect of seed ageing on seed quality and imbibition of chickpea genotypes produced under water stressed or non-stressed condition.

1.4 Dissertation overview

Chapter 1 is the general introduction outlining a review of the study including previous research and relevant background theory, justification for the choice of research, contribution and importance of the study.

Chapter 2 reviews previous literature in an attempt to gain an understanding on the effect of water stress, seed size and storage conditions on seed quality of chickpea genotypes. The review considers previous findings on the effect of water stress on the accumulation of sugars and proteins, the effect of seed ageing and imbibition damage on seed quality in general and focus specifically on chickpea.

Chapter 3 describes the performance of chickpea genotypes with different seed sizes on seedling emergence. The major findings of the objective are discussed in relation to the influence of three chickpea genotypes (Desi-K, Saina-K and ICCV-K) on emergence rate, final hypocotyl and complete emergence.

Chapter 4 describes the effect of seed ageing on seed quality and imbibition of genotypes produced under water stressed and non-stressed conditions. Variation in physiological quality parameters including electrical conductivity, tetrazolium chloride test, germination percentage, mean germination time, weight increase during imbibition and seed coat adherence and their implications as indicators of seed quality in three chickpea genotypes are discussed.

Chapter 5 describes the effect of water stress during seed development on the final content of sugars and proteins, germination and seed vigour of three chickpea genotypes. The final contents of raffinose, sucrose, stachyose and proteins are discussed in relation to water stress as well as the response of chickpea genotypes to physiological quality.

Chapter 6 is general discussion and conclusion, discussing the insights on physiological quality responses in relation to the future improvement of chickpea genotypes with regard to better adaptation to water stress, storage conditions and seed size effect. This chapter also highlights the major limitations to the study and points out directions for further research.

CHAPTER 2: LITERATURE REVIEW

2.1 Chickpea adaptation and utilization

Chickpea can be grown over a wide range of environments. However, it grows well in areas with annual rainfall between 400 and 600 mm (Gaur et al., 2010). The best temperature for germination is between 5 and 15 ºC while temperatures above 29 ºC and frost could be harmful during flowering and pod formation (Gaur et al., 2010). Chickpea requires fertile, sandy-loam soil with good drainage for good performance and does not tolerate water-logged conditions. Apart from its capacity to fix nitrogen in the soil, chickpea can also be used for medicinal purposes and industrially, for textile sizing as well as a fodder in dry areas where grazing vegetation is scarce (Mathews, 2005). Moreover, chickpea is a functional food with low fat; it is cholesterol free and an ideal source of energy for vegetarian populations and those with major food related health problems like diabetes, cancer or coronary heart diseases (Jukanti et al., 2012).

2. 2 Seed quality aspects

The two most important indicators of seed quality are germination capacity and physiological vigour (Tekrony, 2003). Seed vigour gives an indication of the ability of seeds to emerge under a wide range of environmental conditions and is the most informative indicator of physiological seed quality. The maximum potential of seed physiological quality is determined by genetic factors, and is dependent on several external factors which occur throughout seed development on the mother plant, at harvest time, and during storage (Tekrony, 2003; Powell et al., 2005).

2.2.1 Germination

Germination may be defined as the physiological events that occur from the beginning of seed imbibition to the onset of radicle protrusion (Finch-Savage and Leubner-Metzger, 2006). In a study to measure changes in respiratory activity or water uptake, it was shown that the germination process can be divided into a sequence of three phases (Copeland and McDonald, 2001). Generally, seeds that do not have a problem with dormancy and seed coat permeability are the ones that show a three-phase process of water uptake during the process of germination (Figure 2.1). According to Bewley et al. (2013), when a seed is dry during imbibition, the turgor potential is reduced by solutes inside the cell and that results to rapid uptake of water from the surrounding solution (phase I), and as the water potential of seed increases during imbibition, the water content of seed increases. However, the resistance of cell wall to expansion results in a turgor potential increase (Copeland and McDonald, 2001). When the seed water potential increases, the water uptake decreases and the seed enters the lag phase (phase II), in which only a small or negligible

amount of water is absorbed by the seed over a relatively long period of time (Copeland and McDonald, 2001). This allows the seed to complete all its physiological pre-germinative processes and make it ready for radicle emergence (Finch-Savage and Leubner-Metzger, 2006). Finally, when the seed has completed all pre-germinative embryonic processes in phase II, it transfers to phase III, which results in radicle protrusion from the seed coat. In the current study, the effect of water stress and storage conditions on seed quality of three chickpea genotypes was evaluated by assessing germination percentage and mean germination time based on the radicle protrusion.

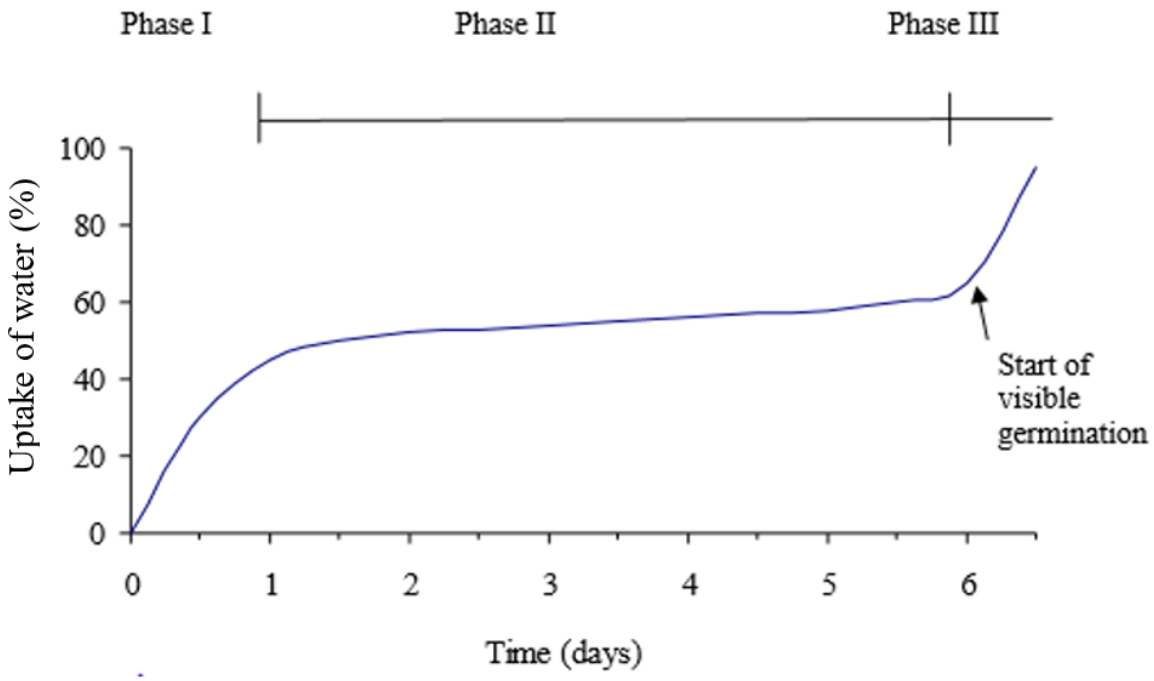


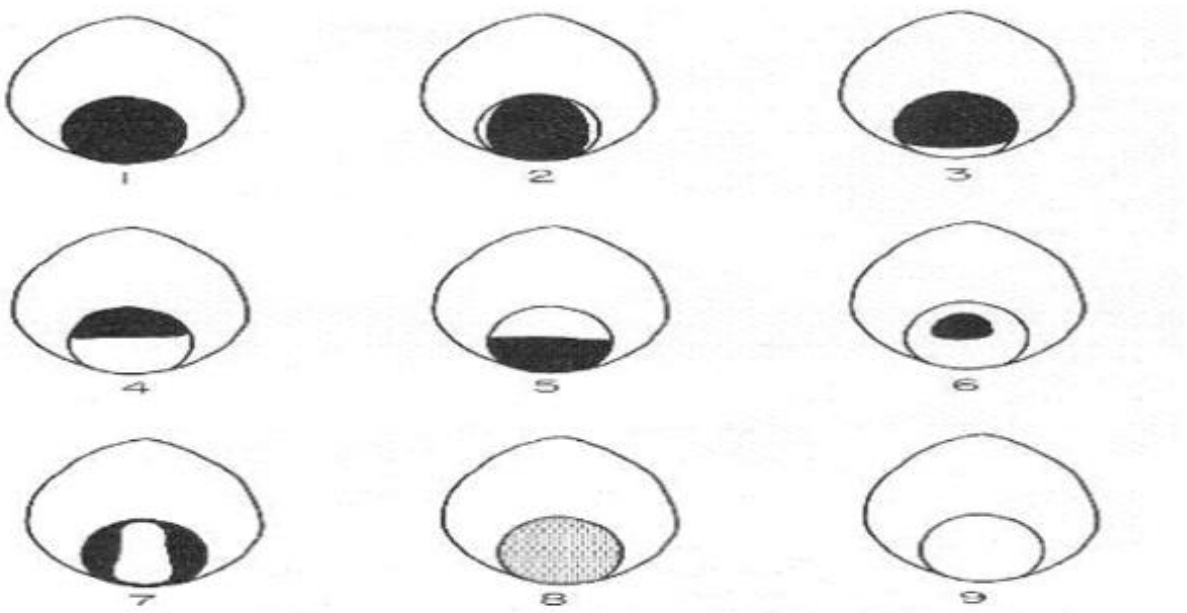
Figure 2.1: Triphasic water uptake pattern of seed germination (Copeland and McDonald, 2001).

2.2.2 Seed viability

Viability is an important element of seed quality that refers to the ability of seeds to germinate and produce normal seedlings. It takes a seed to be viable or alive in order to germinate, grow, develop a new plant, multiply and complete the life cycle (Porter, 2017). However, it is difficult or impossible to determine whether a seed is alive or dead from external physical appearance. For example, two seeds may appear alike and possess the same size, shape and colour, yet one of them may be alive and the other dead. In a study evaluating seed vigour of pea seeds with similar outside appearance, Matthews and Powell (2006) reported a difference in seed vigour and time dependent germination. Therefore, to determine the percentage of viable seed in a given lot, seed

viability testing is widely used. According to Marcos Filho (2015), seed viability is one of the indications of potential performance in the field. Under similar environmental conditions, a seed lot with high viability will produce better seedling emergence as compared to a low viability seed lot. Seed viability indicates that, in the absence of dormancy, a seed contains structures and substances that give it the ability to germinate under favourable conditions.

Germination and tetrazolium test are the most important viability tests available (Patil and Dadlani, 2009). Tetrazolium test was developed by a German scientist, George Lakon in the early 1940s to differentiate between viable and dead tissues of seed embryos on the basis of dehydrogenase enzyme activity (Khajjak et al., 2016). Upon seed hydration, the activity of dehydrogenase enzyme increases, resulting in the release of hydrogen ions, which reduce the colourless tetrazolium salt solution (2,3,5-triphenyl tetrazollium chloride) into a red compound called formazan (Patil and Dadlani, 2009; Sujatha et al.,2011). Thus, it is possible to differentiate the red coloured living tissues from the colourless dead ones and the seeds are classified into viable and non-viable seed classes. In the current study, the staining pattern of seeds in tetrazolium chloride test (Figure 2.2) was used as a criterion to evaluate the percentage of viable and dead seeds for chickpea genotypes produced under water stressed and non-stressed condition as well as for seeds stored under different conditions.



NO. 1 GERMINABLE. Embryo completely stained
NO. 2 GERMINABLE. Margins of scutellum unstained
NO. 3 NON-GERMINABLE. Basal portions of scutellum and radicle unstained
NO. 4 NON- GERMINABLE. Lower half of embryo unstained
NO. 5 NON-GERMINABLE. Upper half of embryo unstained
NO. 6 NON-GERMINABLE. Radicle and scutellum unstained
NO. 7 NON-GERMINABLE. Embryonic axis unstained
NO. 8 NON-GERMINABLE. Embryo stained pink
NO. 9 NON-GERMINABLE. Embryo completely unstained

Figure 2.2: Staining pattern of dicot seeds in TZ test: black areas indicate stained living tissue; white areas represent unstained and dead tissue (Peters, 2000).

2.2.3 Seed vigour

Seed vigour is measured as germination or emergence under or following stress and it is considered as the best measure of potential seedling establishment in the field (Marcos Filho, 2015). According to Ferguson (1995), seed vigour is not a single measurable property but a concept describing the interaction of several characteristics including the rate and uniformity of germination and growth, tolerance to environmental stresses after sowing, and retention of performance capacity after storage. Seed ageing during storage and imbibition damage are the major processes that lead to loss of seed vigour and eventually, seed viability. Seed vigour is also influenced by seed production factors (Ferguson, 1995) as shown in Table 2.1. In addition, the electrical conductivity test has been developed as a vigour test for the prediction of field emergence (Marcos Filho, 2015). The test measures the leakage of solutes, particularly potassium ions, into water of soaked seeds. An increase in the electrical conductivity of seeds

soaked in water indicates high solute leakage. Loss of solutes reflects the presence of dead tissues within the seed. According to Simon and Harun (1972), as seeds dry at maturity, the cell membrane loses its integrity and it is re-established during imbibition but the re-establishment of these membranes is much faster for vigorous seeds with less leakage than less vigorous seeds with high leakage. Furthermore, the incidence of dead tissues and membrane integrity are influenced by the two major factors determining seed vigour in grain legumes; seed ageing during storage and the incidence of imbibition damage, and greater sensitivity of aged seed to imbibition damage (Matthews and Powell, 2006). Thus, the measurement of electrical conductivity assesses the major causes of reduced vigour in grain legumes such as chickpea. In this study electrical conductivity tests were undertaken to evaluate the effect of seed ageing during storage on seed quality and imbibition of chickpea genotypes produced under water stressed and non-stressed conditions.

Table 2.1: Major factors influencing vigour of seed production and storage at various stages (Justice and Bass, 1978).

Stage of seed production	Conditions influencing vigour	Physiological effect	Vigour level
Harvest maturity	Delayed harvest + poor weather	Ageing	Low
⇩	Good harvest conditions	Little ageing	High
Harvest	Testa damage	Imbibition damage	Low
⇩	Little testa damage	No imbibition damage	High
Storage	High seed moisture content and/or temperature	Ageing	Low
	Low seed moisture content and/or temperature	Little ageing	High vigour

* Interaction of seed ageing x imbibition damage = very low vigour

2.3 The effect of storage conditions on seed quality

A number of investigators have reported that the speed of decline in seed quality during storage is largely dependent on temperature and relative humidity, seed moisture content, length of storage, type of seed and seed quality at storage (Yanping et al., 1999; Hung et al., 2001; Amjad and Anjum, 2002). High storage temperature has the potential to hasten the rate of biochemical processes causing more rapid deterioration that results in rapid vigour losses in seeds with high moisture content (Shelar et al., 2008). For example, the rate of decline in germination ability of barley seeds increased as seed moisture content and storage temperature increased (Kong et al., 2014). Earlier, Kibinza et al. (2006) observed that sensitivity of seeds to high temperatures was strongly dependent on their water content. More recently, Gupta et al. (2016) reported that storage temperature of 5 °C was low enough to slow the biochemical and physiological processes that lead to seed deterioration of chickpea. Similarly, Basavegowda and Arunkumar (2013) found that chickpea seeds stored at 5 to 7°C showed high germination quality.

Storage humidity is another key factor affecting seed quality. Alhamdan et al. (2011) observed that seeds stored at the highest relative humidity levels of 75.3% and 84.3% had significantly the lower seed germination percentage and mean germination time compared to those stored at lowest relative humidity levels 11.3% and 22.5%. Basavegowda and Arunkumar (2013) found that 65% to 70 % relative humidity can be effectively used for storing chickpea seeds with reduced deterioration. Also, storage fungi is a major cause of quality losses in stored seeds, and the extent of deterioration is dependent on the relative humidity of the storage environment (Mbofung et al., 2013). It is clear from the foregoing that proper control of storage conditions is essential for maintenance of seed viability.

2.4 The influence of seed deterioration

Seed deterioration refers to the loss of quality, with respect to viability and vigour, due to adverse environmental factors. The rate of deterioration fluctuates critically from one species to another and also among varieties of the same species (Jatoi et al., 2001). For example, small seeded chickpea varieties were found to deteriorate slower than large seeded ones (Sharma and Maheshwari, 2015). Seed deterioration is an undesirable result in chickpea production, and annual losses due to deterioration can be as high as 25% of the harvested crop (Tilebeni and Golpayegani, 2011). Deteriorated seeds have a potential of producing uneven stands, spotty fields, and fewer plants per hectare than healthy seeds of high quality (Biabani et al., 2011). According to Khatun et al. (2009), seeds retained on the mother plant deteriorate after

physiological maturity and physiological changes in a seed may lead to formation of rigid seeds in pulse crops including chickpea.

Biabani et al. (2011) reported that deterioration of seeds during storage can cause significant declines in seedling vigour and chickpea yield. Similarly, a study by Kapoor et al. (2010) showed that seed quality in chickpea deteriorates following the accelerated ageing period. Accelerated ageing has been widely used to study the physiological and biochemical changes in seeds during ageing. Thus Hussein et al. (2011) and Farhadi et al. (2012) reported that all the physiological parameters measured decreased significantly due to deterioration. In addition, changes in seed viability, germination rate and vigour was linked to biochemical changes (decreased soluble proteins and sugar content) associated with seed ageing (Kapoor et al., 2010). For the purpose of this study, both desi and kabuli chickpea seeds were subjected to accelerated ageing test (41°C and 100% RH) to investigate any differences in rate of deterioration.

2.5 The effect of seed ageing on seed quality

Seed ageing is defined as the reduction in vigour and germination capacity as well as increased solute leakage and reduced storage life under adverse conditions (Madah and Abdi, 2003; Gupta and Aneja, 2004). It is a well-recognized cause of differences in seed quality of all species, including grain legumes (Finch-Savage and Bassel, 2015). The rate at which the process of seed ageing takes place depends on the seed's ability to resist changes caused by degradation. Seed ageing and viability are affected by a number of factors during seed production and in seed storage (Bewley et al., 2012). In cases where relative humidity and temperatures are high, seed deterioration is faster as more rapid respiration takes place and in some instances fungal infestation develops (Magan et al., 2004). According to Bewley et al. (2012), low levels of respiration that sustain seed viability tend to deplete seed reserves with time, resulting in reduced seed vigour. Moreover, seeds that have low vigour as a result of ageing during storage are more likely to have poor field emergence (Finch-Savage and Bassel, 2015). This could be due to seed deterioration and increased susceptibility to imbibition damage if soil conditions are wet. Although the effect of seed ageing on seed quality is well documented, there is little evidence in literature on seed ageing and imbibition damage.

2.6 The effect of imbibition damage on seed vigour

Imbibition damage is another cause of low vigour in grain legumes (Matthews and Powell, 2006). It results from rapid uptake of water into the cotyledons which kills some of the surface cells and increases solute leakage. The damage to cells results from physical disruption of cell membranes (Lamichaney et al., 2016). Seeds of low vigour imbibe water more rapidly resulting in imbibition

damage that leads to dead tissue and high leachate conductivity. This is more severe on white seeded lots with loose adherence of testa that allows rapid movement of water through the gap between testa and cotyledons (Chachalis and Smith, 2000; Peksen et al., 2004). In contrast, seeds of high vigour imbibes water slowly due to the brown and black testa that adheres closely to the cotyledons and restrict water movement into the seed; this limits imbibition damage. A similar association of differences in emergence with colour of the testae occurs in chickpea where white seeded kabuli types emerge more poorly in comparison to the desi types that have brown or black testae (Yadav and Sharma, 2001). The white testae of the kabuli types adhere loosely to the cotyledons compared to the close adherence of coloured testae (Yadav and Sharma, 2001). The larger seed size and loose seed coat adherence may predispose the kabuli seed types to imbibition damage. This may considerably affect the germination and establishment of these seeds especially if they are stored under poor conditions.

2.7 The effect of water stress on seed quality

Water stress during seed growth and development on the mother plant may cause different responses depending on the stage at which stress occurs, its duration and severity. Generally, water stress occurring at, or soon after pollination, may reduce pollen viability and induce embryo abortion and hence limit the total number of seeds produced (Fang et al., 2009). According to Egli (2006), at later stages of development during dry matter accumulation, water stress may lead to reduced seed fill duration which results in shriveled seeds with low weight and poor quality. This is because photo-assimilate production and mobilization from the source (leaves) to sink (storage organs to the grain) is normally limited under water-stressed conditions since key processes such as photosynthesis and translocation rely on water availability (Liu et al., 2016). Desclaux et al. (2000) reported yield loss of up to 35-41% in soybean when water stress was imposed during seed filling. The major portion of this reduction was due to fewer number of seeds being produced (Pathan et al., 2014), but some yield loss was also associated with reduction in seed weight (Frederick et al., 2001). Indeed, water stress during seed production does not only reduce yield but also lower seed quality (Frederick et al., 2001). Although chickpea is known for its better drought tolerance than most of the other cool season legumes (Kashiwagi et al., 2015), water stress remains one of the major constraints to chickpea yield, accounting for 50% of yield reduction globally (Turner et al., 2001). Shrestha et al. (2006) and Leport et al. (2006) noted that water stress reduced chickpea yield by 42% to 70% due to pod abortion, reduced pod production and reduced grain-filling process. A reduction in the grain filling duration, which is a plants survival mechanism, ultimately results in less photosynthates that translates to grain weight and seed size. Although the effect of water stress on grain yield in chickpea and other legumes is well

documented, there is a dearth of information in literature on the likely effect of water stress during seed development on chickpea seed quality. The lack of knowledge could be because seed quality is influenced by an interplay of environmental and genotypic characteristics of the plant as well as the interaction between the two. Among the environmental factors, water stress is known to contribute to yield losses and seed deterioration. However, the actual effect of water stress occurring during seed development on seed quality is still limited. Such information will provide knowledge that will lead to better adoption of technologies that can enhance high seed quality production of chickpea.

2.8 The effect of water stress during seed development on accumulation of sugars, protein content and seed quality

The environmental conditions acting on the mother plant during plant growth also interact with seed developmental processes and this may influence the seeds physiological potential (Tekrony, 2003). For example, some studies showed that the interaction of water stress with seed developmental processes may cause impairment of physiological processes during seed development and consequently lead to intermediate levels of desiccation tolerance which may influence seed vigour (Hilhorst and Toorop, 1997). According to Hoekstra et al. (2002), water stress during seed development may limit the production of assimilates required to synthesize the raffinose family of oligosaccharides (RFOs) and cause alterations in the quantities and composition of accumulated storage compounds during seed maturation. These compounds are also a source of energy that subsequently drives the germination process. Thus implications for the acquisition of maximum desiccation tolerance may ultimately influence germination and vigour.

Moreover, water stress and desiccation tolerance are correlated with the accumulation of substantial quantities of compounds such as soluble carbohydrates (sucrose and the raffinose family of oligosaccharides) and specific proteins (such as the late embryogenesis abundant proteins and heat shock proteins) (Hoekstra et al., 2001). These sugars accumulate when desiccation tolerance is acquired and degrade when the tolerance is lost in orthodox seeds that can be dried at low moisture content without losing viability (Hoekstra et al., 2001), probably because sugars and specific proteins protect subcellular surfaces and confer desiccation tolerance (Leopold et al., 1994). Lahuta et al. (2000) reported that the composition of accumulated food reserves in field bean was altered by water stress and seeds produced had less dry matter, less starch but more oligosaccharides and sucrose. Sucrose is the major carbohydrate imported into the developing seed, which acts as a galactosyl acceptor in RFOs biosynthesis. Peterbauer

and Richter (2001) reported that the final content of RFOs deposited in mature seeds can be affected by the concentration of sucrose and myoinositol, which act as initial substrates in RFOs synthesis.

Although Lahuta et al. (2000) attributed changes in relative ratios of RFOs under conditions of water stress to a build-up in desiccation tolerance in field beans, they did not extend their study to examine how this would relate to seed vigour. However, Siddique and Wright (2004) examined differences in environmental conditions experienced by pea plant during development and maturation, the length of seed filling period, concentrations of soluble carbohydrates and proteins as potential causes of differences in seed vigour of peas. The authors concluded that none of these variables could solely explain the variations in seed vigour they observed. On the other hand, agricultural systems in semi-arid regions rely on the ability of orthodox seeds to tolerate desiccation (Lahuta et al., 2000; Bailly et al., 2001). However, despite the widespread phenomenon of desiccation tolerance in orthodox species and its importance to seed quality development, there is no conclusive evidence in the literature on the accumulation of RFOs and proteins in relation to water stress and its effect on seeds reproduction quality.

2.9 The effect of seed size on seed quality

Seed size is one of the major components of seed quality (Adebisi et al., 2011). Large seeds tend to have higher seedling growth and establishment (Jerlin and Vadivelu , 2004). Some studies showed that the large seeds of chickpea recorded superiority in growth traits as compared to small seeds (Emenky and Khalaf, 2010). Similarly, Roozrokh et al. (2005) showed that large seeds of chickpea had higher germination percentage compared to small seeds. Earlier, Vishvanath et al. (2006) reported that large seeds of French bean expressed higher seed quality parameters viz. germination percentage, field emergence and seedling length than small size seeds. Similarly, Mandel et al. (2010) found that larger seeds of *Hypatis suaveolous* showed higher emergence potential than smaller seeds. Moreover, germination and seedling emergence of *Convolvulus arvensis* was significantly affected by seed size, with large seeds having greater potential to germinate as compared to medium and small seeds (Tanveer et al., 2013). Hojjat (2011) reported that large seeds germinated early and showed better germination than small seeds of lentil genotypes.

However, some contrasting results have also been reported. For example, Majnoun Hosseini et al. (2009) observed that the time to emergence in desi chickpea was shorter than that for the kabuli genotype and mean germination rate was higher in the small desi seeds compared to large kabuli seeds. Probably due to the fact that smaller seeds have thinner coats which ensures

greater permeability and consequently, less duration of germination process. However, according to Tanveer et al. (2013), the higher potential of larger and medium sized seeds might be due to higher amount of nutrients available for germination. Moreover, Ogola and Mathews (unpublished) observed that kabuli chickpea generally had poor germination and establishment compared to desi types in both summer and winter sowings (both in the green house and in the field) irrespective of storage conditions. They postulated that this could be due to kabuli's larger sized seeds with light-coloured smooth coats that is prone to mechanical and imbibition damage. It is clear from the foregoing that the effect of seed size on seed vigour can vary greatly and this may need further investigation. Therefore we hypothesized that the larger seed size and loose seed coat adherence of typical kabuli chickpea may predispose the seeds to rapid water uptake, death of surface cells during the first phase of germination and consequently, poor stand establishment in the field as compared to the desi genotype with smaller size and cotyledons that are closely attached to the seed coat.

CHAPTER 3: INVESTIGATING THE PERFORMANCE OF CHICKPEA GENOTYPES DIFFERING IN SEED SIZE WITH RESPECT TO SEEDLING EMERGENCE

ABSTRACT

Agricultural productivity depends mainly on the quality of seeds sown. However, seed size is one of the components of seed quality that affects the performance of a crop. This study evaluated the performance of chickpea genotypes with different seed size on seedling emergence. A pot experiment was conducted twice (2016 and 2017) in a glasshouse, and laid out as a single factor (3 chickpea genotypes: Desi-K, Saina-K and ICCV-K) using a completely randomized design with 6 replications. Each replicate per genotype was represented by six 14L pots, giving a total of 36 experimental units. The emergence rate, final hypocotyl and complete emergence percentage were analysed. Small seeded Desi-K had significantly ($P<0.001$) higher and faster emergence compared to Saina-K and ICCV-K in both experiments. The ICCV-K genotype with large seed size (59.4 mm^2) emerged poorly (34.6% and 30.0%) compared to medium sized Saina-K (52.9 mm^2) which had 74.6% and 72.5%, and the small seeded Desi-K (43.7 mm^2) that had the emergences of 77.5% and 77.5% for final hypocotyl and complete emergence, respectively, in the first experiment. Similar results were obtained from the second experiment, ICCV-K had the lowest emergence (35.8% and 35.0%), followed by Saina-K (57.5% and 57.5%) and Desi-K (71.7% and 71.7%) on final hypocotyl and complete emergence, respectively. In terms of hypocotyl emergence rate, Desi-K took 4 -11 days while it took 4 -13 and 4 - 17 days for Saina-K and ICCV-K to final hypocotyl emergence. However, with respect to complete emergence rate, Saina-K had a minimum of 8 to 13 days followed by Desi-K (8 to 16 days) and ICCV-K (8 to 17 days). It was concluded that the poor and slow emergence of ICCV-K could be due to its larger seed size with loose coat adherence that imbibe more water during the first phase of germination, resulting in imbibition damage, low vigour and poor emergence.

Keywords: Seed quality, hypocotyl, complete emergence, emergence rate, seedling establishment

3.1 INTRODUCTION

Chickpea is an important grain legume with enormous potential for improving the economic status of resource-poor smallholder farmers through its nitrogen fixation capacity. Its seeds are rich in protein. There are two major groups of chickpea, namely microsperma (Desi) and macrosperma (Kabuli) types (Moreno and Cubero, 1978). The seeds of Desi chickpea are usually small, rough and dark coloured while Kabuli seeds are large with a smooth, light coloured seed coat. Kabuli is a popular and valuable genotype for the export that attracts heavy premiums from southern Africa and Middle East markets compared to Desi genotypes that are mainly produced for local consumption (Shiferaw et al., 2007; Varshney et al., 2013). However, seed size is an important physical indicator of seed quality that affects vegetative growth which is normally related to yield, market grade and harvest efficiency. The variation in seed size is caused by genetic variation between genotypes (Rosental et al., 2016). According to Dechaine et al. (2015), both the genotype and environment of a maternal plant may have an effect on seed size. Since chickpea has a narrow genetic pool resulting from domestication (Varshney et al., 2013), it is possible that there are differences in seed quality between the small seeded Desi and the large seeded Kabuli with respect to seedling emergence.

Rapid and uniform germination, which enhance seedling emergence and crop establishment, is considered an important factor that influences the yield potential. Generally, a genotype with large seed size is assumed to provide crops with a competitive advantage (Fenner and Thompson, 2005). The influence of seed size on seedling establishment has been demonstrated in some studies. Mandal et al. (2010) reported that variation in seed size influenced the emergence of pignut (*Hypatis suaveolous*), large seeds showed higher emergence potential than small seeds. Similarly, Nerson (2002) showed that small muskmelon seeds had the lowest germination percentage, emergence and lowest seedling growth demonstrating that there is a relationship between seed physical parameters and seed quality. Gunaga et al. (2007) and Gholami et al. (2009) observed that larger seeds generally germinate quicker and take lesser duration compared to smaller seeds of white dammar (*Viteria indica*) and pinto bean (*Phaseolus vulgaris*), respectively.

Similarly, results obtained by Roozrokh et al. (2005) showed that on average, large seeds of chickpea genotypes germinate faster and have higher germination percentage compared to small seeds. Also, Majnoun Hosseini et al. (2009) observed that the time to emergence of Desi chickpea genotype was shorter than that for the Kabuli genotype and the mean germination rate was higher

in the small Desi seeds compared to large sized Kabuli seeds. However, their studies lacked investigation on the effect of genotype on seedling emergence. Emergence exhibits the potential of seedling establishment compared to germination which is based on radicle protrusion. Furthermore, Kaya et al. (2008) noticed that small seeded chickpea genotypes, that imbibed 41.5% water during the first phase of germination, performed better and took shorter germination time compared to large seeded genotypes. Indeed, literature suggests that chickpea genotypes differ in seed germination (Anuradha et al., 2009).

The general observation is that Kabuli types perform poor compared to Desi types with respect to seed germination (Singh et al., 2017). This can have an effect on seedling emergence and affect yield performance. The Kabuli genotypes are large and have a lose seed coat adherence. This might predispose them to imbibition damage during the imbibition phase of germination. Secondly, there could be genotypic differences among the Kabuli and Desi types that may affect seed performance. These differences are not well understood. However, farmers tend to sow large seeds and have hesitation towards small seeds as they doubt emergence behaviour and uniform stand establishment capability of small seeds. Therefore, a preliminary study was conducted to determine the effect of seed size on seedling emergence based on the final hypocotyl and complete emergences of three chickpea genotypes.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

This study was conducted in 2016/17 under controlled glasshouse conditions at the Research Facility Centre, University of KwaZulu-Natal, Pietermaritzburg Campus (29º37‘30º24‘596).

3.2.2 Experimental design

A pot experiment was conducted twice in a glasshouse. A single factor (3 chickpea genotypes) arranged in a completely randomized design with 6 replications was used in both experiments. The three genotypes were: Desi-K, Saina-K and ICCV- K which had different seed sizes and coat colour (Figure 3.1). Desi-K had an average seed size of 43.7 and 42.2 mm^2 while Saina-K had (52.9 and 55.8.4 mm^2) and ICCV-K (59.4 and 56.2 mm^2) for experiment 1 and 2, respectively. For each experimental unit, 14 L pots with a diameter of 30 cm were used to plant seeds for the test of complete emergence.

3.2.3 Cultural practices

Soil of uniform class was collected from Ukulinga research farm of the University of KwaZulu-Natal. The soil was moistened and mixed using a Jaymix to ensure uniform soil texture. Each pot was filled with 12 kg of soil before 2400 mL water was added and allowed to drain to attain field capacity. Before sowing, seed size for each genotype was measured using a Vernier caliper (OMNI-TKCH®). Two seeds were sown per pot, at the depth of 5 cm using a dibber. An amount of 800 mL water was applied three times a week to promote seedling establishment after sowing.

3.2.4 Data collection

3.2.4.1 Hypocotyl emergence, complete emergence and emergence rate

During crop emergence, data on the appearance of hypocotyl and complete emergence of the seedlings was observed on daily basis from day zero (just after sowing) until fully expanded leaves were observed in each experimental unit (Figure 3.2). Emergence percentage was calculated by dividing the number of emerged seedlings by the total number of seeds sown. From these observations, the time to first day of hypocotyl and complete emergences were recorded for each treatment to evaluate the emergence rate.

3.2.5 Data analysis

Data analysis was executed based on analysis of variance (ANOVA) using GenStat® statistical software (18th edition). Significant differences between the treatment means for each parameter were compared using the least significant difference (LSD) test at $P < 0.05$ level of confidence.

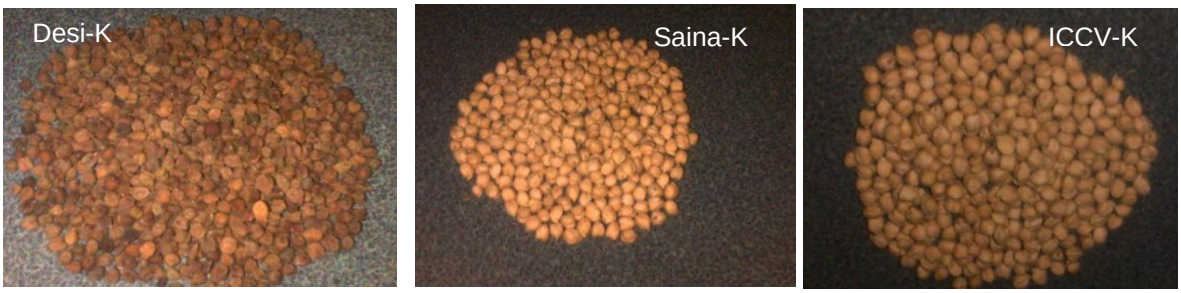


Figure 3.1: Desi-K and Kabuli (Saina-K & ICCV-K) chickpea genotypes differing in seed size and coat colour.

Figure 3.2: The illustration of chickpea hypocotyl (left) and complete emergence (right).

3.3 RESULTS AND DISCUSSION

The difference brought by effect of genotype seed size on seedling emergence was highly significant (P <0.001) in both experiment 1 and 2 (Figure 3.3 and 3.4). ICCV-K had larger seed size (59.4 mm^2) and poorest final hypocotyl (34.6%) and lowest complete emergence (30.0%) compared to Saina-K (52.9 mm^2, 74.6% and 72.5%) and Desi-K (43.7 mm^2, 77.5% and 77.5%), respectively, in experiment 1, (Figure 3.3). Similar trend was observed in experiment 2. ICCV-K with the largest seeds (56.2 mm^2) had the lowest emergence (35.8% and 35.0%), followed by Saina-K with medium seeds (55.8 mm^2, 57.5% and 57.5%) and Desi-K (42.2 mm^2, 71.7% and 71.7%) with smallest seeds and highest complete emergence, respectively (Figure 3.4). Figures 3.5 and 3.6 show the rate of hypocotyl and complete emergence of the three genotypes. In experiment 1, all genotypes showed that hypocotyl emerged 4 days after sowing. Thereafter, the rate of emergence was faster in Desi-K than the other two genotypes and its emergence ended on day 11. It was followed by Saina-K which ended emergence after 12 days and lastly by ICCV-K which took 17 days to end hypocotyl emergence. Similar trend was observed on the number of days to complete emergence as shown in Figure 3.5. All genotypes started their complete emergence 8 days after sowing. Saina-K took a minimum of 13 days to final emergence, followed by Desi-K and ICCV-K which both took 17 days. In experiment 2, the same trend was observed on the number of days to final hypocotyl emergence and complete emergence (Figure 3.6). The hypocotyls of Desi-K, ICCV-K and Saina-K emerged from day 4 to 11, 4 to 17 and 4 to 12, respectively. Similarly, the trend of complete emergence showed that all genotypes started complete emergence after 8 days and the emergence ended on day 13, 16 and 17 for Saina-K, Desi-K and 1CCV-K, respectively.

Overall results showed that both the rate and final percentage of hypocotyl and complete emergence were significantly affected by the seed size. The small seeded Desi-K had the highest and fastest emergence compared to the Kabuli genotypes (Saina-K and ICCV-K) in both experiments. This might be due to the smaller seeds having thinner coats that enabled greater radicle permeability and consequently, less duration of emergence process. This study showed that the Kabuli (ICCV-K with the large seed size) exhibited poor and slow emergence in both experiments. This could be due to its larger seed size with loose coat adherence that imbibe more water during the first phase of germination, resulting in imbibition damage, low vigour and poor emergence (Majnoun Hosseini et al., 2009). These results are in agreement with earlier studies that found that seed size caused by genetic variation between genotypes can affect seedling

emergence percentage of pigeon pea (Aarssen and Burton, 1990; Verma et al., 2005). Similarly, Majnoun Hosseini et al. (2009) observed that the time to emergence was shorter in small Desi seeds compared to larger Kabuli seeds. In contrast, Roozrokh et al. (2005) and Anuradha et al. (2009) showed that large seeded chickpea genotype had higher germination percentage compared to small seeds probably due to high amount of nutrients available for faster germination (Tanveer et al., 2013).

Also, previous studies of mungbean and lentil genotypes showed that larger seeds had higher seedling survival percentage, growth and establishment due to larger endosperm that enhanced emergence ability, through greater supply of stored energy to support early seedling growth and plant tissues compared to smaller seeds (Leishman, 2001; Hojjat, 2011). Soltani et al. (2002) revealed that large seeds had an advantage in producing more vigorous chickpea seedlings under saline or non-saline conditions. It then appears that environmentally or maternally based differences in seed size affect seedling survival differently than genetically based differences in seed size. However, Moles and Westoby (2004) reported that larger seeds tend to produce seedlings that are more likely to survive to maturity than seedlings from smaller seeds, but that is not always the case. Some inconsistency in the effect of seed size on seedling survival may be due to confounding different sources of genetic and environmental variation in seed size (Krannitz et al., 1991). According to Aarssen and Burton (1990), genetically based differences may arise due to differences among embryonic genotypes in their demands for nutritive material, or differences among maternal genotypes in the degree to which they fill their seed.

Although Anuradha et al. (2009) and Soltani et al. (2002) reported the positive influence of chickpea seeds' size on germination and seedling vigour. The results of this study contradicted that on the emergence of seeds. To the best of our knowledge, the present study is the first test of the effects of chickpea genotypes with different seed size on seedling emergence (emergence rate, final hypocotyl and complete emergences). The obtained results suggested that the measurements of seedling establishment at early stages should not be underemphasized since the stand establishment is obviously related to final grain yield. Hence, seedling emergence is generally assumed to play an important role in determining the establishment and yield of the crop. Furthermore, the present study discovered that the sole performance of chickpea genotypes with different seed size would not be expected to describe the seedling vigour of a lot. Therefore, there is need for further investigation to confirm whether the poor performance of the large sized ICCV-K seeds could be explained by coat adherence or other characteristics such as seed chemical composition.

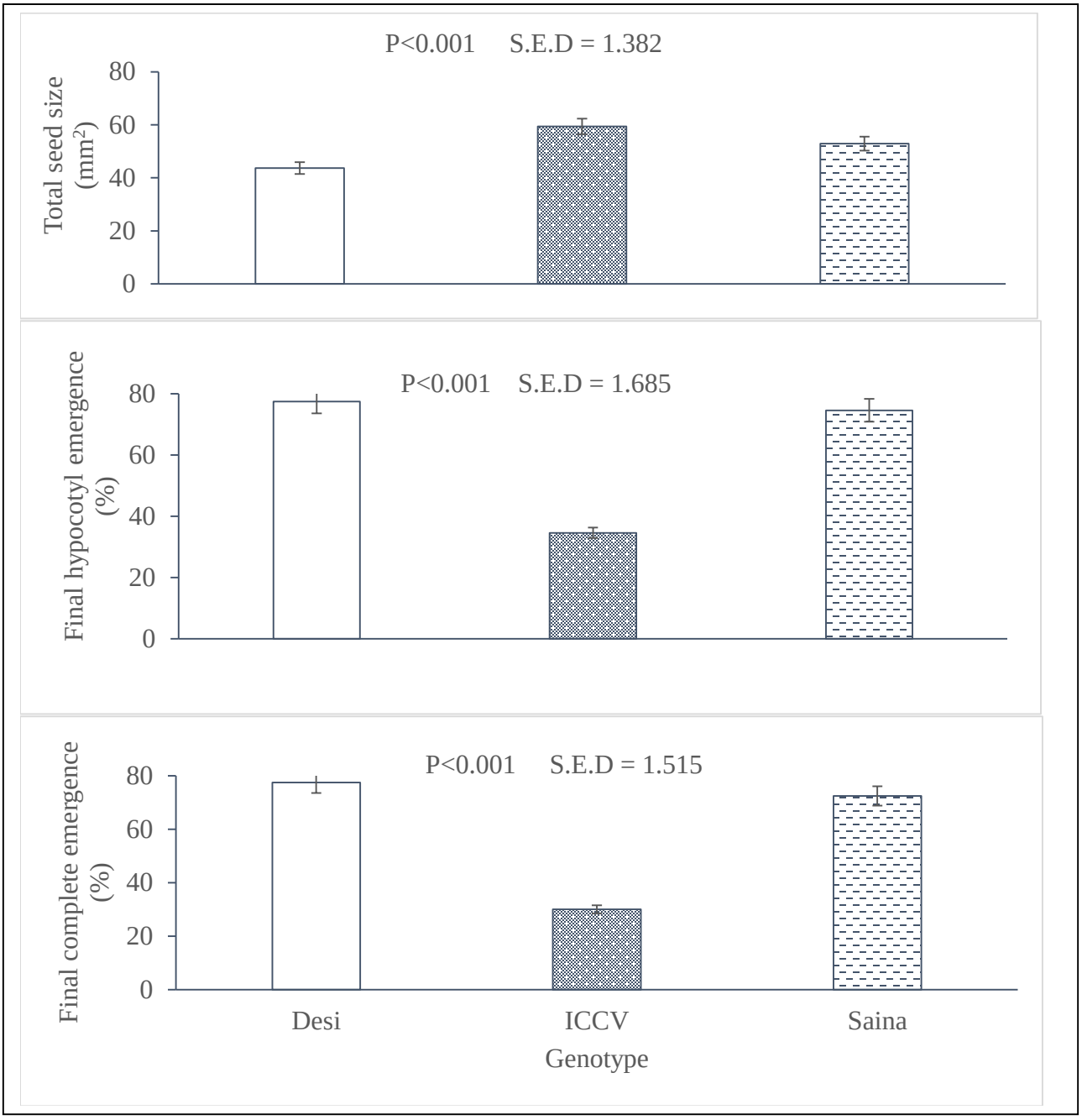


Figure 3.3: The effect of three chickpea genotypes with different seed size on final hypocotyl emergence and complete emergence in experiment 1.

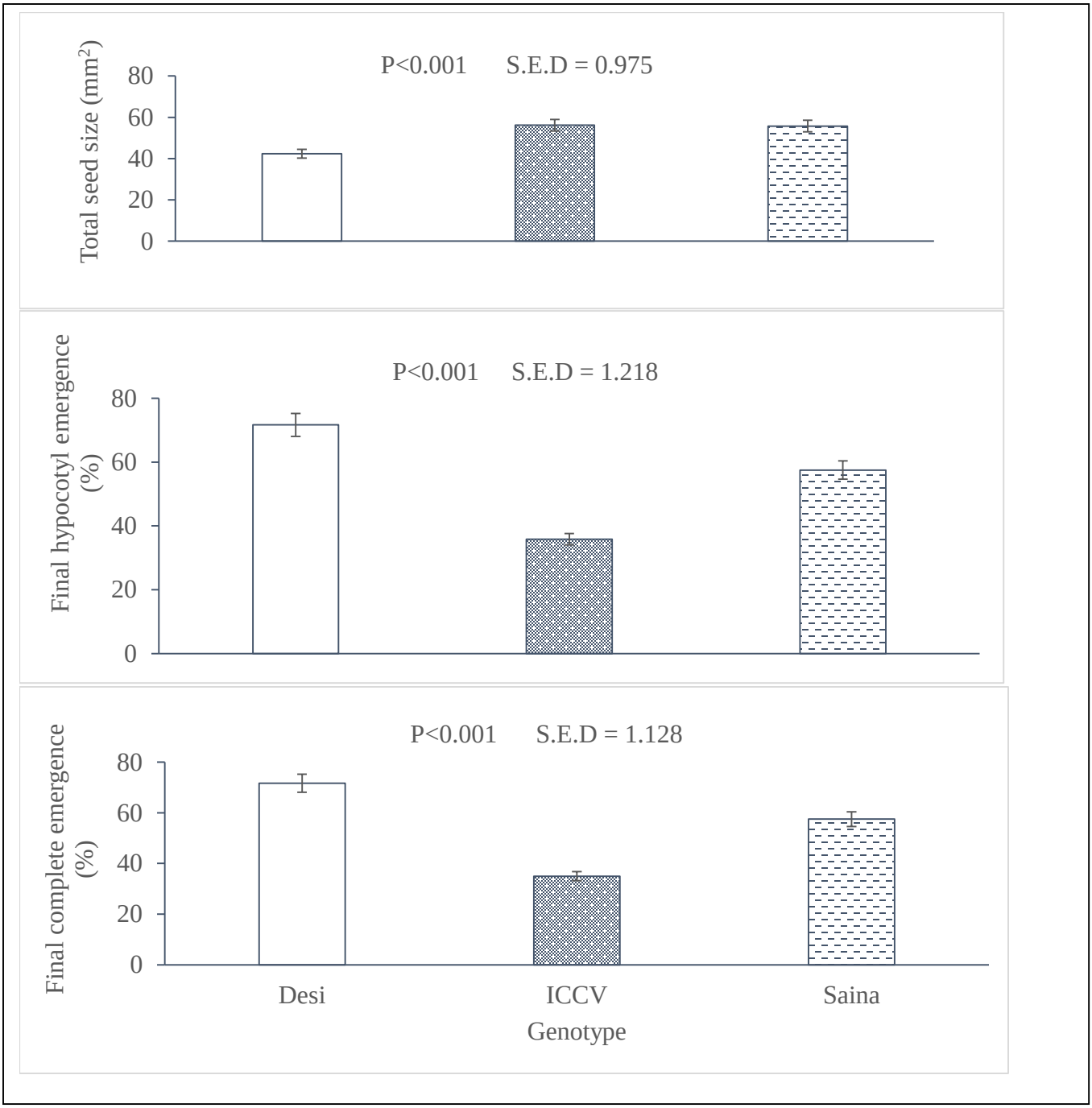


Figure 3.4: The effect of three chickpea genotypes with different seed size on final hypocotyl emergence and complete emergence in experiment 2.

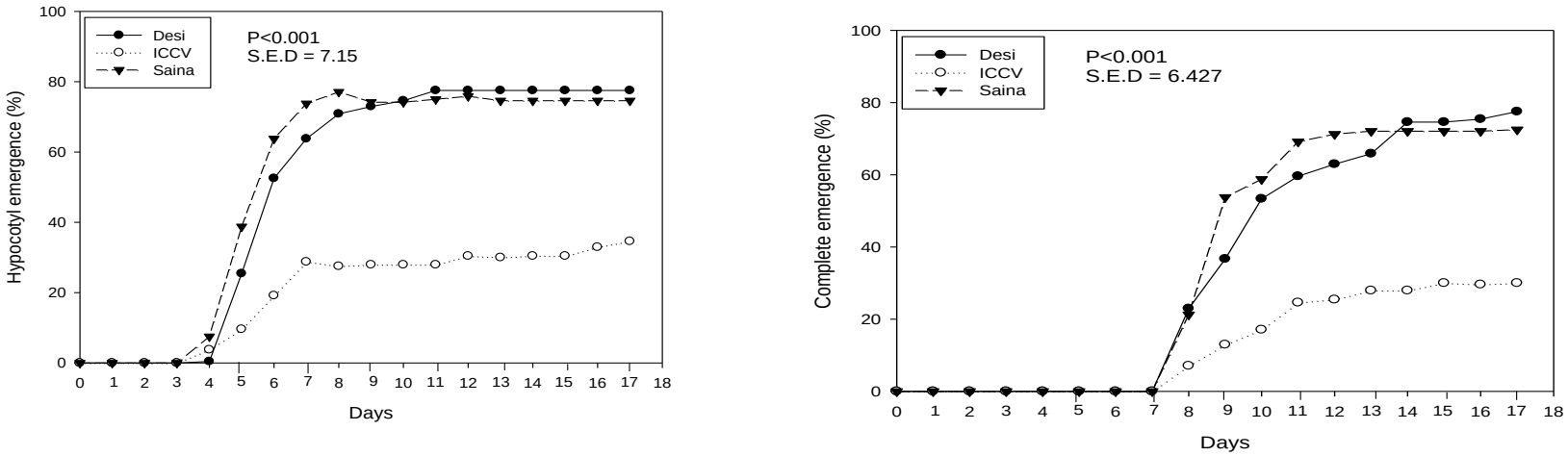


Figure 3.5: The influence of chickpea genotypes with different seed size on hypocotyl and complete emergence rate in experiment 1.

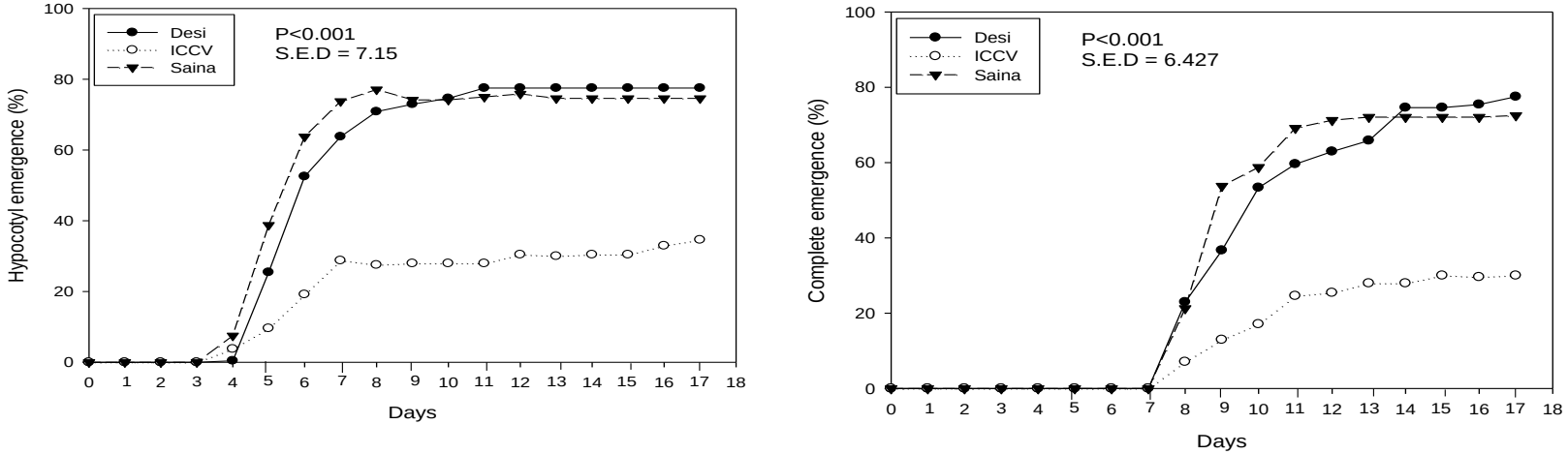


Figure 3.6: The effect of chickpea genotypes with different seed size on hypocotyl and complete emergence rate in experiment 2.

3.4 CONCLUSION

Overall results showed that chickpea genotypes with different seed sizes differ in seedling emergence. The seed size influences both the rate and final percentage of hypocotyl and complete emergence. Although, the small seeded Desi-K showed higher and faster emergence, Saina-K performed better than ICCV-K which exhibited poor and slow emergence in both experiments while both genotypes belongs to the same group (Kabuli). This indicated that the performance of chickpea genotypes with respect to emergence is not only affected by seed size but could be also affected by genetic variation among the genotypes. Therefore, further studies were recommended to investigate effects of genetic variation and biochemical profile on the performance of Desi-K and Kabuli chickpea genotypes (Saina-K and ICCV-K).

CHAPTER 4: THE EFFECT OF SEED AGEING ON SEED QUALITY AND IMBIBITION OF CHICKPEA GENOTYPES PRODUCED UNDER WATER STRESS AND NON-STRESS CONDITIONS

ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important grain legume in smallholder farming systems especially in the arid and semi-arid lands. Its production however is often limited by poor seed quality. The study evaluated the effect of seed ageing on physiological seed quality and imbibition of chickpea seeds harvested from plants grown under water stress and non-stress conditions. This experiment was a factorial combination of 2 water levels (stress and non-stress) and 3 genotypes (Desi-K, Saina-K and ICCV-K) arranged in a CRD with 3 replicates. Seeds obtained from the experiment were artificially aged for 0, 1, 3, 5 or 7 days at 41 ºC and 100% relative humidity. Data was collected on germination percentage (GP), mean germination time (MGT), electrical conductivity (EC), tetrazolium chloride test (TZ) and imbibition weight. The seed coat adherence of 3 genotypes was also viewed under a scanning electron microscope. There was significant ($P< 0.05$) differences in GP. Saina-K showed a 7% reduction (93 to 87%) compared to 29% (93 to 67%) in ICCV-K and 45% (73.3% to 40.0%) in Desi-K. The results are consistent with the observations on MGT (1.6, 1.7 and 1.9 days for Saina-K, ICCV-K and Desi-K, respectively). Desi-K had lower EC ($\mu S\ cm^{-1}g^{-1}$) (3.24) compared to Saina (4.08) and ICCV (4.13). Unaged seeds had greater seed viability (according to TZ test) (81.7%) compared to aged seeds (79.2%, 75.8%, 67.5% and 58.3% for 1, 3, 5 & 7 days, respectively). In terms of imbibition weight, ICCV-K had (0.49 g and 0.52 g) followed by Saina-K (0.38 g and 0.43 g) and Desi-K (0.45 g and 0.42 g) on seeds from non-stressed and stressed conditions, respectively. Moreover, the scanning electron microscope showed ICCV-K seed coat being loosely attached to the cotyledon followed by Saina-K that was slightly-loosely attached and Desi-K being tightly adhered to the seed coat. It was concluded that the higher imbibition, EC and lower GP in ICCV-K was probably due to its larger seed size with smooth coat that is loosely attached to the cotyledon, and seed deterioration caused by seed ageing.

Key words: drought, electrolyte leakage, accelerated ageing, seed deterioration

4.1 INTRODUCTION

Chickpea is an important grain legume that contributes significantly to food security and alleviates poverty amongst the poor resourced communities in semi-arid areas (Jalota et al., 2006). However, its production is insufficient to satisfy the ever increasing demand for food and poor communities that cannot afford expensive animal based proteins (Munirathnam and Sangita, 2009). In Africa, Ethiopia is the leading chickpea producer accounting for 2.5% of world and more than 55% of Africa's chickpea production followed by Tanzania with 2.1% of world production (Korbu et al., 2016). Although chickpea production in Africa shows potential for increase, most developed African countries such as South Africa produce insignificant amounts of it, while increased production can drastically increase food security (Korbu et al., 2016).

Chickpea production is constrained by biotic stresses (e.g. pods borer) and abiotic factors (water stress, soil salinity, high temperatures and low soil fertility) that also affect seed quality (Gowda et al., 2013). Losses in seed quality occur in the field, during harvest and under storage. In the field, environmental factors such as high temperatures during seed development can cause early pod ripening and rapid seed maturation that results into small seeds with poor quality (Thomas et al., 2009). Also, water stress soon after pollination may reduce pollen viability and induce embryo abortion and hence limit the total number of seeds produced (Fang et al., 2009). At later stages of seed development, especially during dry matter accumulation, water stress may lead to reduced seed fill duration (Egli, 2006).

In addition, prolonged seed storage and/or ageing adversely influence the viability of seeds. Seed viability deteriorate during storage (Biabani et al., 2011; Bewley and Black, 2012). Ageing shows reduction in seed vigour and results to germination of weak seedlings (Maity et al., 2000). The reduction in seed vigour is worse if seeds are stored at high temperatures and/or high relative humidity, as seed deterioration is faster where high respiration occurs (Mbofung et al., 2013). According to Magan et al. (2004), one of the main causes of seed deterioration is cell membrane disruption caused by increased free fatty acid levels and free reactive oxygen radicals by lipids. As a result, seed cells may not be able to retain their normal physical condition and function (Goel et al., 2003).

Imbibition damage is another cause of cell membrane damage that results to low vigour in grain legumes (Matthews and Powell, 2006). It results from rapid uptake of water into

the cotyledons, which damages some of the surface cells and increases solute leakage (Lamichaney et al., 2016). The level of leakage is influenced by the degree of seed ageing during storage which is more severe on the white seeded lots with a loose adherence of testa (Yadav and Sharma, 2001). Loose testa allows rapid penetration of water into the seed through a gap between the testa and cotyledon (Chachalis and Smith, 2000), which results to imbibition damage (Peksen et al., 2004). Seeds with brown or black testae have been indicated to have high vigour and slow imbibition because their testa adheres closely to the cotyledons and thus, restrict water penetration into the seed. In grain legumes, seed ageing, imbibition damage and their interaction are the major factors affecting seed quality (Matthews and Powell, 2006).

In previous studies, accelerated ageing was developed as a self-ageing technique to study the physiological and biochemical changes in chickpea seeds during ageing (Kapoor et al., 2010). However, there is currently limited studies investigating seed ageing influence on quality and imbibition of chickpea genotypes grown at different production environments. Therefore, this study evaluated the effect of seed ageing on germination, vigour and imbibition of three chickpea genotypes (Desi-K, ICCV-K and Saina-K) produced under water stress and non-stress conditions.

4.2 MATERIALS AND METHODS

4.2.1 Experimental seeds collection

A pot experiment was conducted in the winter seasons of 2016/2017 at the Controlled Research Facility Center, University of KwaZulu-Natal, Pietermaritzburg (29º 37‘30º 24‘596), South Africa. The experiment was designed as a 3 x 2 factorial in a controlled environment (tunnel), using a completely randomized design (CRD). Three chickpea genotypes: Desi-K, Saina-K and ICCV-K were used for this experiment. Two water levels: stressed (no irrigation after flowering) and non-stressed (irrigation with 800 mL pure water 3 times a week throughout the crop growth) conditions were simulated. Each experimental unit (genotype/water level) was represented by five (14 L) pots replicated three times, giving a total of 90 experimental units. The seeds were harvested at harvest maturity and used for the seed ageing experiment.

4.2.2 Accelerated ageing test

Seeds were exposed to 41 ºC; 100% relative humidity for 0, 1, 3, 5 and 7 days. The non-aged seeds (0 days) were used as a control. The ageing chamber was a plastic container measuring 8.0 x 8.0 x 5.0 cm (length x width x depth, respectively) covered with a lid. The chamber had a wire tray with a 10.0 x 10.0 x 2.0 cm (length x width x depth, respectively) mesh screen and the pore sizes of the mesh screen was 1.89 mm^2 (1.16 mm x 1.63 mm) (Figure 4.1). In each accelerated ageing plastic container, 40 g of sodium chloride and 100 mL of distilled water was added and a dry screen tray was inserted carefully not to splash water on the screen. The chickpea seeds were then weighed and placed on the surface of the screen tray, one layer deep to ensure even uptake of moisture from the humid environment. A lid was placed on each accelerated ageing plastic container, ensuring that edges were not sealed. The plastic accelerated ageing containers with seeds were then placed on a shelf in the ageing chamber, allowing an air space of approximately 2.5 cm between accelerated ageing plastic boxes to ensure temperature uniformity. Seeds were removed according to predetermined intervals (1, 3, 5 or 7 days) to investigate the effect of the accelerated ageing on seed physiological quality and imbibition.

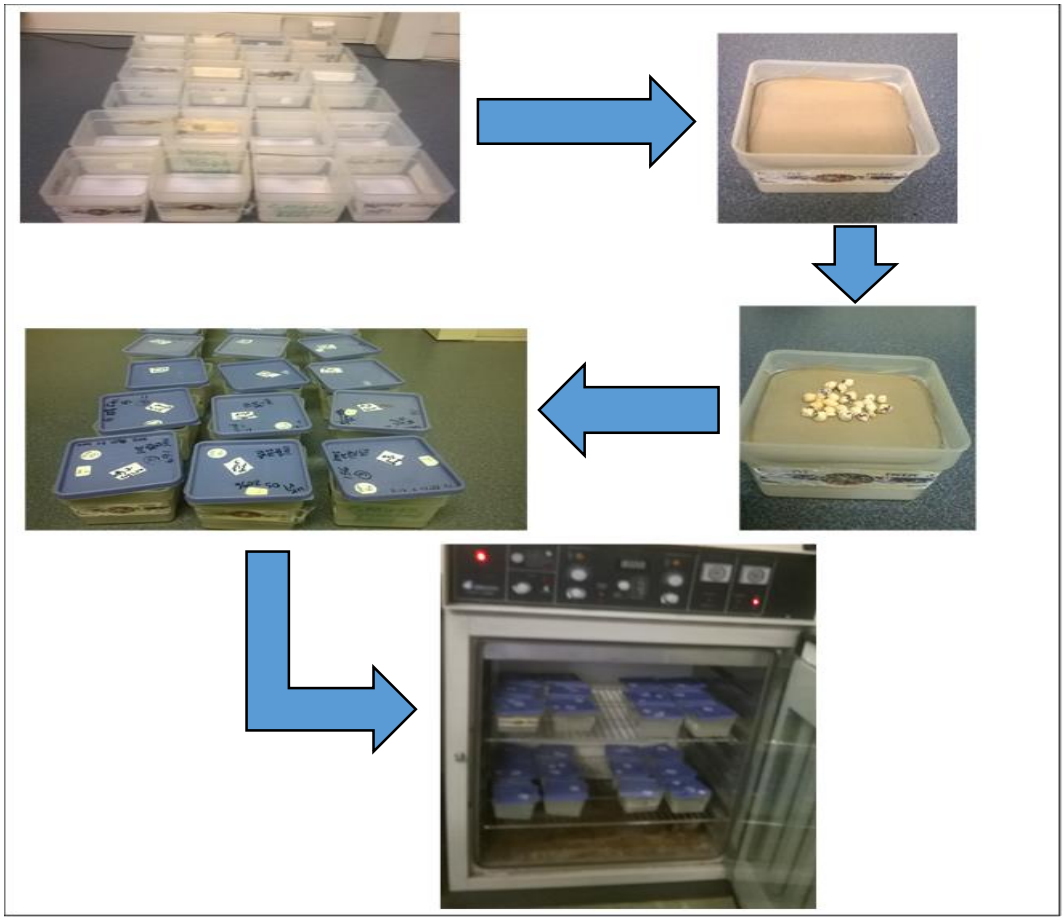


Figure 4.1: An illustration of set-up of containers used during accelerated ageing test.

4.2.3 Data collection

4.2.3.1 Germination percentage

Germination percentage was evaluated according to ISTA (2012). Three replicates of ten seeds obtained from days 0, 1, 3, 5 and 7 of the accelerated ageing test were germinated using the moist brown paper towel method. Seeds were arranged in straight lines midway on the moistened paper towels. The paper towels were then rolled and placed in a zip lock bag to avoid moisture loss. Then bags were placed in a germination chamber set at 25°C. Germination was evaluated by counting, from day 0 to day 8, the number of germinated seeds that had 2mm radicle protrusion. Equation 1 was used to calculate the germination percentage of each treatment.

$$\text{Percentage germination (GP)} = \left(\frac{\text{Seeds germinated}}{\text{Total seeds}} \right) \times 100\% \qquad (1)$$

4.2.3.2 Mean germination time

Mean germination time was assessed on the basis of radicle protrusion and counted daily from the day of planting (Day 0) until there was no germination increase. The data obtained from the assessment was used to calculate the mean germination time according to Heydecker (1968) as shown in equation 2.

$$\text{Mean germination time (MGT)} = \left(\frac{\sum FX}{\sum X} \right) \quad (2)$$

Where F is the number of days from the beginning of the germination test, and X is the number of seeds newly germinated on that day.

4.2.3.3 Electrical conductivity

In order to determine the amount of solute leakage from seeds (µS $cm^{-1}g^{-1}$), the electrical conductivity (EC) of seeds was measured according to ISTA (2012) using EC meter (Jenway, 4510 model). The meter was calibrated using a 0.01 M potassium chloride (KCL) solution that was prepared by dissolving 0.745 g of pure, dry analytical grade potassium chloride in 1 L of distilled water. The calibration and analysis was carried out at room temperature. Ten seeds per experimental unit were put into beakers and immersed with 20 mL of distilled water for 24 hours. After 24 hours, EC was recorded from the imbibed seeds. Equation 3 was used to calculate the conductivity (µS $cm^{-1}g^{-1}$).

$$\text{EC (µS/cm/g)} = \frac{\text{Conductivity reading – Background reading}}{\text{Weight of replicate (g)}} \quad (3)$$

Where background reading was the control/blank.

4.2.3.4 Tetrazolium chloride test

Seed viability was assessed using tetrazolium chloride (TZ) test according to ISTA (2012). An aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride (1.0%) was prepared using distilled water (pH 7.0). Seeds were immersed in the distilled water for 18 hours before they were cut longitudinally through the embryo using a razor blade. The prepared seeds were put in a 90 mm petri dish and fully immersed with the TZ solution for 2 hours. The reaction was kept at room temperature (25 ºC). The preparation room was kept dark the entire time of the experiment because TZ is sensitive to light. Tissues that stained reddish

pink were regarded as viable and those unstained were regarded unviable. The number of viable seeds were counted from each treatment based on the ability of TZ to stain viable embryos. The seed viability was calculated using equation 4.

$$\text{Seed viability}\,\% = \left(\frac{\text{Number of stained embryos}}{\text{Total number of embryos}}\right) \times 100\% \qquad (4)$$

4.2.3.4 Imbibition

Imbibition test was determined using a moist brown paper towel method. The initial weight of individual aged and unaged seeds of three genotypes produced under water stressed and non-stressed conditions was determined using a digital weighing balance. Thereafter, four replicates of ten seeds were labelled per experimental unit. Each replicate was arranged in straight lines midway of three paper towels that were moistened with 150 mL of distilled water. The paper towels were then rolled and placed in a zip lock bag to avoid moisture loss. The duration of seed imbibition was 0.5, 1, 2, 3, 6, 12 or 24 hours. For each time interval, the weight of individual seed per experimental unit was determined to assess the effect of seed ageing on imbibition rate of chickpea genotypes produced under water stress and non- stress condition.

4.2.3.5 Seed coat adherence

The seed coat adherence of three genotypes (Desi-K, ICCV-K and Saina-K) was viewed under a scanning electron microscope at 25 x magnification. The seed samples were mounted on the stub using carbon double sided tape. They were then cut, transferred into a 90 mm petri dish and sputter coated with gold using Quorum (Q150R ES). The seed coat adherence of chickpea genotypes was viewed using Zeiss Evo / LS 15 SEM at high vacuum (Figure 4.2).

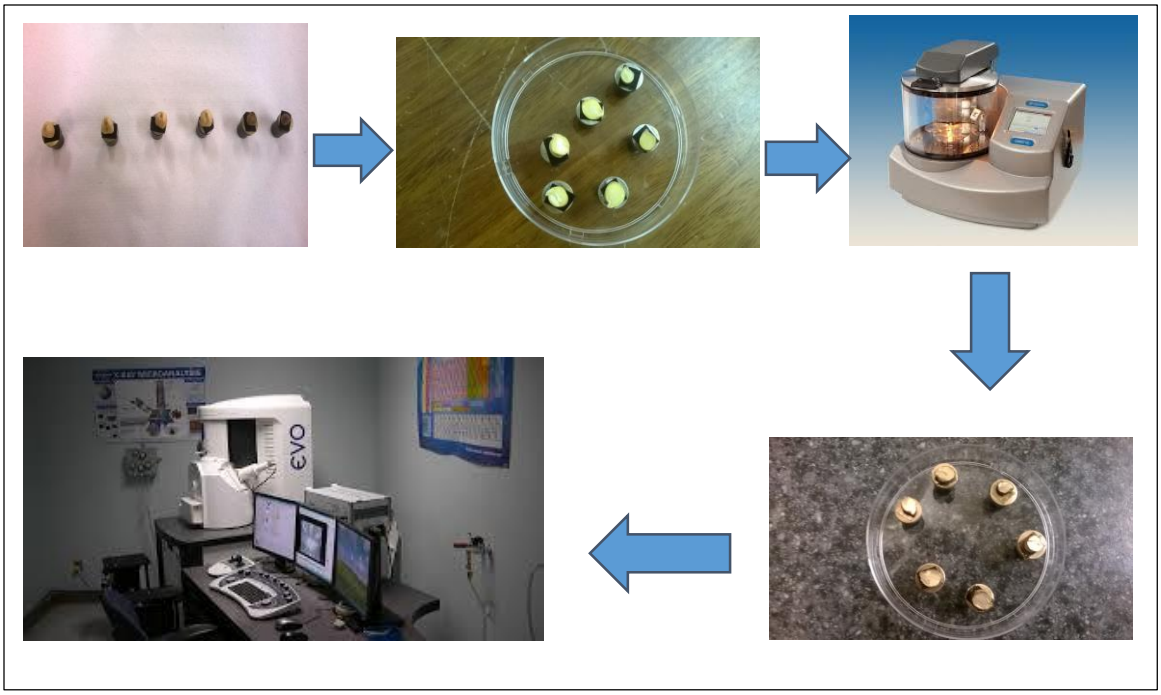


Figure 4.2: An illustration of the process of seed coat adherence viewing using a scanning electron microscope.

4.2.3.6 Data analysis

Data analysis was executed based on analysis of variance (ANOVA) using GenStat® statistical software (18th edition). Significant differences between the treatment means for each parameter were compared using the least significant difference (LSD) test at $P < 0.05$ level of confidence.

4.3 RESULTS AND DISCUSSION

4.3.1 The effect of seed ageing on physiological quality parameters

There was a significant (P<0.05) interaction effect of seed ageing on germination percentage of the three chickpea genotypes produced under water stress and non-stress conditions (Figure 4.3). Genotypic differences were observed among the cultivars after ageing for 7 days. Saina-K showed a 7% reduction (93 to 87%) in germination percentage compared to ICCV-K: 29% (93 to 67%) and Desi-K with 45% (73.3% and 40.0%) under non-stressed and stressed conditions. The results were consistent with those observed for mean germination time showing Saina-K with the lowest (1.6 days) followed by 1.7 and 1.9 days of ICCV-K and Desi-K, respectively (Figure 4.4). However, the interaction of seed ageing, genotype and production environment on mean germination time was not significant (P >0.05).

Generally, a genotype with lowest germination percentage took maximum mean germination time after the accelerated ageing period. This showed that seed ageing slowed the process of germination. Similar results were observed by Ghassemi-Golezani et al. (2014) on lentil seeds. The decrease in germination percentage by accelerated ageing may be a result of progressive loss of seed viability and vigour (Jain et al., 2006; Hussein et al., 2011). According to Bewley et al. (2012), low levels of respiration that sustains seed viability tends to deplete seed reserves with time, resulting in reduced seed vigour when seeds are stored under high temperatures and relative humidity. However, Bijanzadeh et al. (2017) reported that the reduction in germination might be due to degradation of mitochondrial membrane during seed ageing, leading to reduction in energy supply necessary for germination (Pandey and Pati, 2017). Such differences on seed germination capacity were observed on pea seeds (Khan et al., 2003) and in pigeon pea (Kalpana and Madhava, 1995). Also, progressive depreciation of seed viability during seed ageing was previously reported by many studies (Maity et al., 2000; Jatoi et al., 2001; El-Keblawy, 2003).

Moreover, significant differences (P<0.05) among the three genotypes and production environment were observed on electrical conductivity (EC) after seed ageing. ICCV-K had higher EC (4.13 µS $cm^{-1}g^{-1}$) compared to Saina-K (4.08 µS $cm^{-1}g^{-1}$) and Desi-K (3.24 µS $cm^{-1}g^{-1}$) (Figure 4.5). Similarly, seed lots produced under water stressed condition had higher EC (4.31 µS $cm^{-1}g^{-1}$) compared to non-stressed seed lots (3.32 µS $cm^{-1}g^{-1}$) (Figure

4.6). However, the interaction effect of seed ageing on genotypes and the production environment was insignificant (P>0.05). The high electrical conductivity of ICCV-K and seed lots produced under water stressed condition resulted from disruption of cell membrane caused by seed ageing and imbibition damage (Volk et al., 2006; Arun et al., 2017; Gimenez et al., 2017). Similar results were reported on dwarf French beans (Powell et al., 1986) and in cowpeas (Aveling and Powell, 2005). Basra et al. (2003) showed that an increase in cell membrane leakage was due to addition of free fatty acids that increased fusion of plant vesicles. Trawatha et al. (1995) and Navaey et al. (2014), concluded that free fatty acids have a deleterious effect on membranes probably because they are detergents. The results were in agreement with findings of Wojtczak and Więckowski (2016) who reported that in the presence of free fatty acids, isolated plant mitochondria showed swelling and uncoupling of oxidative phosphorylation.

In terms of tetrazolium chloride (TZ) test, seed ageing significantly (P<0.05) reduced seed viability. Unaged seeds (day 0) had the highest seed viability of 81.7% compared to aged seeds which had 79.2%; 75.8%; 67.5% and 58.3% for 1, 3, 5 or 7 days, respectively (Figure 4.7). The finding showed evidence of reduced TZ staining following the accelerated ageing period as previously observed in other grain legumes (Abdullah et al., 1990; Khan et al., 2003; Matthews and Powell, 2006). It is envisaged that the membranes of aged seeds, whose integrity has been reduced by deterioration, are more susceptible to poor vital staining. Thus seeds that have low vigour as a result of seed ageing will be more likely to have poor field emergence due to seed deterioration.

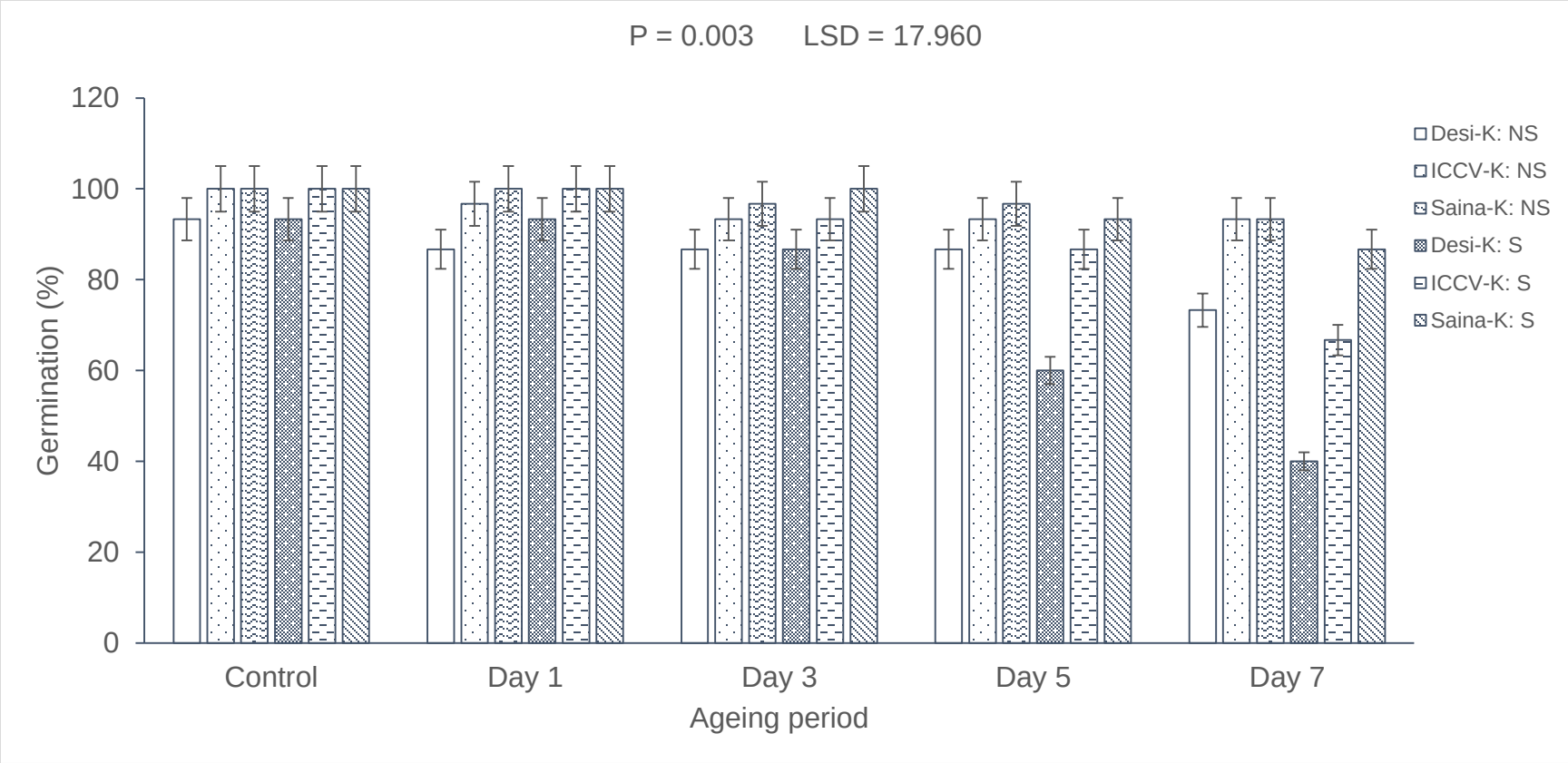


Figure 4.3: The effect of seed ageing period on germination percentage of three chickpea genotypes grown under water stressed and non-stressed conditions.

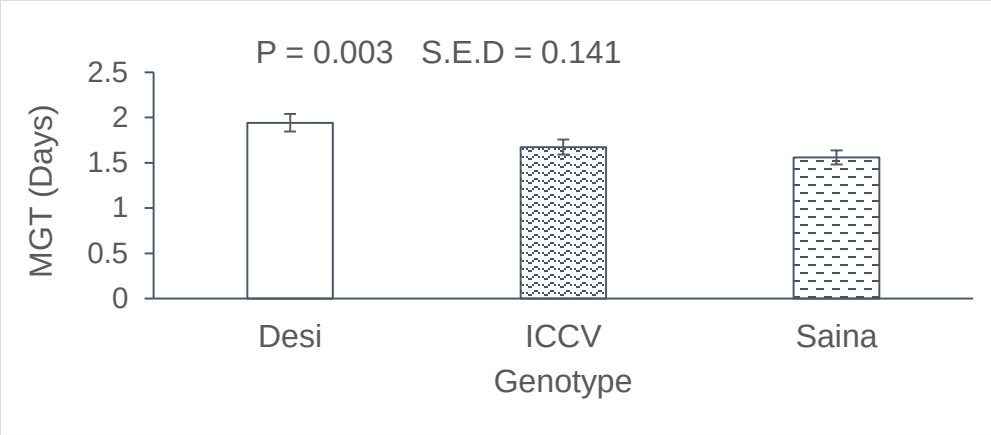


Figure 4.4: The effect of seed ageing on mean germination time of three chickpea genotypes.

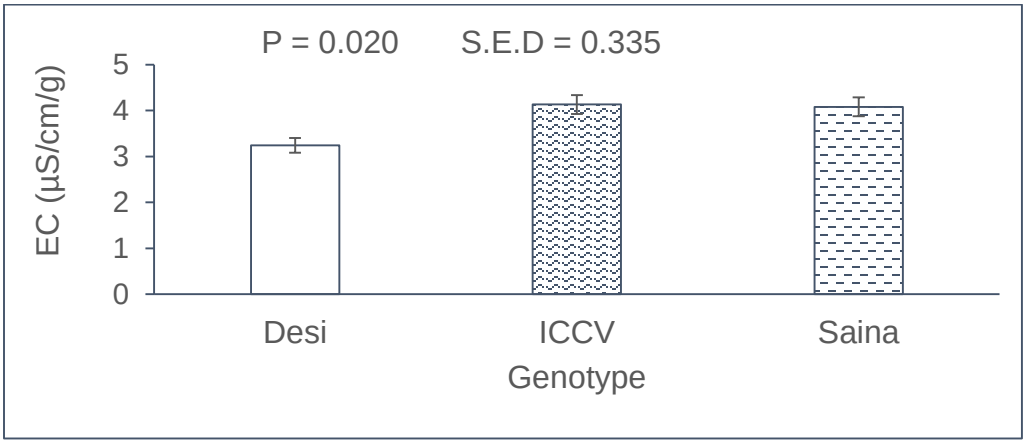


Figure 4.5: The effect of seed ageing on electrical conductivity of three chickpea genotypes.

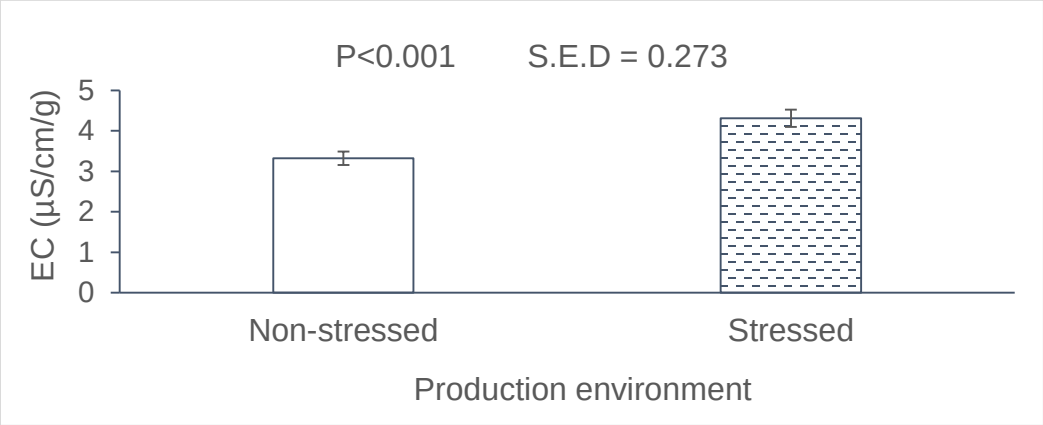


Figure 4.6: The effect of seed ageing on electrical conductivity under different production environment.

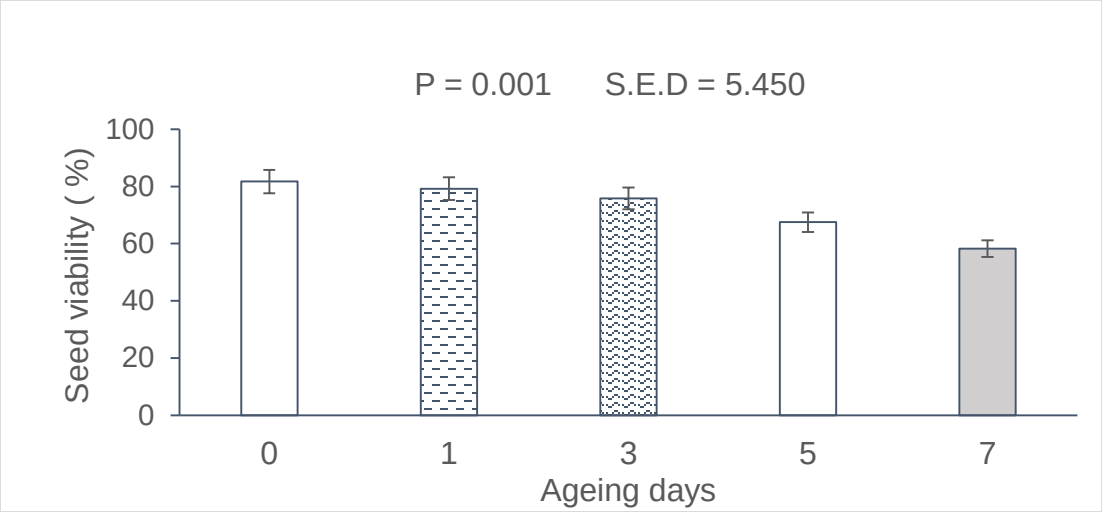


Figure 4.7: The effect of accelerated ageing period on seed viability (TZ %).

4.3.2 The effect of seed ageing on imbibition of chickpea genotypes

There was a highly significant ($P < 0.001$) interaction effect of seed ageing on the imbibition of three genotypes produced under water stressed and non-stressed conditions. Genotypic differences were observed among the cultivars after ageing for 7 days. ICCV-K had higher imbibition weight (0.49 g and 0.52 g) compared to Saina-K (0.38 g and 0.43 g) and Desi-K (0.45 g and 0.42 g) under non-stressed and water stressed conditions, respectively (Figure 4.8). The results were consistent with those observed for weight increase pattern during imbibition time. ICCV-K showed rapid weight increase (0.39, 0.59 and 0.62 g) compared to Saina-K (0.33, 0.52 and 0.55 g) and Desi-K (0.32, 0.49 and 0.51 g) after 30 minutes, 12 and 24 hours, respectively (Figure 4.9 A). Moreover, significant differences ($P < 0.001$) were also observed on the weight increase pattern of unaged and aged seeds (Figure 4.9 B). Unaged (day 0) seeds showed the lowest weight increase (0.33 - 0.50 g) compared to the highest change in seed weight (0.35 - 0.51 g; 0.34 - 0.54 g; 0.34 - 0.56 g and 0.36 - 0.57 g) on seeds aged for 1, 3, 5 and 7 days after imbibition time of 30 minutes to 12 hours, respectively. With respect to the production environment, seed lots from stressed condition showed rapid imbibition with increased weight of 0.35, 0.55 and 0.57 g compared to non-stressed seed lots with 0.34, 0.52 and 0.54 g after 30 minutes, 12 and 24 hours of imbibition, respectively (Figure 4.9C).

Furthermore, the scanning electron microscope showed ICCV-K seed coat being loosely attached to the cotyledon, followed by Saina-K that was slightly- loosely attached to the cotyledon (Figure 4.10A and 4.10B). However, Desi-K showed tightly adherence of seed coat to the cotyledon (Figure 4.10C). This observations revealed that the loose adherence of seed coat to the cotyledon

is the major factor influencing the imbibition rate of chickpea genotypes. Thus, ICCV-K showed rapid and higher imbibition compared to the other genotypes. Similar results were reported on other grain legumes (Powell et al., 1986; Chachalis and Smith, 2000; Matthews and Powell, 2006). According to Peksen et al. (2004), the white or light seed coats tend to loosen rapidly during imbibition while the coloured seed coats remain closely attached to the cotyledons, suggesting that the loose adherence of seed coat to the cotyledons results into rapid movement of water between the testa and cotyledons (Yadav and Sharma, 2001). Previous studies have also shown that pigmented testae in dwarf French beans (Powell et al., 1986) and in long bean (Abdullah et al., 1991) are associated with the slow rate of imbibition. The authors' findings are in agreement with this study. The slow rate of imbibition observed in dark coloured Desi-K resulted from the tightly adherence of seed coat to the cotyledon that restricted water movement into the seeds, hence limiting imbibition damage. It is clear that the low vigour found in ICCV-K or white seeded genotypes could be explained by imbibition damage which is expressed in high solute leakage and poor germination. This study concluded that, the poor seed quality exhibited by kabuli chickpea genotype (ICCV-K) was due to the loose seed coat adherence that allowed rapid water uptake by the seeds resulting to imbibition damage, high electrical conductivity (dead cell tissue) and consequently, low seed vigour and poor germination/ emergence.

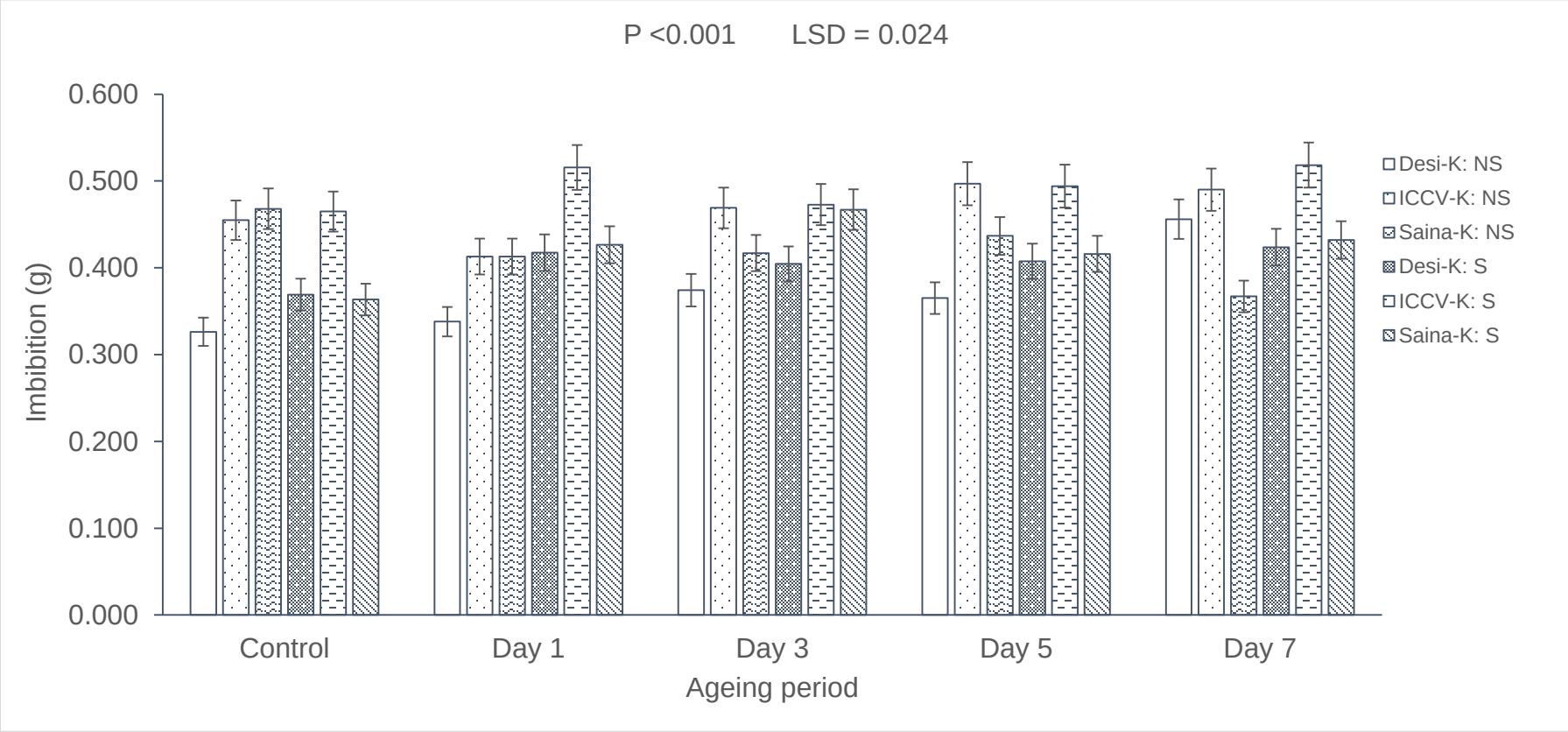


Figure 4.8: The effect of seed ageing on the imbibition of three chickpea genotypes grown under water stressed or non-stressed conditions.

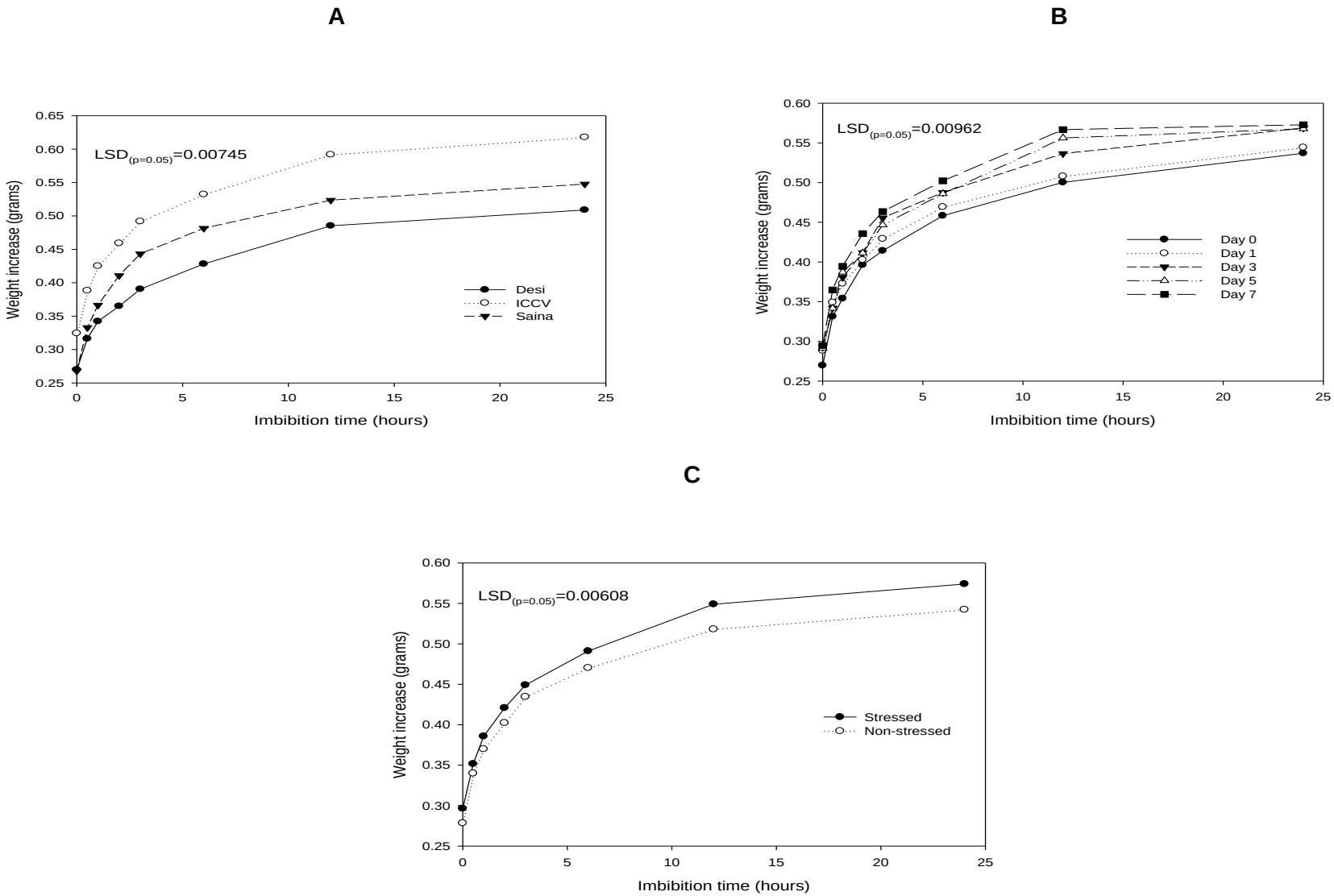
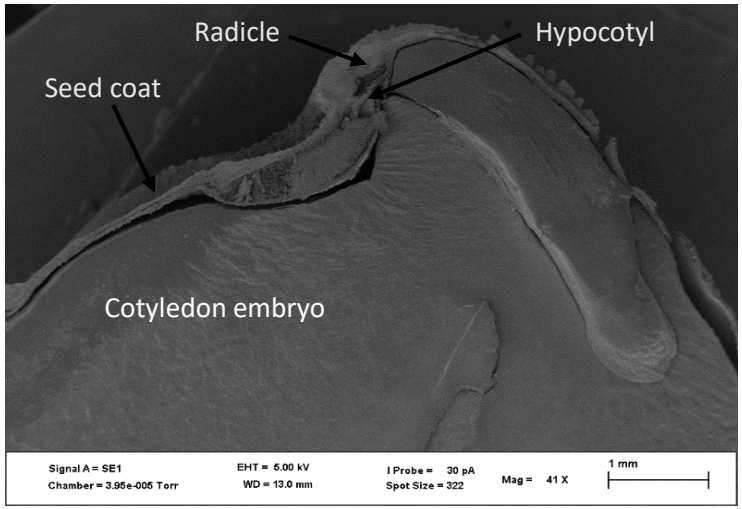
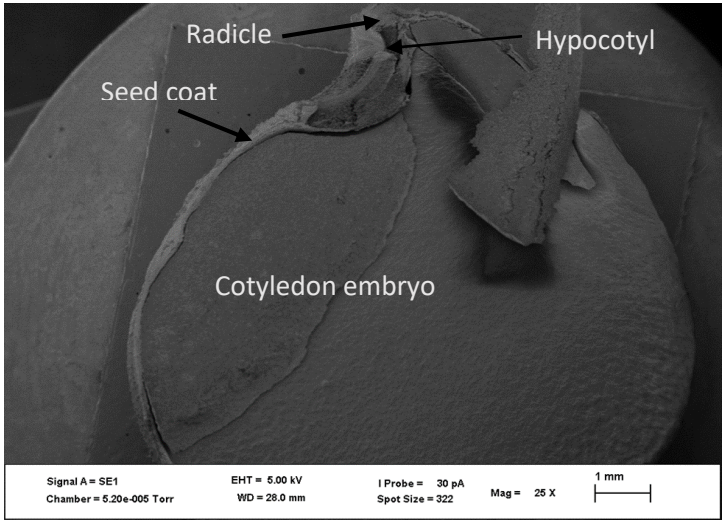


Figure 4.9: The pattern of weight increase during imbibition on three chickpea genotypes (A), unaged (day 0) and aged seeds (B) and the production environment (C).

A - (ICCV-K)



B - (Saina-K)



C - (Desi-K)

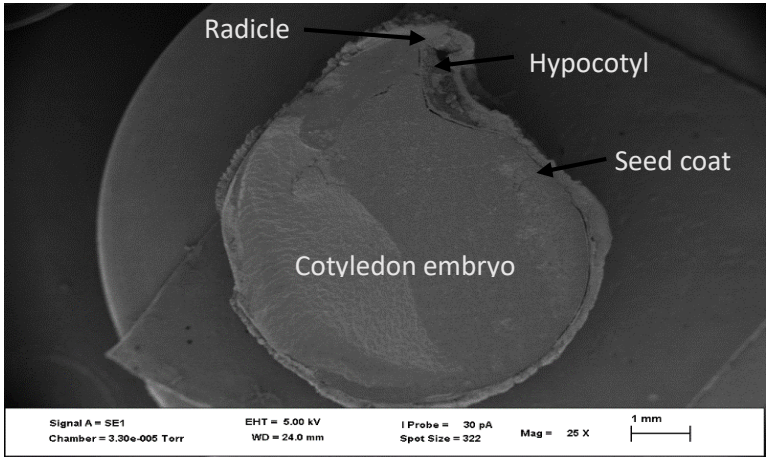


Fig. 4.10: The seed coat adherence of ICCV-K (A); Saina-K (B) and Desi-K (C) viewed under a scanning electron microscope at 25 x magnification

4.4 CONCLUSION

The study showed that seed ageing can have a negative effect on seed quality and imbibition of chickpea genotypes produced under water stress and non-stressed condition. Seed ageing caused progressive loss of seed viability, vigour and increased imbibition rate which resulted to cell death and high solute leakage from the seeds. This research further revealed that the poor performance of Kabuli (ICCV-K) chickpea genotype was not only caused by seed ageing but also the loose seed coat adherence allowed rapid water uptake by the seeds during imbibition, resulting to imbibition damage and consequently, low seed vigour and poor germination. This finding suggest that there are genetic differences among chickpea genotypes with respect to the production environment which could affect storage potential and consequently seed quality. It was recommended that farmers must ensure precise water supply during seed development stage and proper storage conditions after crop harvest. Moreover, further studies on genetic variation, possible mechanisms and regulatory processes that control chickpea seed quality during production and storage are recommended.

CHAPTER 5: THE EFFECT OF WATER STRESS DURING SEED DEVELOPMENT ON SUGARS AND PROTEINS ACCUMULATION, GERMINATION AND SEED VIGOUR OF CHICKPEA

ABSTRACT

Water stress during seed development may affect chickpea seed quality through its effect on seed soluble sugars and proteins. This study evaluated the effect of water stress during seed development on the accumulation of soluble sugars, proteins and physiological variables allied to chickpea establishment. The growth experiment was laid out as a 3 x 2 factorial design with 3 chickpea genotypes (Desi-K, Saina-K and ICCV-K) and 2 production environments: water stress (no irrigation after flowering) and non-stressed (800 mL at 3 days intervals throughout the crop growth). Seeds were harvested at harvest maturity and germination as well as seed vigour were analysed. Stressed seeds had significantly ($P< 0.05$) higher total sugars (208.2 vs. 206.9 µg/g) than non-stressed seeds. However, protein contents of stressed and non-stressed seeds did not differ significantly (11.87 vs. 11.86 µg/g). Non-stressed seeds were larger (45.3 vs. 41.9 mm^2) with higher seed viability (74.2% vs. 55.8%) and germination percentage (63.3 vs. 60.0%), but lower electrical conductivity (648 vs. 833.3 µS/cm/g) and mean germination time (1.3 days vs. 1.8 days) than stressed seeds. It was concluded that irrigation during seed development reduces the final sugars and protein content but increases the seed size and physiological quality parameters allied to production of chickpea seeds. Therefore, water availability to chickpea crop is critical during seed development.

Keywords: Seed quality, drought, legumes, chemometrics, PCA

5.1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an alternative source of proteins with advantages including inhabitation of low production soils while relatively affordable after harvesting (Ma et al., 2005). It plays a distinct role in agricultural ecosystems through nitrogen fixation and is known as an excellent colonizer of low nitrogen soils (Graham and Vance, 2003). However, water stress is one of the major constraints to chickpea production because of resultant low seed quality (Turner et al., 2001). Water stress causes significant economic losses on chickpea smallholder farmers. This stress may arise from environmental conditions such as drought, salinity or extreme temperatures that induce abnormal biochemical and physiological responses from plants. Environmental conditions acting on the mother plant interact with seed developmental processes and this influences the potential of seeds to reproduce vigorously (Tekrony, 2003; Hilhorst and Toorop, 1997).

During seed development, water stress can limit the production of assimilates that are necessary to synthesize soluble carbohydrates (Hoekstra et al., 2001). Soluble carbohydrates are important source of energy for germination and establishment strength (Hoekstra et al., 2003). The major soluble carbohydrates in legume seeds such as chickpea are sucrose, raffinose, stachyose and verbascose, which belong to raffinose family of oligosaccharides (RFOs) (Ma et al., 2005). Previous studies have indicated soluble carbohydrates to play a significant role in the acquisition of desiccation tolerance and have been implicated to protect seeds against disruption of both cell membranes and cytoplasm (Leopold, 1990). Leopold (1990) found that the contents of soluble sugars accumulated in orthodox seeds when desiccation tolerance was acquired and degraded when the tolerance was lost. Moreover, Blackman et al. (1992) also found that the concentration of oligosaccharides increased in soybean seeds during the acquisition of desiccation tolerance. Eventually, loss of desiccation tolerance was observed and that was associated to the aligned reduction of oligosaccharides contents. Moreover, Hoekstra et al. (1994) specified that there is also a strong relationship between water stress resistance and the concentration of oligosaccharides.

Previous studies also demonstrated that a reduction of soluble sugars, specifically the RFOs, results in poor seed quality (Obendorf, 1997). The RFOs accumulate during seed development under normal conditions without water stress (Bailly et al., 2001). Soybean with low raffinose concentration was reported to show low germination and seedling vigour (Meis et al., 2003). Although Lahuta et al. (2000) related changes in relative ratios of RFOs under water stressed conditions to accumulate together with desiccation tolerance in field beans, these authors did not extend their study to examine how this would relate to reproduction quality.

The effect of water stress on soluble carbohydrate concentrations, including RFOs, and how this may relate to seed vigour has not been clearly explained in the literature. Siddique and Wright (2004) examined differences in environmental conditions experienced by pea plant during development and

maturation, the length of seed filling period, concentrations of soluble carbohydrates and proteins as potential causes of differences in seed vigour of peas. The authors concluded that none of these variables could solely yield the variations in seed vigour they observed. On the other hand, agricultural systems in semi-arid regions rely on the ability of orthodox seeds to tolerate desiccation (Lahuta et al., 2000; Bailly et al., 2001). However, despite the widespread phenomenon of desiccation tolerance in orthodox species and its importance to seed quality development, there is no conclusive evidence in the literature on the accumulation of RFOs and proteins in relation to water stress and its effect on seed vigour.

Therefore, correlation of the effect of environmental conditions during seed development with concentration of soluble sugars and protein contents, and the seed vigour of under-produced leguminous crops such as chickpea is necessary to pinpoint ways of increasing their production. This study was conducted to investigate the effect of water stress during seed development on the final contents of sugars (sucrose, raffinose and stachyose), and soluble proteins, germination and seed vigour of three chickpea genotypes (Saina-K, Desi-K and ICCV-K). For chickpea farmers in water stressed regions, results of this study could bring insight of water provision towards seed development for accurate and efficient irrigation regime of chickpea.

5.2 MATERIALS AND METHODS

5.2.1 Seed collection

The chickpea seeds used in this study were collected as a harvest from a previous season in Kenya from subsistence farmers. Fresh seeds, free of pest damages, abnormalities or pathogen infections were visually evaluated and selected for the trials.

5.2.2 Growth experiment

A pot experiment was conducted in winter seasons of 2016/2017 at the Controlled Research Facility Center, University of Kwa-Zulu Natal, Pietermaritzburg (29º 37‘30º 24‘596), South Africa. The experiment was designed as a 3 x 2 factorial in a controlled environment (tunnel), using a completely randomized design (CRD) arranged in a split plot with three replications. Three chickpea genotypes: Desi-K, Saina-K and ICCV-K were used for this experiment. Two water levels: stressed (no irrigation after flowering) and non-stressed (irrigation with 800 mL pure water 3 times a week) conditions were simulated. Each experimental unit was represented by ten 14 L pots. The seeds were harvested at harvest maturity and taken for the assessment of average seed size in terms of seed area (length x breath), sugars, proteins, physiological quality (electrical conductivity and tetrazolium chloride test) and performance (germination percentage and mean germination time).

5.2.3 Data collection

5.2.3.1 Determination of soluble sugars

The extraction and determination of soluble sugars were carried out using standard high performing liquid chromatography (HPLC) method. Sugars were extracted from 0.5 g of seeds powder using 80% v/v methanol (10 mL). The samples were left to stand for 1 h with occasional agitation at room temperature, filtered through Whatman™ filter paper to obtain clean liquid extracts, and evaporated in Genvac evaporator (Genevac® EZ 2.3; IPSWICH; England) to remove methanol. The methanol was replaced by 10 mL distilled water before samples were filtered into glass HPLC vials using 0.25 µm syringe nylon filter.

Concentrations of stachyose, raffinose and sucrose were determined using a HPLC binary pump system (Agilent Technologies, UK). Sample extracts were injected into a Rezex RCM monosaccharide Ca+ (8%) column of 7.8 mm diameter x 300 mm (Phenomenex, Torrance, CA, USA). The column temperature was set at 86 °C using a thermo-stated column compartment (G1316A, Agilent). The mobile phase used was HPLC-grade water at a flow rate of 0.6 mL/min. The presence and concentration of the selected sugars were calculated by comparing peak area of samples against

peak area of known standard concentrations using formulae from known standard curves (0.05-1.25 mg/mL; R^2 = 0.995).

5.2.3.2 Determination of proteins

The soluble proteins content was determined using the Bradford assay method which is characterized by less interference from common reagents and non-protein compounds of biological samples (Bradford, 1976; Kruger, 2009). A standard curve of absorbance versus µg proteins was developed using bovine serum albumin (BSA) at concentrations of 0 –100 µg BSA per 100 µl of distilled water. 0.25 ml of 1M NaOH was added in each concentration. The absorption of dye reagent was measured at 590 nm using one point absorption spectrophotometer (Shimadzu UV-1800, Shimadzu Scientific Instruments INC., Columbia, USA). Activity of 5 ml prepared BSA against 2.5 ml dye reagent was tested by incubation at 60 ºC for 5 min and measuring absorbance for developing a linear standard curve (R^2 = 0.9457; y = 0.0291x + 0.3359). For sample analysis, 0.5 g of seed powder was homogenised in 5 ml of 100mM TRIS with Ultra-Turrax stirrer before it was filtered using filter paper. The filtered sample was centrifuged at 10 000 RPM for 15 min at 2 ºC. The decant supernatant was then suspended into 2 ml TRIS buffer before added dye reagent. Samples were left to stand for 30 min at room temperature before their absorption was measured at 590 nm. The absorbance value was substituted into a standard curve to calculate the soluble protein concentration.

5.2.3.3 Determination of seed size, viability and electrical conductivity

The parameters were assessed using the same materials, methods and formulas as described in the previous chapter. Seed size was measured based on total seed size (length x breadth) using a vernier caliper (OMNI-TKCH®). Seed viability was assessed using tetrazolium chloride (TZ) test according to ISTA (2012). An aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride (1.0%) was prepared using distilled water (pH 7.0). Seeds were immersed in distilled water for 18 hours before being cut longitudinally through the embryo using a razor blade. The prepared seeds were put in a 90 mm petri dish and fully immersed with the TZ solution for 2 hours at room temperature (21 ºC). The preparation room was kept dark the entire time of the experiment because TZ is sensitive to light. Tissues that stained reddish pink were regarded as viable and those unstained were regarded unviable. The number of viable seeds were counted from each treatment based on the ability of TZ to stain viable embryos. The seed viability was calculated using equation 1.

$$\text{Seed viability}(\%) = \left(\frac{\text{Number of stained embryos}}{\text{Total number of embryos}} \right) \times 100\% \qquad (1)$$

In order to determine the amount of solute leakage from seeds (µS $cm^{-1}g^{-1}$), the electrical conductivity (EC) of seeds was measured according to ISTA (2012) using the EC meter (Jenway, 4510 model). The meter was calibrated using 0.01 M potassium chloride (KCL) solution. The calibration and

analysis was carried out at room temperature. Ten seeds per experimental unit were put into 80 mL beakers and immersed with 20 mL of distilled water for 24 hours. After 24 hours, EC was recorded from the imbibed seeds solution. Equation 2 was used to calculate the conductivity (µS $cm^{-1}g^{-1}$).

$$\text{EC (µS/cm/g)} = \frac{\text{Conductivity reading – Background reading}}{\text{Weight of replicate (g)}} \quad (2)$$

Where, leachate conductivity was the EC of soaked seeds solution and the blank conductivity was the EC of a clear prism/ ultra-pure water used to soak the seeds.

5.2.3.4 Determination of germination percentage and mean germination time

Germination percentage was evaluated according to ISTA (2012). Four replicates of ten seeds each were germinated using the moist brown paper towel method. Seeds were arranged in straight lines midway on the moistened paper towels. The paper towels were then rolled and placed in a zip lock bag to avoid moisture loss. Then the bags were placed in a germination chamber set at 25°C. Germination was evaluated by counting, from day 0 to day 8, the number of germinated seeds that had 2mm radicle protrusion (ISTA, 2012). Equation 3 was used to calculate germination percentage.

$$\text{Percentage germination (GP)} = \left(\frac{\text{Seeds germinated}}{\text{Total seeds}}\right) \times 100\% \quad (3)$$

Mean germination time was assessed daily from the day of planting (Day 0) until there was no increase in germination. The data obtained from the assessment was used to calculate the mean germination time according to Heydecker (1968) as shown in equation 4.

$$\text{Mean germination time (MGT)} = \left(\frac{\sum FX}{\sum X}\right) \quad (4)$$

Where F is the number of days from the beginning of the germination test, and X is the number of seeds newly germinated on that day.

5.2.3.5 Data analysis

All the data collected were subjected to analysis of variance (ANOVA) using GenStat® software (18^{th}) edition. Significant differences between the treatment means for each parameter were compared using the least significant difference (LSD) test at $P \leq 0.05$. Principal component-based analysis (PCA) was also used to determine principal contributors to the differences observed on samples.

5.3 RESULTS AND DISCUSSION

5.3.1 Comparing quality and performance of stressed and non-stressed seeds

In terms of size (length x breath), non-stressed seeds were significantly ($P < 0.01$) larger than stressed seeds (45.3 vs. 41.9 mm^2) (Table 5.1). The difference was associated to water being needed by the mother plant to nurse its developing seeds. Water is a main component of photosynthesis, a process of building reserves such as carbohydrates and eventually proteins (Hibberd, 2002; Blankenship, 2013). However, non-stressed seeds had lower concentrations of proteins and sugars than stressed seeds (11.86 and 206.9 µg/g and 11.87 and 208.2 µg/g, respectively). This was hypothesized to be a resultant of water shortage causing higher concentrations in stressed seed. However, this did not necessarily mean higher contents. The proteins and sugars analysis was done on the same size (0.5 g) of seed powder, meaning the results depicts concentrations of sugars or proteins in the seeds. The difference in size would have an effect if the total contents per seed was analysed. The difference between stressed and non-stressed seeds in proteins was insignificant ($P = 0.54$) while sugars had significant ($P < 0.05$) difference. This was associated with the fact that carbohydrates are the primary product of photosynthesis (Fulkerson and Donaghy, 2001; Long et al., 2015) while proteins are secondary forms of peptides (Branden, 1999; Dobson, 2003; Whitmore and Wallance, 2008). Therefore, the water shortage affected carbohydrates more than protein concentrations because carbohydrates are closer to the photosynthesis reaction.

Non-stressed seeds had significantly ($P < 0.01$) lower electric conductivity (EC) than stressed seeds (648.0 vs. 833.3 µs/cm/g, respectively). It was hypothesized that stressed seeds were harder because they had lower moisture contents than non-stressed seeds (data not shown). Their hardness could have caused partial loss of cell membrane functioning which led to higher water permeability during imbibition prior measurement of EC. Rapid water permeability into dried orthodox seeds causes imbibition damage (Chachalis and Smith, 2000; Peksen et al., 2004), which accelerates EC. The non-stressed seeds, that had lower concentration of sugars, had higher seed viability (74.2%) than stressed seeds (55.8%). This was linked to the fact that carbohydrates are known as the first seed storage reserves to be degraded when seeds need energy (Nonogaki et al., 2010; Hartmann and Trumbore, 2016; Izquierdo et al., 2017). The non-stressed seeds also had higher germination percentage (63.3 vs. 60.0%) at a shorter period (1.3 vs. 1.8 days) which was aligned with their higher viability compared to stress seeds.

Table 5.2: Comparison of quality and performance of stressed and non-stressed chickpea seeds.

Production environment	Physiological parameters						
	Total seed size (mm^2)	Proteins (µg/g)	Sugars (µg/g)	Electrical conductivity (µs/cm/g)	Seed viability (%)	Germination (%)	Mean germination time (day)
Non-stressed	45.3±8.7	11.86±0.1	206.9±12.5	648.0±272.89	74.2± 9.9	63.3±23.7	1.3±0.6
Stressed	41.9±5.5	11.87±0.1	208.2±11.1	833.3±187.5	55.8±13.8	60.0±19.2	1.8±0.3
P	<0.01	0.541	<0.05	<0.001	0.003	0.656	0.004
LSD	1.46	0.10	2.67	36.40	11.04	15.65	0.35
CV%	2.1	0.0	16.0	2.9	2.1	11.5	8.8

Data presented as mean ± standard deviation

5.3.2 The effect of water stress on final contents of soluble sugars and proteins on three chickpea genotypes

Highly significant differences ($P < 0.001$) between genotypes were observed from raffinose contents. Saina-K had the highest raffinose content (63.2 µg/g), followed by Desi-K (58.2 µg/g) and ICCV-K (52.5 µg/g) (Fig. 5.1). However, there was no significant difference brought by the interaction between genotypes and the production environment. Similar trend was observed on sucrose content as well. Saina-K had the highest sucrose content (81.2 µg/g) compared to Desi-K (74.9 µg/g) and ICCV-K (66.9 µg/g). The differences in stachyose contents were not significant ($P > 0.05$) for both between chickpea genotypes and genotype x production environment interaction.

Generally, Desi-K had lower contents of sugars, lowest germination percentage and highest mean germination time compared to other cultivars. Similar results were observed by Meis et al. (2003) in soybean. The authors deduced that the concentration of raffinose have positive relationship with lower seed quality (germination and vigour). The results showed that there are genetic differences among chickpea genotypes with respect to their response to water stress, and these responds maybe associated with the differences in accumulation of the sugars. The differences in conteresponse nt of soluble sugars were associated with an expression of specific genes in pea seeds (Peterbauer et al., 2001). In this study, it was postulated that Saina-K may have specific genes that have been enhanced by water stress altering the course of seed development resulting to the higher content of raffinose and sucrose content that was observed. Saina-K belongs to a unique group called Kabuli.

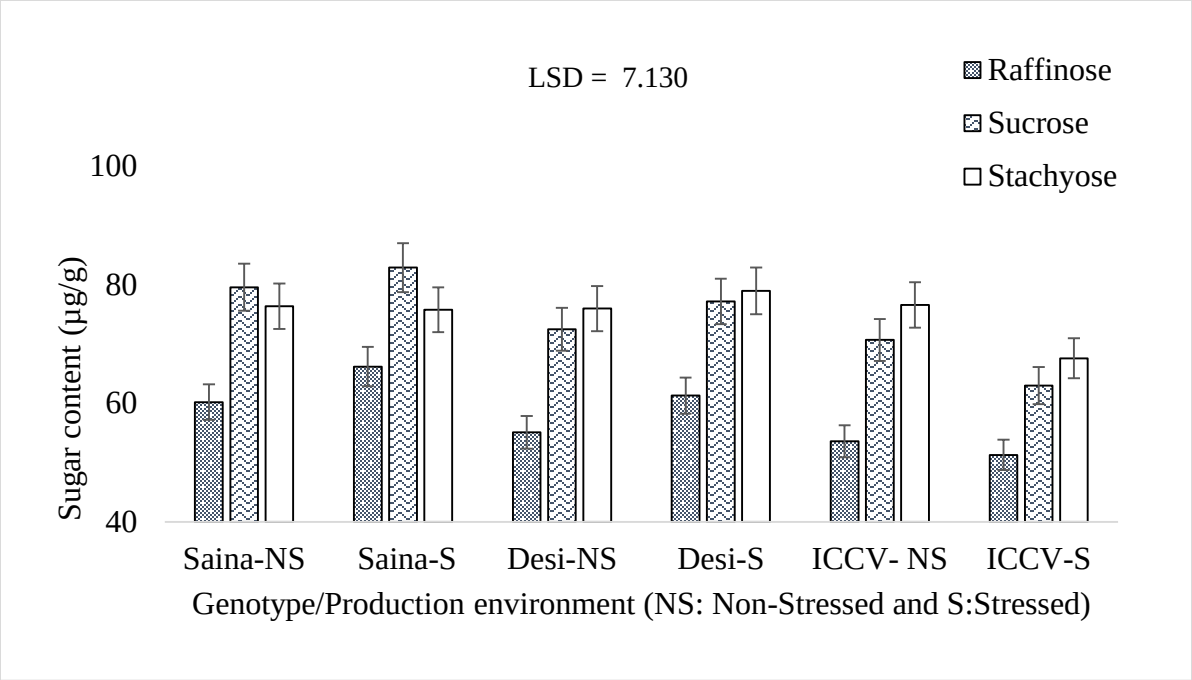


Figure 5.1: The effect of water stress on contents of three soluble sugar of three chickpea genotypes.

There was insignificant (P > 0.05) difference in protein content of Desi-K and ICCV-K under water stress and non- stressed conditions (Figure 5.2). However, Saina-K significantly (P < 0.05) had higher protein content (11.91 µg/g) compared to Desi-K (11. 87 µg/g) and ICCV-K (11.85 µg/g) under water stress condition. The increase in protein content under water stress has been reported earlier by Rai and Singh (1982) on chickpea. Several studies indicated that water stress induces accumulation of proteins (Hoekstra et al., 2001; Svensson et al., 2002; Marian et al., 2004). According to Hoekstra et al. (2002), the proteins accumulate when desiccation tolerance is acquired and degrade when the tolerance is lost in orthodox seeds that can be dried at low moisture content without losing viability, probably because they protect subcellular surfaces and confer desiccation tolerance (Leopold et al., 1994). The results however contradict previous studies. Decreased protein levels under water stress have been reported (Pierre and Savoure, 1990; Roy-Macauley et al., 1992), attributed to numerous physiological and biochemical changes that occur in response to water stress in different plant species (Cheng et al., 2002). For example, water stress is reported to inhibit the incorporation of amino acids into proteins and to cause a decrease in the protein content of the tissues. In addition, it has been previously suggested that the primary action of water stress might be to disrupt membrane and where there is extensive membrane damage, it restricts resumption of protein synthesis (Kaur and Sharma, 2014).

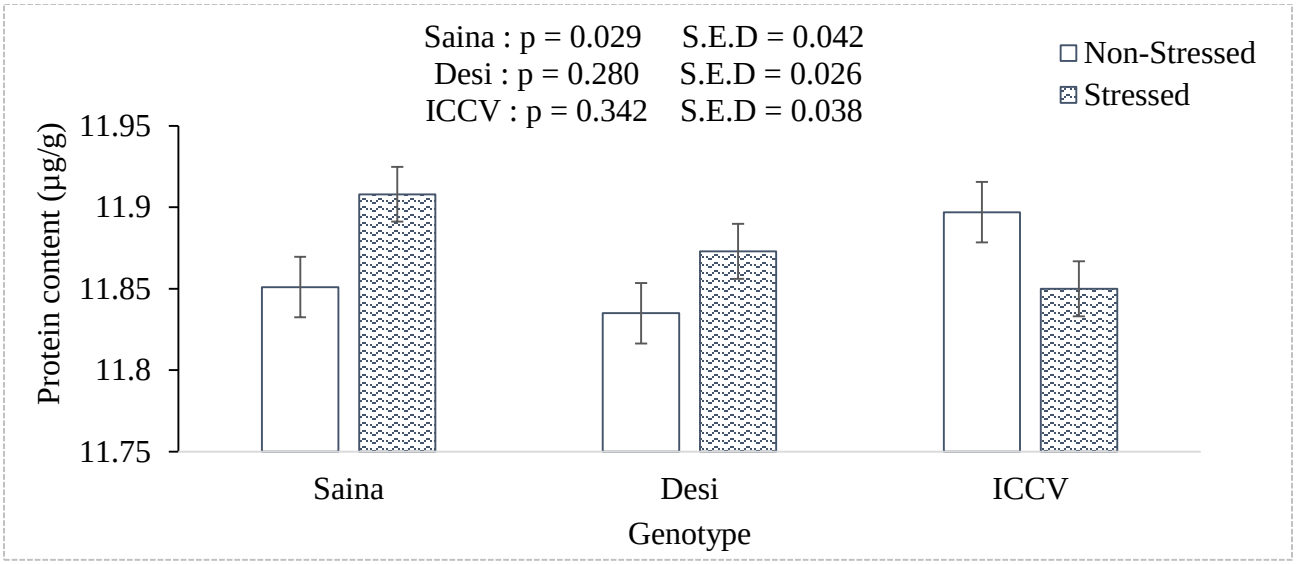


Figure 5.2: The effect of water stress on protein content of chickpea genotypes (Saina, Desi & ICCV).

5.3.3 The effect of water stress on seed size, electric conductivity, and viability on three chickpea genotypes

Significant differences ($P < 0.01$) were observed between genotypes, production environment and the interaction between the two factors on seed size (Figure 5.3A). Saina-K had the largest total seed size (47.9 mm^2) compared to ICCV-K (47.2 mm^2) and Desi-K (40.6 mm^2) under non-stressed condition. For stressed seeds, Desi-K had the smallest seed size (36.5 mm^2) followed by Saina-K (42.2 mm^2) and ICCV-K (47.2 mm^2). Water stress reduced seed size. This might be due to reduced seed fill duration caused by water stress during dry matter accumulation as was reported by Egli (2006). This study results agreed with previous reports on chickpea (Leport et al., 1999) and in soybean (De Souza et al., 1996). According to De Souza et al. (1996) and Leport et al. (1999), the smaller seed size of stressed seeds resulted from shortened seed filling period and accelerated plant's leaf senescence which was associated with reduced manufacturing of storage compounds. Davies et al. (1999) associated the effect of water stress on seed size with the source/sink relations. However, Behboudian et al. (2001) observed that chickpea seed size was not reduced by water stress.

There were significant differences ($P < 0.001$) observed on electrical conductivity (EC) between genotypes, production environment and the interaction between the two factors (Figure 5.3B). Saina-K had the lowest electrical conductivity (546 µS/cm/g) compared to ICCV-K (607 µS/cm/g) and Desi-K (791 µS/cm/g) under non-stressed condition. Similar trend was observed except an

increased level of EC under stressed condition. Saina-K had the lowest electrical conductivity (613 µS/cm/g) compared to Desi-K (935 µS/cm/g) and ICCV-K (952 µS/cm/g).The differences observed on electrical conductivity and low seed viability were believed to have been attributed by the destroyed integrity of cell membrane that was differently induced by water stress in each genotype. It was hypothesized that water stress led to disruption of compartments within the seed cells resulting in a mixing of enzymes (Walters et al., 2002). According Walters et al. (2002), membrane function is damaged when seeds are exposed to water stress and this is more likely to be responsible for poor seed development and seed performance in germination. However, Ghassemi-Golezani and Mazloomi-Oskooyi (2012) found that water stress had no significant effect on maximum seed vigor of common bean cultivars. The authors obtained minimum electrical conductivity of seed leachates on stressed seeds. Their results were in agreement with previous reports on maize and sorghum (Ghassemi-golezani et al., 1997) and soybean (Vieira et al., 1992). Therefore, this clarifies that there is a difference in damaged membrane function and it differs with species or legumes.

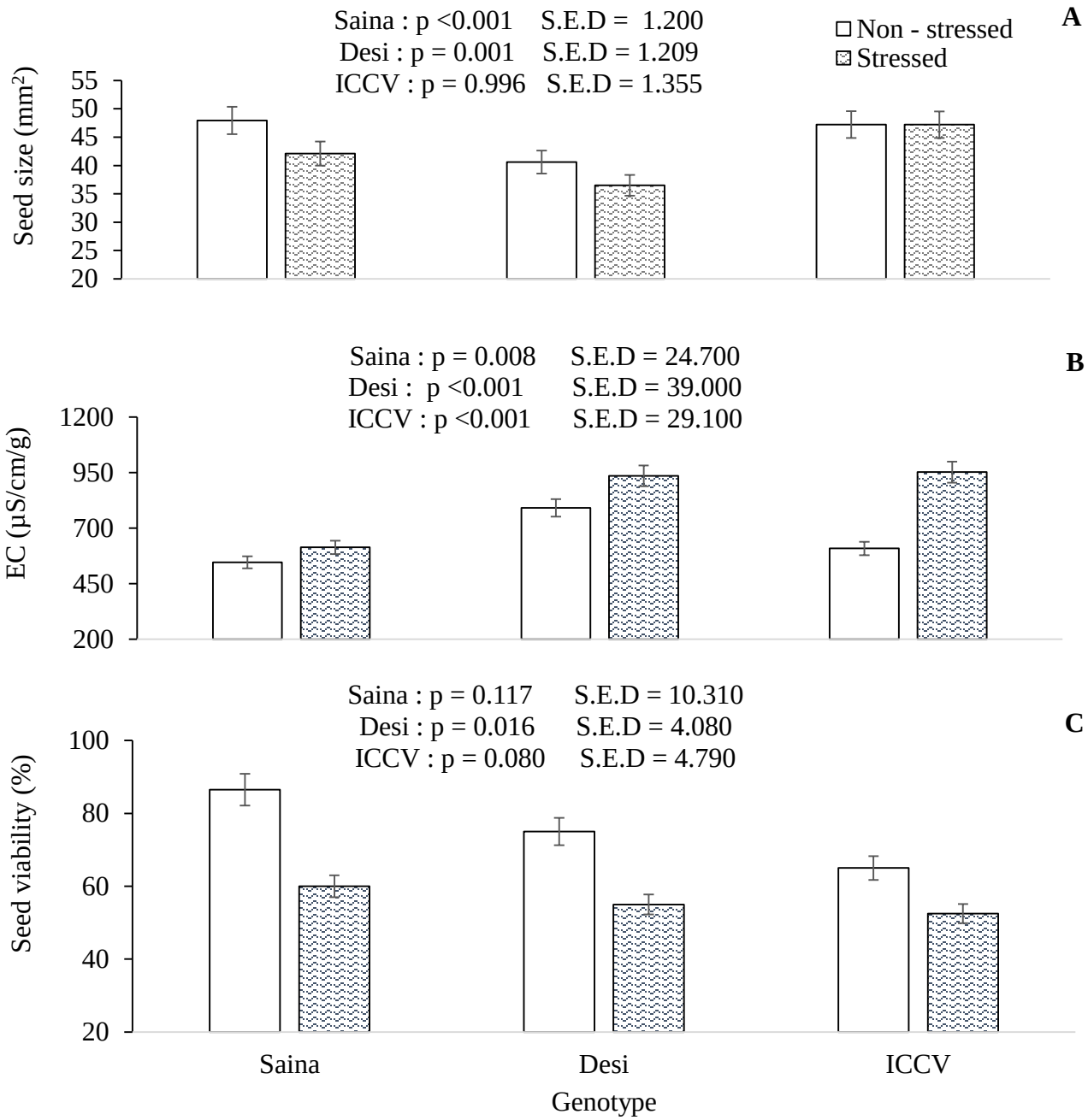


Figure 5.3: The effect of water stress on seed size (A), electric conductivity (B), and seed viability (C) of three chickpea genotypes.

5.3.4 The effect of water stress on germination of three chickpea genotypes

Significant differences (P< 0.05) in germination performance were observed between chickpea genotypes (Figure 5.4). Saina-K showed highest germination percentage (77.5%) compared to Desi-K (58.8%) and ICCV-K (48.8%). Saina-K had the lowest mean germination time (1.2 days) followed by 1.6 and 1.7 days of ICCV-K and Desi-K, respectively. Water stress greatly affected seed germination, but the response intensity and adverse effect of stress was dependent on the

genotype. Thus, ICCV-K was very sensitive to water stress as compared to other genotypes. The reduction and delay in germination resulted from changes in membrane permeability and water uptake due to altered endogenous hormonal levels caused by water stress. These results are in close agreement with previous studies (Sadeghian and Yavari, 2004; Almaghrabi and Abdelomoneim, 2012; Llanes et al., 2016). Water stress was previously reported to adversely affect the seed germination, plant growth and development (Almaghrabi and Abdelomoneim, 2012) and seedling growth (Ashraf et al., 2002). According to Dodd and Donovan (1999), water stress can reduce germination either by limiting water absorption by the seeds, by affecting the mobilization of stored reserves (Bouaziz and Hicks, 1990; Chutia and Borah, 2012) or by directly affecting the structural organization or synthesis of proteins in germinating embryos (Ramagopal, 1990).

In contrast, Ghassemi-Golezani and Ghassemi (2013) observed that water stress had no significant effect on seed quality of chickpea as measured by germination percentage and rate, although maximum germination percentage and rate slightly decreased with decreasing water supply. These results were in agreement with those reported for maize and sorghum (Ghassemi-Golezani et al., 1997), common bean (Ghassemi-Golezani and Mazloomi-oskooyi, 2008), faba bean (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009) and chickpea (Ghassemi-Golezani et al., 2010). However, although chickpea is known for its better drought tolerance than most of the other cool season legumes (Kashiwagi et al., 2015), the study proved that water stress remains one of the major constraints to chickpea seed quality as measured by germination percentage and mean germination time.

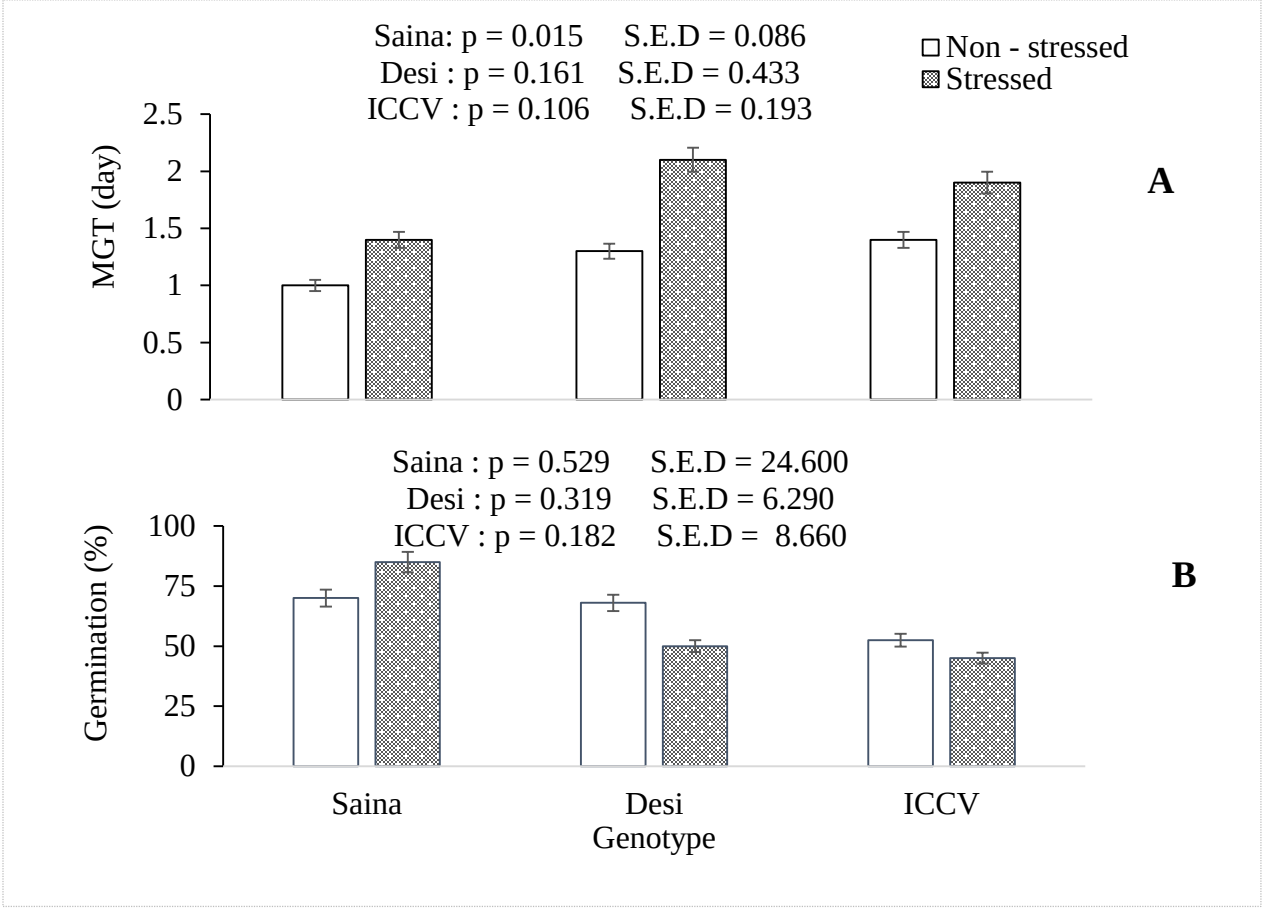


Figure 5.4: The effect of water stress on germination percentage and mean germination time (MGT) of three chickpea genotypes.

5.3.5 The multivariate classification of stressed and non-stressed chickpea seeds as affected by biochemical compounds and germination performance

Principal component–based analysis (PCA) is a multivariate chemometric procedure of determining principal contributors to the differences observed on samples (Kano et al., 2001; Abdi and Williams, 2010). The PCA method also examine the relationships (correlations) of each parameter of principal components (PC). The technique simplifies the source of differences from a big number of factors by dividing the components responsible for differences into fewer groups (Wold et al., 1987; Jollifer and Morgan, 1992; Abdi and Williams, 2010). Each sample is then projected based on the created components (normally two) contributing to their similarity or correlation (Abdi and Williams, 2010). In this study, Principal component-based analysis (PCA) was used to classify seeds from water stressed and non-stressed conditions based on their

concentrations of biochemical compounds (proteins, sugars, SV and EC) and their germination performances (GP and MGT). The closeness of growth condition to a biochemical compound illustrates a positive relationship. The contribution of each parameter to seed performance was measured by its proximity to the performance factor. The first two principal components contributed a total of 98% in the accuracy of the model to determine differences of the chickpea seeds (Fig. 5.5A).The first PC contributed 36% and the second PC contributed 20% towards the growth-based classification and the biochemical contribution to germination performance (Fig.5.5B). The overall model holds 56% confidence that parameter contributed to the seeds germination performance. The model was considered successful because there are many other parameters contributing to seed difference. Therefore, getting 56% accuracy using only 5 parameters was considered successful.

Water stress contributed positively to the concentrations of raffinose, sucrose, stachyose and electric conductivity. The irrigation (non-stressed condition) contributed positively in seed size and seed viability. The correlation agreed with the results obtained from ordinary data analysis, displayed tables and graphs above. There was insignificant difference between protein contents of stressed and non-stressed seeds. This could be due to the low accumulation of proteins under stressed condition while chickpea seeds have high protein content in nature. It has been previously suggested that the primary action of water stress might be to disrupt membrane and where there is extensive membrane damage, it restricts resumption of protein synthesis (Kaur and Sharma, 2014). This indicates that water stress inhibited the incorporation of amino acids into proteins and this resulted to low protein content (Cheng et al., 2002).

Germination percentage (GP) was more affected by electrical conductivity (EC) of the seeds due to destroyed integrity of cell membrane that allowed rapid water uptake by seeds resulting to imbibition damage, high solute leakage (dead cell tissue) and consequently, low seed vigour and poor germination (Volk et al., 2006; Arun et al., 2017; Gimenez et al., 2017). The model found a stronger correlation of GP and EC compared to the effect of stress to GP. As a result, GP was projected on stressed seeds although non-stressed seeds had higher GP occurrence. This indicated that water stress affected seed germination through inducing changes in cell membrane permeability, limiting water absorption by the seeds while electrical conductivity induced severe damage in cell membrane of chickpea seeds (Sadeghian and Yavari, 2004; Chutia and Borah, 2012; Llanes et al., 2016).

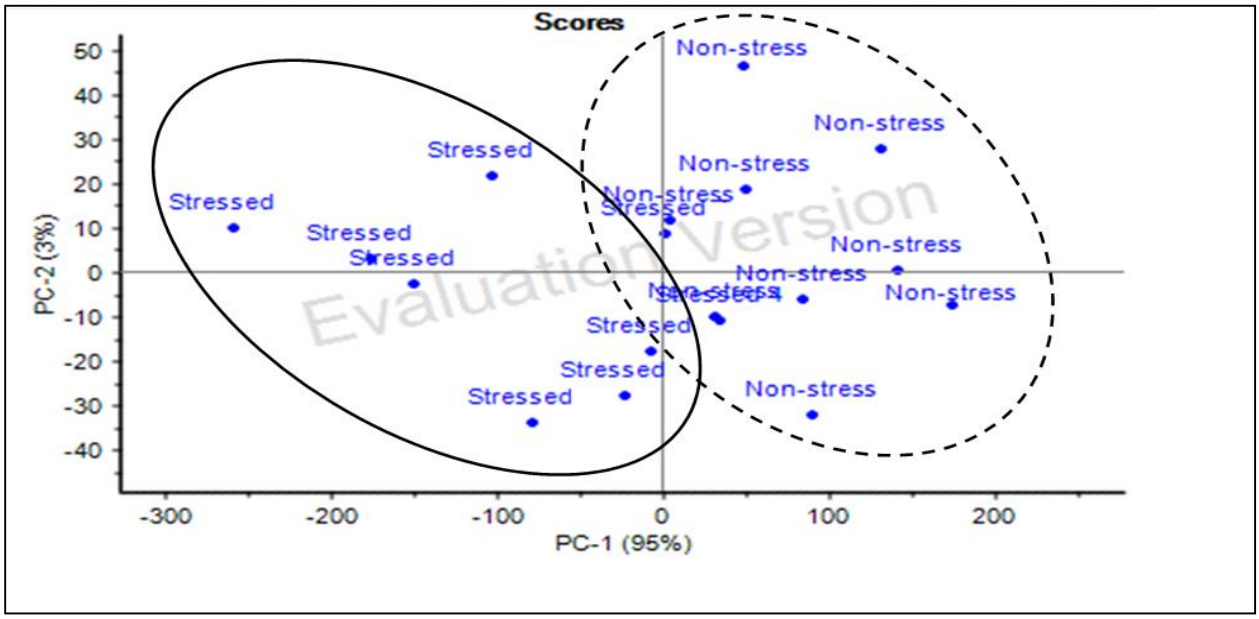


Fig. 5.5A: Water regimes classification using principal components analysis

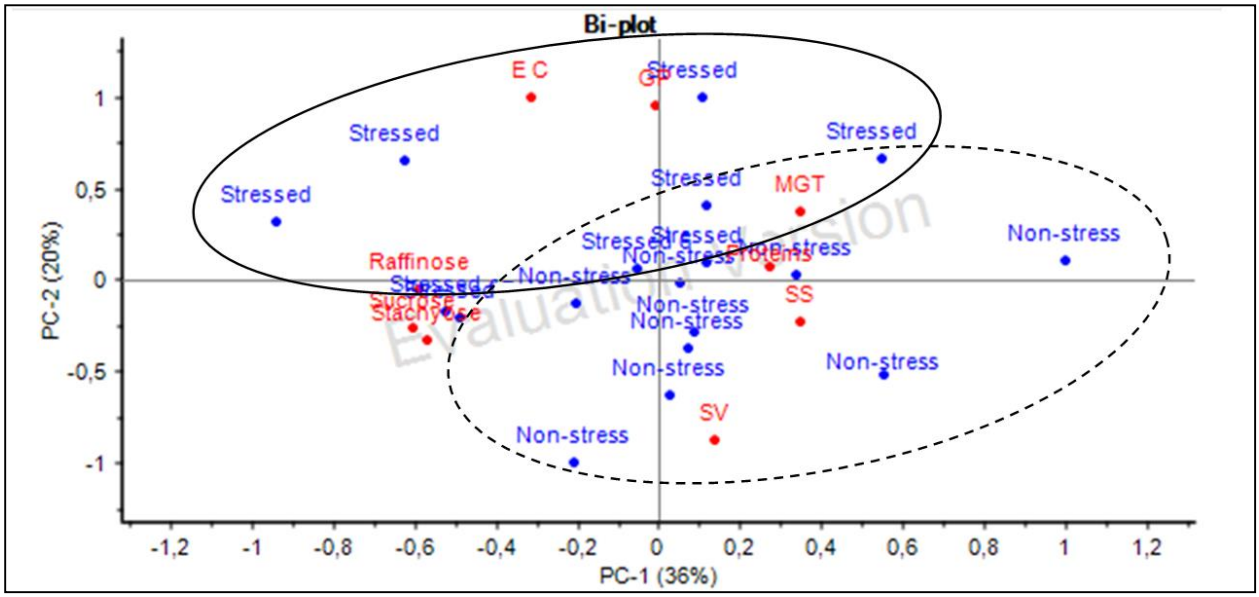


Fig. 5.5B: The classification of samples based on their stress condition using principal components analysis to examine contributions of biochemical compounds.

Model developed using leave-one-out full cross validation with NIPALS algorithm (7 components) at 0.05 level of significance. SV, seed viability; SS, seed size; EC, electric conductivity; GP, germination percentage; MGT, mean germination time

5.4 CONCLUSION

This study revealed that water stress have a positive relationship with the final seed contents of soluble proteins, sucrose, raffinose and stachyose. The stressed seeds had higher contents of sugars and proteins but high electrical conductivity, low seed viability and delayed germination at lower percentage than non-stressed seeds. Genotypic or micro-structural research on the lower performance of seeds with higher concentrations of sugars was recommended since this study, together with the previous studies, had proven this for chickpea. Also, it was recommended that chickpea farmers in dry areas should ensure sufficient water supply at seed development stage of seeds for planting in the next seasons. They can compromise irrigation regime with water availability when producing seed for consumption since water-stressed seeds have higher concentrations of sugars and proteins.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND FUTURE RESEARCH

6.1. Background

For successful crop production, the use of good quality seed is essential to alleviate food security threats. However, this requires comprehensive exploration and in-depth understanding of the factors responsible for poor seed quality. Water stress and storage conditions are currently the most important abiotic factors responsible for low seed quality in grain legumes (Biabani et al., 2011; Gowda et al., 2013). Although chickpea is considered as a fairly drought tolerant crop due to its deep rooting system, water stress can reduce chickpea yield by 42 to 70% (Shrestha et al., 2006). Water stress further limits the production of assimilates required to synthesize sugars (raffinose family of oligosaccharides: RFOs) (Hoekstra et al., 2003). However, a reduction in RFOs may result in poor seed quality since these sugars play a significant role in the acquisition of desiccation tolerance and have been shown to protect cell membranes and cytoplasm. Also, the RFOs are the source of energy that subsequently drives the germination process. Moreover, poor storage conditions characterized by high relative humidity and temperature may lead to a rapid deterioration of seed viability and vigour. Therefore, identification of the factors that have a negative impact on seed quality of chickpea genotypes is needed in order to obtain high and stable yield. The main aim of the study was to evaluate the effect of water stress, seed size and storage conditions on seed quality of different chickpea genotypes. In order to achieve this, three objectives were set out. Firstly, to examine the performance of chickpea genotypes with different seed size on seedling emergence; secondly, to investigate the effect of seed ageing on seed quality and imbibition of genotypes produced under water stress and non-stress condition; and thirdly, to determine the effect of water stress during seed development on sugar and proteins accumulation, germination and seed vigour.

6.2. The effect of seed size on the emergence and germination of three chickpea genotypes

It was discovered in this work that the differences in seed size among genotypes affect the seed quality as measured by germination and seedling emergence. Kabuli chickpea genotypes exhibited low seed viability, poor and slow emergence, low germination percentage, delayed mean germination time and high electrical conductivity. These resulted from disruption of cell membrane caused by larger seed size with loose coat adherence that imbibed more water during the first phase of germination, resulting in imbibition damage, low vigour and poor emergence. The disruptions were also observed in previous studies of seed germination (Chachalis and Smith, 2000; Matthews and Powell, 2006; Majnoun Hosseini et al., 2009; Arun et al., 2017;

Gimenez et al., 2017). However, the findings from the seed size experiment contradicted with previous studies by Roozrokh et al. (2005) and Anuradha et al. (2009). Large seeded chickpea genotypes were reported to have higher germination and seedling survival percentage, growth and establishment due to larger endosperm that enhanced emergence ability through greater supply of stored energy to support early seedling growth and plant tissues compared to smaller seeds (Leishman, 2001; Hojjat, 2011). Similar results were also reported by Soltani et al. (2002) in chickpea seeds produced under saline and non-saline conditions. Inconsistency in the effect of seed size on seedling survival may be due to confounding different sources of genetic and environmental variation in seed size. Thus in the study, it was postulated that chickpea performance with respect to emergence is not only affected by seed size but also genetic variation among the genotypes. Further research on investigating the effects of genetic variation and biochemical profile on the performance of Desi-K and Kabuli chickpea genotypes (Saina-K and ICCV-K) should be undertaken. In addition, the amount of nutrients available for germination and seedling emergence should be also determined in chickpea seeds, as the finding of Tanveer et al. (2013) postulated that large seeds have high amount of nutrients available for faster germination.

6.3. The effect of storage conditions on the seed quality (germination and vigour) of three chickpea genotypes

Seed ageing caused progressive loss of seed viability, vigour and increased imbibition rate which was hypothesized to have resulted to cell death and high solute leakage from the seeds produced under water stress and non-stressed conditions. These results were also obtained by Hussein et al. (2011) and Gimenez et al. (2017). In this study, this trend was more severe on water stressed seed lots, indicating that there might be minor genetic differences among chickpea genotypes but more to the production environment that could affect storage potential and consequently, seed quality at priming and germination. It was hypothesised that the membranes of aged seeds, whose integrity has been reduced by deterioration, are more susceptible to poor vital staining. As a result, chickpea seeds that have low vigour which attributed from seed ageing are more likely to exhibit poor germination and seedling establishment after planting, thus resulting in poor plant population and consequently low yields. According to Bewley et al. (2012), the low vigour caused by seed ageing attributes from low levels of respiration that deplete seed reserves which sustain seed viability. However, Bijanzadeh et al. (2017) reported that the low vigour might be due to degradation of mitochondrial membrane during seed ageing, leading to reduction in energy supply necessary for germination (Pandey and Pati, 2017). It was recommended that farmers must ensure proper storage conditions after crop harvest. Moreover, further studies on possible

mechanisms and regulatory processes that control chickpea seed quality during production and storage were recommended.

6.4. The effect of water stress during seed development on the seed quality and performance

This study demonstrated that water stress can cause significant economic losses on chickpea smallholder farmers. Stressed seeds had high electrical conductivity, low seed viability and delayed germination at lower percentage than non-stressed seeds. The differences observed on electrical conductivity and low seed viability were believed to have been attributed by the destroyed integrity of cell membrane that was induced by water stress in the genotypes. It was hypothesized that water stress led to disruption of compartments within the seed cells resulting in a mixing of enzymes (Walters et al., 2002). According to Walters et al. (2002), membrane function is damaged when seeds are exposed to water stress and this is more likely to be responsible for poor seed development and seed performance in germination. Llanes et al. (2016) reported that water stress induce changes in membrane permeability and water uptake due to altered endogenous hormonal levels, and this results to the reduction and delay in germination. Therefore, the water stress was also hypothesized to cause seed cell membrane damages in this study.

Moreover, water shortage affected carbohydrates more than protein contents, and that was associated with the fact that carbohydrates are the first product of the photosynthesis reaction. There was a reduction of soluble sugars, specifically the RFOs, which resulted to poor chickpea seed quality. Chickpea seeds with lower contents of sugars had the lowest germination percentage and highest mean germination time. Similar results were observed by Meis et al. (2003) in soybean. The authors deduced that the content of raffinose have negative relationship with seed quality (germination and vigour). However, in this study it was hypothesized that Saina-K may have specific genes that have been enhanced by water stress altering the course of seed development resulting to the higher content of raffinose and sucrose content that was observed. The study further observed that non-stressed seeds with lower concentration of sugars had higher seed viability than stressed seeds. This incidence contradicted with what was hypothesized because it is feasible to align sugar contents of seeds with their viability. Therefore, possible mechanisms of the accumulation and final content of sugars (RFOs compounds) in the seed during water stress should also be investigated to provide insights of regulatory processes that control chickpea seed quality.

REFERENCES

Aarssen, L.W. and Burton, S.M. 1990. Maternal effects at four levels in *Senecio vulgaris* grown in a soil nutrient gradient. American Journal of Botany, 77, 1231-40.

Abdi, H. and Williams, L.J. 2010. Principal component analysis. Wiley Interdisciplinary Reviews: Computational Statistics, 2, 433-459.

Abdullah, W., Powell, A. and Matthews, S. 1991. Association of differences in seed vigour in long bean (*Vigna sesquipedalis*) with testa colour and imbibition damage. The Journal of Agricultural Science, 116, 259-264.

Adebisi, M., Kehinde, T., Ajala, M., Olowu, E. and Rasaki, S. 2011. Assessment of seed quality and potential longevity in elite tropical soybean (*Glycine Max* L.) grown in Southwestern Nigeria. Nigerian Agricultural Journal, 42, 94-103.

Alhamdan, A. M., Alsadon, A. A., Khalil, S. O., Wahb-allah, M. A., Nagar, M. E. and Ibrahim, A. A. 2011. Influence of storage conditions on seed quality and longevity of four vegetable crops. American-Eurasian Journal of Agriculture and Environmental Science, 11, 353-359.

Almaghrabi, O. A. and Abdelomoneim, T. S. 2012. Using of arbuscular mycorrhizal fungi to reduce the deficiency effect of phosphorous fertilization on maize plants (*Zea mays* L.). Life Science Journal, 9, 48-54.

Amjad, M. and Anjum, M. 2002. Effect of relative humidity and ageing period on the quality of onion seed. International Journal of Agriculture and Biology, 4, 291-296.

Anuradha, R., Balamurugan, P., Srimathi, P. and sumathi, S. 2009. Influence of seed size on seed quality of chickpea (*Cicer arietinum* L.). Legume Research, 32, 133-135.

Arun, M.N., Bhanuprakash, K., Hebbar, S.S. and Senthivel, T. 2017. Effects of seed priming on biochemical parameters and seed germination in cowpea (*Vigna unguiculata* L.). Legume Research, 40, 3.

Ashraf, M. Y., Sarwar, G., Ashraf M., Afaf, R. and Sattar, A. 2002. Salinity induced changes in 4-amylase activity during germination and early cotton seedling growth. Biologia Plantarum, 45, 89-91.

Aveling, T. and PowelL, A. 2005. Effect of seed storage and seed coat pigmentation on susceptibility of cowpeas to pre-emergence damping-off. Seed Science and Technology, 33, 461-470.

Bailly, C., Audigier, C., Ladonne, F., Wagner, M. H., Coste, F., Corbineau, F. and Come, D. 2001. Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. Journal of Experimental Botany, 52, 701-708.

Basavegowda, G. S. and Arunkumar, H. 2013. Effect of commercial cold storage conditions and packaging materials on seed quality of chickpea (*Cicer arietinum* L.). Global Journal of Science Frontier Research and Agricultural Veterinary Science, 13, 2.

Basra, S.M.A., Ahmad, N., Khan, M.M., Iqbal, N. and Cheema, M.A. 2003. Assessment of cotton seed deterioration during accelerated ageing. Seed Science and Technology, 31, 531-540.

Basu, P., ALI, M. and Chaturvedi, S. 2009. Terminal heat stress adversely affects chickpea productivity in northern India-strategies to improve thermo-tolerance in the crop under climate change. W3 Workshop Proceedings: Impact of Climate Change on Agriculture, 23-25.

Behboudian, M. H., M.A. Q., Turner, N. C. and Palta, J. A. 2001. Reactions of chickpea to water stress: yield and seed composition. Journal of the Science of Food and Agriculture, 81, 1288-1291.

Bewley, J.D. and Black, M. 2012. Physiology and biochemistry of seeds in relation to germination. International Journal of Plant Physiology and Biochemistry, 2, 276-339.

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M. and Nonogaki, H. 2013. Seeds physiology of development, germination and dormancy. International Journal of Plant Physiology and Biochemistry, 141, 1644-1652.

Biabani, A., Carpenter Boggs, L., Katozi, M. and Sabouri, H. 2011. Effects of seed deterioration and inoculation with mesorhizobium cicerion on yield and plant performance of chickpea. Australian Journal of Crop Science, 5, 66.

Bijanzadeh, E., Naderi, R., Nosrati, K and Egan, T.P. 2017.Effects of accelerated ageing on germination and biochemistry of eight rice cultivars. Journal of Plant Nutrition, 40, 155-164.

Blackman, S. A., Obendorf, R. L. and Leopold, A. C. 1992. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. Plant Physiology, 100, 225-230.

Blankenship, R.E. 2013. Molecular mechanisms of photosynthesis. John Wiley and Sons. 1-13.

Bouaziz, A. and Hicks, D.R. 1990.Consumption of wheat seed reserves during germination and early growth as affected by soil water potential. Plant and Soil, 128, 161-165.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Branden, C.I. 1999. Introduction to protein structure. Garland Science, London, UK, 2nd edition, 13-33.

Brevedan, R. and Egli, D. 2003. Short periods of water stress during seed filling, leaf senescence, and yield of soybean. Crop Science, 43, 2083-2088.

Chachalis, D. and Smith, M. 2000. Imbibition behavior of soybean (*Glycine max* L.) accessions with different testa characteristics. Seed Science and Technology, 28, 321-331.

Cheng, Z., Targolli, J., Huang, X. and Wu, R. 2002. Wheat LEA genes, PMA80 and PMA1959, enhance dehydration tolerance of transgenic rice (*Oryza sativa* L.). Molecular Breeding, 10, 71-82.

Chutia, J. and Borah, S. 2012. Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* L.) genotypes of Assam, India. II. Protein and

proline status in seedlings under PEG induced water stress. American Journal of Plant Sciences, 3, 71-80.

Copeland, L. O. and Mcdonald, M. B. 2001. Seed germination. Principles of Seed Science and Technology. Springer, 72-123.

Davies, S., Turner, N., Siddique, K., Leport, L. and Plummer, J. 1999. Seed growth of desi and kabuli chickpea (*Cicer arietinum* L.) in a short-season Mediterranean-type environment. Australian Journal of Experimental Agriculture, 39, 181-188.

De Souza, P., Egli, D. B. and Bruening, W. P. 1997. Water stress during seed filling and leaf senescence in soybean. Agronomy Journal, 89, 807-812.

Dechaine, J., Brock, M. and Weinig, C. 2015. Maternal environmental effects of competition influence evolutionary potential in rapeseed (*Brassica rapa*). Evolutionary Ecology, 29, 77-91.

Desclaux, D., Huynh, T.T. and Roumet, P. 2000. Identification of soybean plant characteristics that indicate the timing of drought stress. Crop Science, 40, 716-722.

Dobson, C.M. 2003. Protein folding and misfolding. Nature, 426, 884-890.

Dodd, G. L .and Donovan, L. A. 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. American Journal of Botany. 86, 1146-1153.

Egli, D. 2006. The role of seed in the determination of yield of grain crops. Australian Journal of Agricultural Research, 57, 1237-1247.

El-Keblawy, A. 2003. Effects of artificial accelerated aging on germination behaviour of the cosmopolitan weed: Implications for weed control. Pakistan Journal of Weed Science Research, 9, 125-134.

Emenky, F. A. O. and Khalaf, A. S. 2010. Effect of sowing depths and seed size of some winter cultivars of chickpea (*Cicer arietinum* L.) on field emergence and vegetative growth. Research Journal of Seed Science, 3, 170-177.

Fang, X., Turner, N. C., Yan, G., LI, F. and Siddique, K. H. 2009. Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. Journal of Experimental Botany, 61, 335-345.

FAOSTAT, 2016. Food and Agricultural Organization Statistical Database of United Nation.

Farhadi, R., Rahmani, M. R., Salehi balashahri, M. and Sadeghi, M. 2012. The effect of artificial ageing on germination components and seedling growth of Basil (*Ociumum basilicm* L.) seeds. Journal of Agriculture and Food Technology, 2, 69-72.

Fenner, M. and Thompson, K. 2005. The ecology of seeds, Cambridge University Press.

Ferguson, J.1995. An introduction to seed vigour testing. Seed vigour testing seminar, Copenhagen, Zurich: International Seed Testing Association, 1-10.

Finch-Savage, W. E. and Bassel, G. W. 2015. Seed vigour and crop establishment: extending performance beyond adaptation. Journal of Experimental Botany, 67, 567-591.

Finch-savage, W. E. and Leubner-Metzger, G. 2006. Seed dormancy and the control of germination. New Phytologist, 171, 501-523.

Frederick, J. R., Camp, C. R. and Bauer, P. J. 2001. Drought-stress effects on branch and main stem seed yield and yield components of determinate soybean. Crop science, 41, 759-763.

Fulkerson, W.J. and Donaghy, D.J. 2001. Plant-soluble carbohydrate reserves and senescence-key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. Australian Journal of Experimental Agriculture, 41, 261-275.

Gaur, P. M., Tripathi, S., Gowda, C. L., Ranga Rao, G., Sharma, H., Pande, S. and Sharma, M. 2010. Chickpea seed production manual. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502, 1-28.

Ghassemi-Golezani, K., Soltani, A. and Atashi, A. 1997. The effect of water limitation in the field on seed quality of maize and sorghum. Seed Science and Technology, 25, 321-323.

Ghassemi-Golezani, K. and Mazloomi-Oskooyi, R. 2008. Effect of water supply on seed quality development in common bean (*Phaseolus vulgaris*). International Journal of Plant Production, 2,117-124.

Ghassemi-Golezani, K. and Hosseinzadeh-Mahootchy, A. 2009. Changes in seed vigour of faba bean (*Vicia faba* L.) cultivars during development and maturity. Seed Science and Technology, 37, 713-720.

Ghassemi-Golezani, K., Khomari, S., Dalil, B., Hosseinzadeh-Mahootchy, A. and Chadordooz-Jeddi, A. 2010. Effects of seed aging on field performance of winter oilseed rape. Journal of Food Agriculture and Environment, 8,175-178.

Ghassemi-Golezani, K. and MazloomI-Oskooyi, R. 2012. Effect of water supply on seed quality development in common bean (*Phaseolus vulgaris*). International Journal of Plant Production, 2, 117-124.

Ghassemi-Golezani, K. and Ghassemi, S. 2013. Effects of water stress on some physiological traits and grain yield of chickpea (*Cicer arietinum* L.) cultivars. International Journal of Biosciences, 3, 62-70.

Ghassemi-Golezani, K., Chadordooz-JeddI, A., Zehtab-SalmasI, S. and Oustan, S. 2014. Influence of seed size and aging on seedling growth and field establishment of lentil (*Lens culinaris* Medik). Plant Breeding and Seed Science, 67, 27-36.

Gholami, A., Sharafi, S., Sharafi, A., Ghasemi, S., Hierro, J. L., Eren, O., Khetsuriani, L., Diaconu, A., Török, K. and Montesinos, D. 2009. Germination of different seed size of pinto bean cultivars as affected by salinity and drought stress. International Journal of Food, Agriculture and Environment, 7, 555-558.

Gimenez, J.I., Esteves Amaro, A.C., Machado, S.R. and Ferreira, G. 2017. Slow imbibition of *Annona emarginata* seeds: metabolic and ultrastructural evaluations. Botany, 95, 1033-1040.

Goel, A., Goel, A. K. and Sheoran, I. S. 2003. Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. Journal of plant physiology, 160, 1093-1100.

Gowda, C. L., Srinivasan, S., Gaur, P. and Saxena, K. 2013. Enhancing the productivity and production of pulses in India. National institute of Advanced studies, Bangalore and Indian council of Agricultural Research, New Delhi, 145-159.

Graham, P. H. and Vance, C. P. 2003. Legumes: importance and constraints to greater use. Plant physiology, 131, 872-877.

Gunaga, R., Hareesh, T. and Vasudeva, R. 2007. Effect of fruit size on early seedling vigour and biomass in white dammer (*Vateria indica*): a vulnerable and economically important tree species of the Western Ghats. Journal of Non-Timber Forest Products, 14, 197-200.

Gupta, A. and Aneja, K. 2004. Seed deterioration in soybean varieties during storage-physiological attributes. Seed Research, 32, 26-32.

Gupta, A., Punia, R. and Dhaiya, O. 2016. Physiological and biochemical changes in pearl millet seeds under different storage conditions and containers. An International Quarterly Journal of Life Sciences, 33, 1455-1460.

Hartmann, H. and Trumbore, S. 2016. Understanding the roles of nonstructural carbohydrates in forest trees-from what we can measure to what we want to know. New Phytologist, 211, 386-403.

Heydecker, W. 1968. Two approaches to the study of germination data. International Seed Testing Association, 33, 340-351.

Hibberd, J.M. and Quick, W.P. 2002. Characteristics of C4 photosynthesis in stems and petioles of C3 flowering plants. Nature, 415, 451-454.

Hilhorst, H. W. and Toorop, P. E. 1997. Review on dormancy, germinability, and germination in crop and weed seeds. Advances in agronomy, 61, 111-165.

Hoekstra, F., Haigh, A., Tetteroo, F. and Van Roekel, T. 1994. Changes in soluble sugars in relation to desiccation tolerance in cauliflower seeds. Seed Science Research, 4, 143-147.

Hoekstra, F. A., Golovina, E. A. and Buitink, J. 2001. Mechanisms of plant desiccation tolerance. Trends in Plant Science, 6,431-438.

Hoekstra, F. A., Golovina, E. A. and Nijsse, J. 2003. What do we really know about desiccation tolerance mechanisms. The Biology of Seeds, Recent Research Advances, 259-270.

Hojjat, S. S. 2011. Effects of seed size on germination and seedling growth of some Lentil genotypes (*Lens culinaris* Medik.). International Journal of Agriculture and Crop Sciences, 3, 1-5.

Hung, L., Hong, T. and Ellis, R. 2001. Constant, fluctuating and effective temperature and seed longevity: a tomato (*Lycopersicon esculentum* Mill.) exemplar. Annals of Botany, 88, 465-470.

Hussein, H. J., Shaheed, A. I. and Yasser, O. M. 2011. Effect of accelerated aging conditions on viability of sunflower (*Helianthus annus* L.) seeds. Euphrates Journal of Agriculture Science, 3, 1-9.

ISTA. 2012. International rules for seed testing rules. Seed Science and Technology, 27, 27-31.

Izquierdo, N., Benech-Arnold, R., Batlla, D., Belo, R.G. and Tognetti, J. 2017. Seed composition in oil crops: Oilseed Crops: Yield and Adaptations under Environmental Stress, 34-51.

Jain, N., Koopar, R. and Saxena, S. 2006. Effect of accelerated ageing on seed of radish (*Raphanus sativus* L.). Asian Journal of Plant Sciences, 5, 461-464.

Jalota, S.K., Sood, A. and Harman, W.L. 2006. Assessing the response of chickpea (*Cicer arietinum* L.) yield to irrigation water on two soils in Punjab (India): a simulation analysis using the CROPMAN model. Agricultural Water Management, 79, 312-320.

Jatoi, S. A., Afzal, M., Nasim, S. and Anwar, R. 2001. Seed deterioration study in pea, using accelerated ageing techniques. Pakistan Journal of Biological Sciences, 4, 1490-1494.

Jerlin, R. and Vadivelu, K. 2004. Effect of fertilizer application in nursery for elite seedling production of Pungam (*Pongamia pinnata* L.). Journal of Tropical Agricultural Research Extension, 7, 69-71.

Joliffe, I.T. and Morgan, B.J.T. 1992. Principal component analysis and exploratory factor analysis. Statistical methods in medical research, 1, 69-95.

Jukanti, A. K., Gaur, P. M., Gowda, C. and Chibbar, R. N. 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. British Journal of Nutrition, 108, S11-S26.

Justice, O.L. and Bass, L.N. 1978. Principles and practices of seed storage. USA Department of Agriculture, Science and Education Administration.

Kalpana, R. and Madhava, R. 1995. On the ageing mechanism in pigeonpea (*Cajanus cajan* L.) seeds. Seed Science and Technology, 23, 1-9.

Kano, M., Hasebe, S., Hashimoto, I. and Ohno, H. 2001. A new multivariate statistical process monitoring method using principal component analysis. Computers and chemical engineering, 25, 1103-1113.

Kapoor, N., Arya, A., Siddiqui, M., Amir, A. and Kumar, H. 2010. Seed deterioration in chickpea (*Cicer arietinum* L.) under accelerated ageing. Asian Journal of plant sciences, 9, 158-162.

Kashiwagi, J., Krishnamurthy, L., Purushothaman, R., Upadhyaya, H., Gaur, P., Gowda, C., Ito, O. and Varshney, R. 2015. Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.). Field Crops Research, 170, 47-54.

Kassie, M., Shiferaw, B., Asfaw, S., Abate, T., Muricho, G., Ferede, S., Eshete, M. and Assefa, K. 2009. Current situation and future outlooks of the chickpea sub-sector in Ethiopia. ICRISAT and EIAR (http: //www. icrisat. org/tropicallegumesII/pdfs/Current_Situation. pdf).

Kaur, S. and Sharma, P. 2014. Biochemical characterization of Indian mustard (*Brassica juncea* L.) genotypes in response to moisture stress and irrigation modules. Journal of Oilseed Brassica, 1, 265-272.

Kaya, M., Kaya, G., Kaya, M. D., Atak, M., Saglam, S., Khawar, K. M. and Ciftci, C. Y. 2008. Interaction between seed size and NaCl on germination and early seedling growth of some Turkish cultivars of chickpea (*Cicer arietinum* L.). Journal of Zhejiang University Science B, 9, 371.

Khajjak, M.H., Raza, A.M., Luqman, M., Yousafzai, A., Naudhani, S., Ali, G.M. and Saeed, M. 2016. Evaluation of seed viability of *Juniperus excelsa* found in balochistan by tetrazolium assay. Journal of Applied and Emerging Sciences, 6, 31-35.

Khan, M., Iqbal, M., Abbas, M. and Usman, M. 2003. Effect of aging on viability, vigour and chromosomal damage in pea (*Pisum sativum* L.) seeds. Pakistan Journal of Agricultural Sciences, 40, 1-2.

Khatun, A., Kabir, G. and Bhuiyan, M. 2009. Effect of harvesting stages on the seed quality of lentil (*Lens culinaris* L.) during storage. Bangladesh Journal of Agricultural Research, 34, 565-576.

Kibinza, S., Vinel, D., Côme, D., Bailly, C. and Corbineau, F. 2006. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. Physiological Plantarum, 128, 496-506.

Kong, Q., Mao, P., Yu, X. and Xia, F. 2014. Physiological changes in oat seeds aged at different moisture contents. Seed Science and Technology, 42, 190-201.

Korbu, L., Damte, T. and Fikre, A. 2016. Harnessing chickpea value chain for nutrition security and commercialization of smallholder agriculture in Africa.

Krannitz, P.G., Aarssen, L.W. and Dow, J.M. 1991. The effect of genetically based differences in seed size on seedling survival in Brassicaceae (*Arabidopsis thaliana*). American Journal of Botany, 78, 446-450.

Kruger, N.J. 2009. The Bradford method for protein quantitation. The protein protocols handbook, 15-21.

Lahuta, L., Login, A., Rejowski, A., Socha, A. and Zalewski, K. 2000. Influence of water deficit on the accumulation of sugars in developing field bean (*Vicia faba*) seeds. Seed Science and Technology, 28, 93-100.

Lamichaney, A., Katiyar, P., Natarajan, S. and Sripathy, K. 2016. Relationship among some seed characters, laboratory germination and field emergence in chickpea (*Cicer arietinum* L.) genotypes differing in testa colour. Food Legumes, 29, 29-32.

Leishman, M.R. 2001. Does the seed size/number trade-off model determine plant community structure? An assessment of the model mechanisms and their generality. Oikos International, 93,294-302.

Leopold, A. 1990. Coping with desiccation. In RG Alscher, JR cumming, eds, stress responses in plants: Adaptation and Acclimation Mechanisms. Wiley-Liss, New York, 37-56.

Leopold, A. C., Sun, W. Q. and Bernal-Lugo, I. 1994. The glassy state in seeds: analysis and function. Seed Science Research, 4, 267-274.

Leport, L., Turner, N. C., Davies, S. and Siddique, K. 2006. Variation in pod production and abortion among chickpea cultivars under terminal drought. European Journal of Agronomy, 24, 236-246.

Leport, L., Turner, N.C., French, R., Barr, M., Duda, R., Davies, S., Tennant, D. and Siddique, K. 1999. Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. European Journal of Agronomy, 11, 279-291.

Liu, E., Mei, X., Yan, C., Gong, D. and Zhang, Y. 2016. Effects of water stress on photosynthetic characteristics, dry matter translocation and water use efficiency in two winter wheat genotypes. Agricultural Water Management, 167, 75-85.

Llanes, A., Andrade, A., Masciarelli, O., Alemano, S. and Luna, V. 2016. Drought and salinity alter endogenous hormonal profiles at the seed germination phase. Seed Science Research, 26, 1-13.

Long, S.P., Marshall-Colon, A. and Zhu, X.G. 2015. Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell, 161, 56-66.

Ma, J. M., Horbowicz, M. and Obendorf, R. L. 2005. Cyclitol galactosides in embryos of buckwheat stem-leaf-seed explants fed D-chiro-inositol, myo-inositol or D-pinitol. Seed Science Research, 15, 329-338.

Madah, A. H. and Abdi, N. 2003. Study of variation and seed deterioration of festua ovaina germplasm in natural resources gene-bank. Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 11, 105-125.

Magan, N., Sanchis, V. and Aldred, D. 2004. Role of spoilage fungi in seed deterioration. Fungal Biotechnology in Agricultural Food, and Environmental Applications, 311-323.

Maity, S., Banerjee, G., Roy, M., Pal, C., Pal, B., Chakrabarti, D. and Bhattacharjee, A. 2000. Chemical induced prolongation of seed viability and stress tolerance capacity of mung bean seedlings. Seed Science and Technology, 28, 155-162.

Majnoun Hosseini, N., Siddique, K., Palta, J. and Berger, J. 2009. Effect of soil moisture content on seedling emergence and early growth of some chickpea (*Cicer arietinum* L.) genotypes. Journal of Agricultural Science and Technology, 11, 401-411.

Mandal, S. M., Dipjyoti, C. and Kajal, G. 2010. Seed size variation: influence on germination and subsequent seedling performance in lamiaceae (*Hyptis suaveolens*). Research Journal of Seed Science, 3, 185-192.

Marcos Filho, J. 2015. Seed vigor testing: an overview of the past, present and future perspective. Scientia Agricola, 72, 363-374.

Marian, C.O., Krebs, S.L. and Arora, R. 2004. Dehydrin variability among rhododendron species: a 25-kDa dehydrin is conserved and associated with cold acclimation across diverse species. New Phytologist, 161, 773-780.

Mathews, C. 2005. Minor edible legumes in Mpumalanga, South Africa. Proceedings of first International edible legume conference in conjunction with the fourth world cowpea congress, University of Pretoria, Pretoria, Published in IELC website.http://www.up.ac.za/conferences/ielc/ held at Durban, 17-21.

Matthews, S. and Powell, A. 2006. Electrical conductivity vigour test: physiological basis and use. Seed Testing International, 131, 32-35.

Mbofung, G. C., Goggi, A. S., Leandro, L. F. and Mullen, R. E. 2013. Effects of storage temperature and relative humidity on viability and vigor of treated soybean seeds. Crop Science, 53, 1086-1095.

Meis, S. J., Fehr, W. R. and Schnebly, S. R. 2003. Seed source effect on field emergence of soybean lines with reduced phytate and raffinose saccharides. Crop Science, 43, 1336-1339.

Moles, A. T. and Westoby, M. 2004. Seedling survival and seed size: a synthesis of the literature. Journal of Ecology, 92, 372-383.

Moreno, M.T. and Cubero, J. 1978. Variation in chickpea (*Cicer arietinum* L.). Euphytica, 27, 465-485.

Munirathnam, P. and Sangita, M. 2009. Influence of sowing dates and irrigations on growth and yield of chickpea. Legume Research International Journal, 32, 209-211.

Navaey, H.N., Hzari, M.Y., Seraji, R.A.N. and Eslami, H. 2014. Germination reduce in borage (*Borago officinalis* L.) seed under seed deteriorating conditions. International Journal of Farming and Allied Sciences, 3, 358-361.

Nerson, H. 2002. Relationship between plant density and fruit and seed production in muskmelon. Journal of the American Society for Horticultural Science, 127, 855-859.

Nonogaki, H., Bassel, G.W. and Bewley, J.D. 2010. Germination still a mystery. Plant Science, 179, 574-581.

Obendorf, R. L. 1997. Oligosaccharides and galactosyl cyclitols in seed desiccation tolerance. Seed Science Research, 7, 63-74.

Ogola, J.B.O. and Thangwana, M.N. 2013. Water use efficiency of summer sown chickpea in a tropical environment: Response to genotype and planting density. Journal of Food Agriculture and Environment, 11, 2689-2695.

Pandey, V. and Pati, A. K. 2017. Limitation of improvement in germination by osmopriming of differentially aged non-orthodox neem (*Azadirachta indica*) seeds. Journal of Physiology and Biochemistry, 6, 2.

Pathan, S., Lee, J. D., Sleper, D., Fritschi, F., Sharp, R., Carter, T., Nelson, R., King, C., Schapaugh, W. and Ellersieck, M. 2014. Two soybean plant introductions display slow leaf wilting and reduced yield loss under drought. Journal of Agronomy and Crop Science, 200, 231-236.

Patil, V. and Dadlani, M. 2009. Tetrazolium test for seed viability and vigour. Handbook of Seed Testing, 209-241.

Peksen, A., Peksen, E. and Bozoglu, H. 2004. Relationships among some seed traits, laboratory germination and field emergence in cowpea (*Vigna unguiculata* L.) genotypes. Pakistan Journal of Botany, 36, 311-320.

Peterbauer, T. and Richter, A. 2001. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Science Research, 11, 185-197.

Peters, J. 2000. Tetrazolium testing handbook. Contribution No. 29 to the handbook on seed testing. Lincoln, NE: Association of Official Seed Analysts, 1-18, 151-154.

Pierre, M. and Savoure, A. 1990. Effect of water stress and SO2 pollution on spruce endopeptides. Plant Physiology and Biochemistry, 28, 95-104.

Porter, R. H. 2017. Testing the quality of seeds for farm and garden. Research Bulletin (Lowa Agriculture and Home Economics Experiment Station), 27, 1.

Powell, A. A., Oliveira, M. D. A. and Matthews, S. 1986. The role of imbibition damage in determining the vigour of white and coloured seed lots of dwarf French beans (*Phaseolus vulgaris*). Journal of Experimental Botany, 37, 716-722.

Powell, A., Corbineau, F., Franca-neto, J., Léchappé, J., Mesterhazy, A., Noli, E., Pritchard, H. and Tarp, G. 2005. Towards the future in seed production, evaluation and improvement. Seed Science and Technology, 33, 265-281.

Pukacka, S., Ratajczak, E. and Kalemba, E. 2009. Non-reducing sugar levels in beech (Fagus sylvatica) seeds as related to withstanding desiccation and storage. Journal of Plant Physiology, 166, 1381-1390.

Rai, R., Singh, S.N. and Prasad, V. 1982. Effect of press-mud amended pyrite on symbiotic N2-fixation, active iron contents of nodules, grain yield and quality of chickpea (*cicer arietinum* L.) genotypes in calcareous soil. Journal of Plant Nutrition, 5, 905-913.

Ramagopal, S. 1990. Inhibition of seed germination by salt and its subsequent effect on embryonic protein synthesis in barley. Journal of Plant Physiology, 136, 621-625.

Roozrokh, M., Shams, K. and Vghar, M. 2005. Effects of seed size and seedling depth on seed vigor of chickpea. First National Legume Congress. Mashhad Ferdowsi University, Mashhad, Iran.

Rosental, L., Perelman, A., Nevo, N., Toubiana, D., Samani, T. and Batushansky, A. 2016. Environmental and genetic effects on tomato seed metabolic balance and its association with germination vigor. BMC Genomics, 17, 1047.

Roy-Macauley, H., Zuily-Fodil, Y., Kidric, M., Thi, A.T. and Silva, J.V. 1992. Effect of drought stress on proteolytic activities in phaseolus and vigna leaves from sensitive and resistant plants. Physiologia Plantarum, 85, 90-96.

Sadeghian, S.Y. and Yavari, N. 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. Journal of Agronomy and Crop Science, 190, 38-144.

Sharma, S. N. and Maheshwari, A. 2015. Expression patterns of DNA repair genes associated with priming small and large chickpea (*Cicer arietinum* L.) seeds. Seed Science and Technology, 43, 250-261.

Shelar, V., Shaikh, R. and Nikam, A. 2008. Soybean seed quality during storage: a review. Agricultural Reviews, 29, 125-131.

Shiferaw, B., Jones, R., Silim, S., Teklewold, H. and Gwata, E. 2007. Analysis of production costs, market opportunities and competitiveness of desi and kabuli chickpeas in Ethiopia, IPMS Working Paper, 3, 48.

Shrestha, R., Turner, N., Siddique, K., Turner, D. and Speijers, J. 2006. A water deficit during pod development in lentils reduces flower and pod numbers but not seed size. Australian Journal of Agricultural Research, 57, 427-438.

Siddique, A. and Wright, D. 2004. Effects of date of sowing on seed yield, seed germination and vigour of peas and flax. Seed Science and Technology, 32, 455-472.

Simon, E. and Harun, R. R. 1972. Leakage during seed imbibition. Journal of Experimental Botany, 23, 1076-1085.

Singh, G., Ram, H. and Aggarwal, N. 2017. Growth, productivity and economics of kabuli chickpea (*Cicer arietinum* L.) Genotypes in response to seed rate in northern India. International Journal of Current Microbiology and Applied Sciences, 6, 3917-3930.

Soltani, A., Galeshi, S., Zeinali, E. and Latifi, N. 2002. Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. Seed Science and Technology, 30, 51-60.

Sujatha, K., Sivasubramaniam, K., Selvarani, K., Padma, J. and Sripunitha, A. 2011. Quick test for viability determination in cashew seed nut. International Symposium on Cashew Nut, 1080, 321-323.

Svensson, J., Ismail, A.M., Palva, E.T. and Close, T.J. 2002. Sensing, signaling and cell adaptation. Elsevier Science, 155-171.

Tanveer, A., Tasneem, M., Khaliq, A., Javaid, M. and Chaudhry, M. 2013. Influence of seed size and ecological factors on the germination and emergence of field bindweed (*Convolvulus arvensis*). Planta Daninha, 31, 39-51.

Tekrony, D. 2003. Precision is an essential component in seed vigour testing. Seed Science and Technology, 31, 435-447.

Thomas, J., Prasad, P., Boote, K. and Allen, L. 2009. Seed composition, seedling emergence and early seedling vigour of red kidney bean seed produced at elevated temperature and carbon dioxide. Journal of Agronomy and Crop Science, 195, 148-156.

Tilebeni, H. and Golpayegani, A. 2011. Effect of seed ageing on physiological and biochemical changes in rice seed (*Oryza sativa* L.). International Journal of Agricultural Science, 1, 138-143.

Trawatha, S.E., Tekrony, D.M. and Hildebrand, D.F. 1995. Relationship of soybean seed quality to fatty acid and C6-aldehyde levels during storage. Crop Science, 35, 1415-1422.

Turner, N. C., Wright, G. C. and Siddique, K. 2001. Adaptation of grain legumes (pulses) to water-limited environments. Advances in Agronomy, 71, 193-231.

Varshney, R. K., Song, C., Saxena, R. K., Azam, S., Yu, S., Sharpe, A. G., Cannon, S., Baek, J., Rosen, B. D. and Taran, B. 2013. Draft genome sequence of chickpea (*Cicer arietinum* L.) provides a resource for trait improvement. Nature biotechnology, 31, 240-246.

Verma, S., Bajpai, G. C., Tewari, S. and Singh, J. 2005. Seedung index and yield as influenced by seed size in pigeonpea. Legume Research, 28, 143-145.

Vieira, R. D., Tekrony, D. and Egli, D. 1992. Effect of drought and defoliation stress in the field on soybean seed germination and vigor. Crop Science, 32, 471-475.

Vishwanath, K., Kalappa, V. and Prasad, S. 2006. Standardization of screen sizes for French bean seed processing. Seed Research-New Delhi, 34, 77.

Volk, G.M., Crane, J., Caspersen, A.M., Hill, L.M., Gardner, C. and Walters, C. 2006. Massive cellular disruption occurs during early imbibition of cuphea seeds containing crystallized triacylglycerols. International Journal of Plant Biology, 224, 1415.

Walters, C., Farrant, J. M., Pammenter, N. W. and Berjak, P. 2002. Desiccation stress and damage. Desiccation and survival in plants: drying without dying. CABI publishing, Oxford and New York, 263-291.

Wang, J., Gan, Y., Clarke, F. and Mcdonald, C. 2006. Response of chickpea yield to high temperature stress during reproductive development. Crop Science, 46, 2171-2178.

Whitmore, L. and Wallace, B.A. 2008. Protein secondary structure analyses from circular dichroism spectroscopy: methods and reference databases. Biopolymers, 89, 392-400.

Wojtczak, L. and Więckowski, M.R. 2016. From mitochondrial large amplitude swelling to the permeability transition-a short historic overview. Postepy Biochemii, 62, 298.

Wold, S., Esbensen, K. and Geladi, P. 1987. Principal component analysis. Chemometrics and intelligent laboratory systems, 2, 37-52.

Yadav, S. and Sharma, S. 2001. Seed coat cracking and its effect on seed quality characteristics in kabuli gram (*Cicer arietinum* L.). Seed Research-New Delhi, 29, 7-12.

Yadav, S. K., Yadav, S., Kumar, P. and Kant, K. 2005. A critical overview of chickpea seed technological research. Seed Research-New Delhi, 33, 1.

Yanping, Y., Rongqi, G., Qingquan, S. and Shengfu, L. 1999. Effects of storage temperature and container type on the vigour of welsh onion seeds with low moisture content. Australian Journal of Experimental Agriculture, 39, 1025-1028.

APPENDIX 1: LIST OF ANOVA'S FOR CHAPTER 3

Variate: Total seed size (experiment 1)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	108.21	21.64	1.06	
rep.*Units* stratum					
Genotype	2	1407.71	703.85	34.59	<.001
Residual	28	569.69	20.35		
Total	35	2085.60			

Variate: Total seed size (experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	23.49	4.70	0.17	
rep.*Units* stratum					
Genotype	2	1617.67	808.84	28.65	<.001
Residual	28	790.35	28.23		
Total	35	2431.52			

Variate: Hypocotyl emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	13.73534	2.74707	29.86	
Rep.*Units* stratum					
Day	17	112.57793	6.62223	71.98	<.001
Genotype	2	52.33951	26.16975	284.45	<.001
Day. Genotype	34	24.57716	0.72286	7.86	<.001
Residual	1237	113.80633	0.09200		
Total	1295	317.03627			

Variate: Complete emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	5	5.47840	1.09568	14.44	
Rep.*Units* stratum					
Day	17	104.45988	6.14470	80.99	<.001
Genotype	2	23.64969	11.82485	155.85	<.001
Day.Genotype	34	26.18364	0.77011	10.15	<.001
Residual	1237	93.85494	0.07587		
Total	1295	253.62654			

APPENDIX 2: LIST OF ANOVA'S FOR CHAPTER 4

Variate: Mean Germination Time (day)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.079	0.039	0.22	
Rep.*Units* stratum					
genotype	2	2.304	1.152	6.43	0.003
water_level	1	0.565	0.565	3.15	0.081
Day	4	1.234	0.309	1.72	0.158
genotype.water_level	2	0.227	0.113	0.63	0.535
genotype.Day	8	2.352	0.294	1.64	0.133
water_level.Day	4	0.429	0.107	0.6	0.666
genotype.water_level.Day	8	0.762	0.095	0.53	0.828
Residual	58	10.397	0.179		
Total	89	18.348			

Variate: TZ_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	81.7	81.7	0.46	
rep.*Units* stratum					
genotype	2	670	335	1.88	0.171
water_level	1	1.7	1.7	0.01	0.924
day	4	4383.3	1095.8	6.15	0.001
genotype.water_level	2	443.3	221.7	1.24	0.303
genotype.day	8	2596.7	324.6	1.82	0.113
water_level.day	4	223.3	55.8	0.31	0.867
genotype.water_level.day	8	556.7	69.6	0.39	0.917
Residual	29	5168.3	178.2		
Total	59	14125			

Variate: EC - µS/cm/g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	7.196	7.196	6.42	
rep.*Units* stratum					
genotype	2	10.075	5.038	4.5	0.02
water_level	1	14.571	14.571	13	0.001
day	4	2.228	0.557	0.5	0.738
genotype.water_level	2	1.601	0.801	0.71	0.498
genotype.day	8	12.837	1.605	1.43	0.225
water_level.day	4	3.178	0.794	0.71	0.592
genotype.water_level.day	8	6.879	0.86	0.77	0.634
Residual	29	32.493	1.12		
Total	59	91.059			

Variate: Germination_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	126.7	63.3	0.52	
Rep.*Units* stratum					
Day	4	2728.9	682.2	5.65	<.001
genotype	2	4486.7	2243.3	18.57	<.001
water_level	1	810	810	6.71	0.012
Day.genotype	8	2491.1	311.4	2.58	0.018
Day.water_level	4	1840	460	3.81	0.008
genotype.water_level	2	326.7	163.3	1.35	0.267
Day.genotype.water_level	8	3273.3	409.2	3.39	0.003
Residual	58	7006.7	120.8		
Total	89	23090			

Variate: Imbibition Weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	1	0.596926	0.596926	103.45	
rep.*Units* stratum					
genotype	2	3.359	1.679	291.02	<.001
water_level	1	0.245	0.245	42.53	<.001
day	4	0.490	0.122	21.22	<.001
Time	7	18.099	2.586	448.08	<.001
genotype.water_level	2	0.128	0.064	11.08	<.001
genotype.day	8	0.607	0.076	13.16	<.001
water_level.day	4	0.447	0.112	19.36	<.001
genotype.Time	14	0.189	0.014	2.34	0.003
water_level.Time	7	0.029	0.004	0.71	0.664
day.Time	28	0.115	0.004	0.71	0.864
genotype.water_level.day	8	0.812	0.102	17.59	<.001
genotype.water_level.Time	14	0.009	0.001	0.11	1
genotype.day.Time	56	0.105	0.002	0.33	1
water_level.day.Time	28	0.028	0.001	0.18	1
genotype.water_level.day.Time	56	0.057	0.001	0.18	1
Residual	2159	12.458	0.006		
Total	2399	37.775			

APPENDIX 3: LIST OF ANOVA'S FOR CHAPTER 5

Analysis of variance

Variate: Total seed size

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	148.54	49.51	1.49	
Rep.*Units* stratum					
genotype	2	3245.41	1622.7	48.99	<.001
water_level	1	647.28	647.28	19.54	<.001
genotype.water_level	2	349.03	174.51	5.27	0.006
Residual	231	7651.96	33.13		
Total	239	12042.21			

Variate: TZ_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	3	33.3	11.1	0.07	
replication.*Units* stratum					
genotype	2	625	312.5	1.94	0.178
water_level	1	2016.7	2016.7	12.52	0.003
genotype.water_level	2	108.3	54.2	0.34	0.72
Residual	15	2416.7	161.1		
Total	23	5200			

Analysis of variance

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	137470	68735	1.86	
rep.*Units* stratum					
genotype	2	6124000	3062000	82.67	<.001
water_level	1	3706559	3706559	100.08	<.001
genotype.water_level	2	1477813	738906	19.95	<.001
Residual	424	15704007	37038		
Total	431	27149848			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.3294	0.1098	0.68	
rep.*Units* stratum					
genotype	2	1.2615	0.6307	3.93	0.043
water_level	1	1.884	1.884	11.73	0.004
genotype.water_level	2	0.1758	0.0879	0.55	0.59
Residual	15	2.4094	0.1606		
Total	23	6.0601			

Variate: Germination_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	900	300	0.93	
rep.*Units* stratum					
genotype	2	3408.3	1704.2	5.27	0.018
water_level	1	66.7	66.7	0.21	0.656
genotype.water_level	2	1108.3	554.2	1.71	0.214
Residual	15	4850	323.3		
Total	23	10333.3			

Variate: stachyose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	747.23	249.08	6.66	
rep.*Units* stratum					
genotype	2	126.24	63.12	1.69	0.218
water_level	1	28.52	28.52	0.76	0.396
genotype.water_level	2	151.07	75.53	2.02	0.167
Residual	15	561.13	37.41		
Total	23	1614.18			

Variate: raffinose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	348.33	116.11	9.15	
rep.*Units* stratum					
genotype	2	464.5	232.25	18.3	<.001
water_level	1	65.15	65.15	5.13	0.039
genotype.water_level	2	92.56	46.28	3.65	0.051
Residual	15	190.37	12.69		
Total	23	1160.91			

Variate: sucrose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	980.56	326.85	9.71	
rep.*Units* stratum					
genotype	2	829.57	414.78	12.33	<.001
water_level	1	0.07	0.07	0	0.964
genotype.water_level	2	182.62	91.31	2.71	0.099
Residual	15	504.8	33.65		
Total	23	2497.62			

Variate: Protein content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.000162	0.000081	0.03	
rep.*Units* stratum					
genotype	2	0.002138	0.001069	0.38	0.694
water_level	1	0.001133	0.001133	0.4	0.541
genotype.water_level	2	0.009261	0.00463	1.64	0.242
Residual	10	0.028211	0.002821		
Total	17	0.040905			