

Response of primed soybean (*Glycine max* L.) to storage duration and conditions

by

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PREFACE

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

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

A handwritten signature in black ink, appearing to be 'A.O. Odindo', written over a horizontal line.

Signed: Professor A.O. Odindo

Date: 09 February 2022

DECLARATION: PLAGIARISM

I, Zuzumuzi Sizwe Buthelezi, declare that:

- (i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;
- (ii) this thesis has not been submitted in full or in part for any degree or examination to any other university;
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Signed: Zuzumuzi Sizwe

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GENERAL ABSTRACT

One of the most valuable leguminous crops in the world is soybean (*Glycine max*). However, loss of seed quality (germination, viability, and vigour) during storage can be a primary constraint in soybean production. Seed priming is one of the techniques that can be applied to improve the quality of low vigour seeds with poor germination. However, there is little information on the application of seed priming on seed germination and vigour of stored soybean seeds. It is also unknown how long primed soybean seeds can be stored. Therefore, this study aimed at (i) determining the effect of seed priming on germination of low vigour soybean seeds and (ii) investigating the effect of storage duration and conditions on the viability, germination, vigour, and seedling establishment of primed soybean seeds.

For objective 1, seed ageing was used to simulate low vigour seeds. The experiment was arranged using a complete randomized design (CRD) with the following factors: Cultivars – 3 levels (DM5953RSF, LS6851R, PAN1521R); Seed ageing – 2 levels (Aged and Unaged); Seed priming – 3 levels (Control, hydropriming, and osmopriming) giving a 3 x 2 x 3 factorial treatment structure with four replications totaling to 72 experimental units. For objective 2, a three-factor experiment was undertaken using a completely randomized design (CRD) with the following factors: Cultivars – 3 levels (DM5953RSF, LS6851R, PAN1521R); Seed priming – 3 levels (Control, hydropriming, osmopriming); Storage duration – 8 levels (0, 1, 3, 7, 30, 60, 90, 120 days) giving a 3×3×8 factorial treatment structure with three replications totaling to 216 experimental units. Variables measured include final germination percentage (FGP), mean germination time (MGT), germination index (GI), coefficient of the velocity of germination (CVG), seed moisture content (SMC), electrical conductivity (EC), viability percentage, root length (RL), shoot length (SL), seedling length (SLL), fresh weight (FW), dry weight (DW), seedling vigour index (SVI). Data collected were subjected to the analysis of variance (ANOVA) using GenStat®, 20.1 Edition (VSN International, Hamel Hampstead, UK, 2020) at the 5% level of significance. The means of significantly different variables were separated using Tukey's test with GenStat® at the 5% significance level.

The results showed a highly significant interaction effect ($p < 0.001$) between seed ageing, cultivar, and priming treatments with respect to seed quality. The FGP was 93% before ageing, then 62 % after ageing. Osmopriming of aged seeds improved FGP (78%), whereas hydropriming decreased FGP (48%). The results further indicated a highly significant interaction effect ($p < 0.001$) between storage duration, cultivar, and priming treatments. Osmoprimed seeds maintained the highest GP (91-94%) 0- 30 days of storage compared to

hydroprimed seeds, which maintained the highest GP (92-88%) for 0-7 days. Hydroprimed seeds at the storage of 120 days recorded the lowest seed viability (68 %), SLL (5.69 cm), compared to osmoprimed (16.26 cm) and unprimed seeds (15.37 cm). The EC of primed seeds remained lower for most storage (0-90 days) than for unprimed seeds. However, an increase in EC was evident after 60 days. As a result, EC of all treatments was similar [osmopriming ($14 \mu\text{S cm}^{-1} \text{ g}^{-1}$), hydropriming ($16 \mu\text{S cm}^{-1} \text{ g}^{-1}$), control ($15 \mu\text{S cm}^{-1} \text{ g}^{-1}$)] after 120 days. From these results, it is concluded that (i) germination of low vigour can be improved through osmopriming, (ii) Hydroprimed and osmoprimed seeds can be stored for 0 and 30 days, respectively, without any significant germination and vigour loss, (iii) increase in storage duration negatively affect the germination, viability, vigour, and seedling establishment of primed soybean seeds, regardless of priming treatment.

Keywords: Seed quality, priming, storage

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DEDICATION

This MSc dissertation is dedicated to my late grandmother, Mrs Magogwane "MaNdwandwe" Buthelezi.

TABLE OF CONTENTS

PREFACE	i
DECLARATION: PLAGIARISM.....	ii
GENERAL ABSTRACT	iii
ACKNOWLEDGEMENTS	v
DEDICATION	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	xi
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS/ACRONYMS	xiv
CHAPTER 1: INTRODUCTION	1
1.1 Background.....	1
1.2 Problem statement.....	4
1.3 Aims and objectives.....	5
1.4 Justification	5
1.5 Dissertation structure	5
1.6 References.....	7
CHAPTER 2: LITERATURE REVIEW	11
2.1 Origin and distribution.....	11
2.2 Production	11
2.3 Farm-saved seeds and soybean production.....	13
2.4 Seed quality.....	14
2.4.1 Seed vigour	15
2.4.2 Seed viability	16
2.4.3 Seed germination	17
2.5 Seed storage and deterioration	18

2.6 Factors influencing seed deterioration during storage	20
2.6.1 Temperature and relative humidity	20
2.6.2 Storage duration	20
2.6.3 Genetic	20
2.6.4 Seed moisture content	21
2.7 Seed priming	21
2.7.1 Mechanism of seed priming.....	22
2.7.2 Priming methods	23
2.7.3 Factors affecting seed priming.....	24
2.7.4 Storage potential of primed seeds	25
2.8 Summary and conclusions	26
2.9 References.....	27
CHAPTER 3 EFFECT OF SEED PRIMING ON GERMINATION OF LOW VIGOUR SOYBEAN (<i>GLYCINE MAX</i> L.) SEEDS.....	35
Abstract.....	35
3.1 Introduction.....	35
3.2 Methods and Materials.....	37
3.2.1 Experimental site	37
3.2.2 Experimental material.....	37
3.2.3 Experimental design and treatment structure.....	37
3.2.4 Experimental set-up	38
3.2.5 Statistical analysis.....	40
3.3 Results.....	40
3.3.1 Final germination percentage.....	40
3.3.2 Germination index	42
3.3.3 Mean Germination Time.....	43
3.3.4 Coefficient of the velocity of germination.....	43
3.3.5 Shoot, root, and seedling length.....	43

3.3.6 Seedling Fresh and Dry Weight.....	45
3.3.7 Seedling vigour index	47
3.3.8 Correlation of seed germination and seedling growth parameters.....	48
3.4 Discussion	49
3.4.1 Seed germination	49
3.4.2 Seedling growth	51
3.5 Conclusion	52
3.6 References.....	53
CHAPTER 4 EFFECT OF STORAGE DURATION AND CONDITIONS ON SEED GERMINATION, VIABILITY, VIGOUR, AND SEEDLING ESTABLISHMENT OF PRIMED SOYBEAN SEEDS	57
Abstract	57
4.1 Introduction.....	57
4.2 Methods and materials	59
4.2.2 Experimental material.....	59
4.2.3 Experimental design.....	59
4.2.4 Experimental set-up	59
4.2.5 Statistical analysis.....	62
4.3 Results.....	62
4.3.1 Final germination percentage.....	62
4.3.2 Mean germination time	65
4.3.3 Shoot, root, and seedling length.....	65
4.3.4 Seedling fresh and dry weight.....	69
4.3.5 Accelerated ageing test	72
4.3.6 Tetrazolium Test	73
4.3.7 Electrical Conductivity	74
4.3.8 Seed moisture content.....	76
4.3.9 Correlation of seed germination, viability, and vigour parameters measured	77

4.4 Discussion	79
4.5 Conclusion	81
4.6 References.....	82
CHAPTER 5 GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS	85
5.1 General overview and discussion.....	85
5.2 Conclusion	86
5.3 Recommendations for future research	86
5.4 References.....	88
APPENDICES	89
Appendix 1 Analysis of variance tables for chapter 3	89
Appendix 2 Analysis of variance tables for chapter 4	93

LIST OF TABLES

Table 3.1 Analysis of variance showing mean squares and significance test for FGP, GI, MGT, and CVG of three cultivars subjected to aging and priming.....	41
Table 3.2 Effect of aging, cultivar, and priming on FGP, GI, MGT and CVG	42
Table 3.3 Analysis of variance showing mean squares and significance test for shoot length, and seedling length of three cultivars subjected to aging and priming.....	44
Table 3.4 Effect of aging, cultivar, and priming on shoot length, root length, and seedling length	45
Table 3.5 Analysis of variance showing mean squares and significance test for FW, DW, SVI I, and SVI II of three cultivars subjected to aging and priming.....	46
Table 3.6 Effect of aging, cultivar, and priming on FW, DW, SVI I, and SVI II	47
Table 4.1 Analysis of variance showing mean squares and significance test for FGP, MGT, and AA of three cultivars subjected to priming and storage treatments.....	63
Table 4.2 Analysis of variance showing mean squares and significance test for SL, RL, and SLL of three cultivars subjected to priming and storage treatments	66
Table 4.3 Effect of cultivar, priming, and storage duration on the shoot length	67
Table 4.4 Effect of cultivar, priming, and storage duration on the root length	68
Table 4.5 Effect of cultivar, priming, and storage duration on seedling length	69
Table 4.6 Analysis of variance showing mean squares and significance test for FW and DW of three cultivars subjected to priming and storage treatments.....	70
Table 4.7 Effect of cultivar, priming, and storage duration on fresh weight.....	71
Table 4.8 Effect of cultivar, priming, and storage duration on seedling weight.....	72
Table 4.9 Analysis of variance showing mean squares and significance test for electrical conductivity, viability (TZ), and moisture content for the seed of three cultivars subjected to different priming and storage duration treatments.....	74
Table 4.10 Effect of cultivar, priming, storage duration on seed moisture content.....	77

LIST OF FIGURES

Figure 2.1 Top six soybean-producing countries globally (Food & Agriculture Organization, 2020).	12
Figure 2.2 Top six soybean-producing countries in Africa (FAOSTAT; 2020).....	12
Figure 2.3: Average soybean yield (t/ha) for major producing countries (Brazil, USA, Argentina) and South Africa (FAOSTAT, 2020).	13
Figure 2.4: The time course of major events associated with germination and subsequent post-germinative growth (Bewley, 1997; Nonogaki et al., 2010).....	18
Figure 2.5: Relationship between seed viability and vigour over time (Hampton, 2000).	19
Figure 2.6 Schematic representation of normal germination and seed priming process (Bose et al., 2018) (Phase I; imbibition phase, phase II; germination phase, phase III; post-germination phase).	23
Figure 3.1 Schematic illustration for the accelerated aging experiment set-up.	38
Figure 3.2 Pearson correlation coefficients (r) for seed germination and seedling growth parameters. **GP=final germination percentage, GI=germination index, MGT=mean germination time, CVG=coefficient of the velocity of germination. SL=Shoot length; RL=Root length, SLL=Seedling length, FW=Fresh weight; Dry weight; SVI I= Seedling vigour index I; SVI II=Seedling vigour index II	49
Figure 4.1 Effect of cultivar on final germination percentage (FGP) and mean germination time (MGT).	63
Figure 4.2 Effect of priming on final germination percentage (FGP) and mean germination time (MGT).	64
Figure 4.3 Effect of storage duration on germination percentage and mean germination time (MGT). DAP = days after priming.	64
Figure 4.4 Effect of storage duration and priming on germination percentage and mean germination time (MGT). DAP = days after priming.	65
Figure 4.5 Germination percentage after the accelerated aging test. DAP= days after priming	73
Figure 4.6 Effect of priming and storage duration on the viability of soybean cultivars. DAP=days after priming	74
Figure 4.7 Effect of priming and cultivar on the electrical conductivity of seed leachate	75
Figure 4.8 Effect of storage duration and its interaction with priming on the electrical conductivity of seed leachate. DAP = days after priming.	76

Figure 4.9 Pearson correlation coefficients (r) for seed germination and seedling growth parameters. *Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ns, Not Significant. GP= final germination percentage; MGT=mean germination time; AA=seed vigour based on accelerated ageing test; SL=Shoot length; RL=root length; SLL=seedling length; FW=fresh weight; DW=dry weight; TZ%=seed viability percentage based on tetrazolium test; EC=electrical conductivity of seed leachate, SMC=seed moisture content. 78

LIST OF ABBREVIATIONS/ACRONYMS

AA – Accelerated ageing

CVG – Coefficient of the velocity of germination

DW – Dry weight

EC – Electrical conductivity

FAO – Food and Agriculture Organisation of the United Nations

F/GP- final/germination percentage

FW – Fresh weight

GI – Germination index

GP – Germination percentage

H₂O - Water

MGT – Mean germination time

NAMC – National Agricultural Marketing Council

PEG – Polyethylene Glycol

RH – Relative humidity

RL – Root length

SL – Shoot length

SLL – Seedling length

SMC – Seed moisture content

SVI – Seedling vigour index

TZ - Tetrazolium

CHAPTER 1: INTRODUCTION

1.1 Background

Soybean [*Glycine max* (L) Merrill] is a leguminous crop that originated from China (Hymowitz, 2008; Qiu & Chang, 2010). The species belongs to the Fabaceae family, subfamily Papilionoideae, and is regarded as one of the most valued crops globally (Wijewardana et al., 2019). It provides a valuable source of protein and oil and a range of nutraceutical and pharmaceutical uses (Kering & Zhang, 2015). Soybean is one of the oldest cultivated crops consumed by the Chinese before 2500BC (Dlamini, 2015).

The cultivated soybean (*Glycine max*) was domesticated between 6000-9000 years ago in China from its wild relative (*Glycine soja*) (Kim et al., 2012). Egypt's first production in Africa was reported in 1858, while the first recorded cultivation in Sub-Saharan Africa in South Africa in 1903 (Diers & Scaboo, 2019; Shurtleff & Aoyagi, 2009). Subsequently, in countries like Tanzania, Nigeria, Malawi, and Sudan in 1907, 1908, 1909, and 1912, respectively (Khojely et al., 2018).

Recently, soybean production occupied approximately 6% of the world's arable land (Goldsmith, 2008). The major soybean-producing countries are the United States of America, Brazil, Argentina, China, and India. These countries contribute approximately 84% of the total world production (Simpson, 2020). While all other countries, including South Africa, contribute to the remaining portion (16%). Despite observed yield improvements (De Beer, 2016), the local yield remains low compared to the top soybean-producing countries. For instance, the U.S.A.'s average yield is around 3.1 t ha⁻¹, while in South Africa, it is 1.8 t ha⁻¹ (Food & Agriculture Organization, 2020). According to Schulze et al. (2007), South Africa contributes less than 1% of global soybean production.

The low yields are linked to several factors such as the lack of improved and well-adapted varieties, use of retained/farm-saved seed with low quality, incorrect fertilizer application, and rhizobia inoculation in soils with no history of soybean production (Diers & Scaboo, 2019; Khojely et al., 2018; Mapuwei, 2014).

Like any crop production enterprise, the growth is dependent on the availability of good quality seeds (Dlamini et al., 2014). The term 'seed quality' refers to multiple criteria that include several seed attributes: genetic and chemical composition, physical condition, physiological germination and vigour, size appearance, and presence of seed-borne, crop and varietal purity, weed and crop contaminants, and moisture content (Šimic et al., 2006; Surki et al., 2012). Other contributors to seed quality include specific chemical compositions and resistance to pests and

diseases (Sabry, 2018). The seed of good quality is essential because the seed is the determinant of maximum yield potential and can increase yield by 20% (Ambika et al., 2014). However, South Africa's soybean industry is characterized by the use of farm-saved seeds, which are often of low germination and vigour (Scholtemeijer, 2017). Soybean farmers often save and store seeds on the farm for the next plant planting season (Wambugu et al., 2009). PANNAR (2013) estimates that 85% of annual plantings in South Africa are through farm-saved seeds. According to the National Agricultural Marketing Council (NAMC, 2011), 75% of commercial farmers use recycled soybean seed.

There are various reasons why farmers retain grain as seeds. The primary goal of storing seeds at a farm level is to preserve seed stocks for sowing next season (FAO, 2018). In general, farmers save their seeds for the next planting season due to financial constraints (Mahlangu et al., 2018). Whenever costs of production increase, farmers search for ways to decrease costs. One strategy is to save and clean seed from a current harvest for the following year's planting (Clayton et al., 2009). Retained seeds begin to lose viability and vigour when harvested, processed, or stored (El-Abady et al., 2012).

Seed germination, viability, and vigour are essential characteristics of seed quality because they influence seedling emergence, plant stand establishment, and yield potential (Hlatshwayo, 2018; Rajala et al., 2011). The first step in the plant's life cycle is seed germination, which occurs when a dry seed imbibes water and finishes with radicle protrusion through the seed coat (Makhaye et al., 2021; Nonogaki et al., 2010). Seed viability refers to an embryo's capacity to germinate under ideal conditions. Seed vigour refers to the traits that define the capacity for normal seedlings to emerge and develop quickly and uniformly under various field conditions and is influenced by both pre-and post-harvest factors (Bradbeer, 1988; Ghaderi-Far et al., 2010; Shaban, 2013). According to

Elias and Copeland (1994) and Singh et al. (2016), factors that affect seed quality during storage include environmental conditions during seed production, kind of seed, initial seed quality, pests, diseases, seed oil and moisture content, mechanical injuries during seed processing, storage materials, air temperature and relative air humidity in storage. The physiological changes in the seed that lead to loss of viability are referred to as deterioration. Deterioration refers to the physiological changes in the seed that cause it to lose viability (Delouche, 2016). Seed deterioration during storage is the primary reason for low soybean productivity (Jaya et al., 2014; Kandil et al., 2013). Seeds deteriorate over time, lose vigour during storage, become sensitive to stresses during germination, and eventually die (Nguyen et al., 2012). Deterioration can progress to the extent that seeds are unacceptable for planting

(Byrd & Delouche, 1971). Seed viability and vigour loss during storage under an uncontrolled environment are significant limitations to soybean production in the tropics (Isaac et al., 2016). Fluctuating air temperature, relative humidity, and storage period are critical factors affecting soybean seed quality during storage (Kandil et al., 2013; Pradhan & Badola, 2012). Undesirable storage conditions, such as air temperature and relative humidity, promote seed degeneration during storage, regardless of initial seed quality. (Singh et al., 2016). Hendges et al. (2017) reported that storage temperature of 10 °C provides better seed conservation, whereas temperatures above 30 °C promote higher rates of deterioration and reduced vigour. Miah et al. (2006) also observed decreased germination and vigour with increased storage relative humidity (R.H). The maximum germination occurred under 50% storage R.H., while at 80% R.H., no germination was observed after two months of storage. Singh et al. (2016) assessed the effect of the storage period on the germination of soybean seeds. Results showed that there was a decline in germination over time. Seeds from short-term (1-3 years) storage had 40-70% germination, while seeds with mid-term (4-6 years) had 0-17% germination. Kandil et al. (2013) also observed a decline in germination with increased storage.

The degree of quality loss on seed preserved using various storage procedures varies between plant species and within plant species (Bortey et al., 2016). A wide variation has also been observed in seed quality loss among different soybean cultivars during storage (Wien & Kueneman, 1981). The observed difference among cultivars may be due to genetic factors (El-Abady et al., 2012).

Although quality losses during storage are inevitable (Hendges et al., 2017), the rate of seed germination for many species can be improved through seed priming techniques (Argerich et al., 1989). Priming is a water-based approach that permits controlled seed hydration to initiate pre-germinative metabolism but prevents the seed from progressing to complete germination (Dutta, 2018).

Several types of priming include hydro-priming, osmopriming, halo-priming, matrix priming, bio-priming, nutri-priming, priming with hormones, plant regulators, and other organic sources (Waqas et al., 2019). The widely used methods are osmopriming, hydropriming, and matrix priming. Hydropriming is the most basic and inexpensive method for boosting seed germination and seedling emergence (Pirmani et al., 2013). Osmopriming can be a sustainable way of improving crop establishment, uniform emergence, and field performance. Both osmopriming and hydro-priming can be used to improve field performance (Singh et al., 2012).

Priming is a form of conditioning that can lead to rapid and uniform germination, resulting in superior stand establishment (Mielezrski et al., 2016). The authors also reported that priming

could lower steep water conductivity by a factor of 2 to 5. The author further reported that it could also increase the germination percentage of low vigour seeds. Singh et al. (2012) found that priming could increase the germination of sorghum seeds by up to 25%. Similar improvement was reported in chickpea (Farhoudi & Tafti, 2012; Ghassemi-Golezani & Hosseinzadeh-Mahootchi, 2013), sunflower (Pirmani et al., 2013), and soybean (Miladinov et al., 2018; Weerasekara et al., 2021).

Seed priming success is determined by several elements, including plant species, primer water potential, duration, temperature, seed vigour, dehydration, and primed seed storage conditions (Miladinov et al., 2018). Seeds that have been primed have a shorter shelf life than seeds that have not been primed and should be stored under ideal conditions before planting (Surendra, 2018). The literature reveals that much work has been on storage conditions, packaging materials, and their effects on seed germination and vigour of different soybean cultivars. However, there is little information on the application of seed priming on the seed germination and vigour of stored soybean cultivars.

1.2 Problem statement

Soybean farmers often save and store seeds on the farm for the next planting season. The seeds are, in many cases, stored under uncontrolled environmental conditions characterized by fluctuating temperatures and relative humidity. The seed moisture content of the stored seeds is often unknown at the time of harvesting, and this can affect the storage potential, especially if the moisture content is high. Furthermore, the harvested seed is threshed to remove it from the pods, and such post-harvest handling activities such as mechanical threshing can damage the seed. Seed storage potential can also be influenced by species differences and possibly by cultivar differences with respect to seed chemical composition in relation to phytic acid. These factors acting singly or in interaction can lead to low storage potential and affect seed germination and vigour, consequently, seedling emergence and establishment. This can lead to poor emergence and low plant populations, resulting in poor yields. Although it has been established that seed vigour can be improved using techniques such as priming (Hydro-priming and Poly-Ethylene Glycol). However, there is little information on the application of seed priming on seed germination and vigour of stored soybean cultivars. It is also unknown how long primed soybean seeds can be stored; whether seed vigour tests such as mean germination time and tetrazolium can be used to predict seedling emergence and establishment under sub-optimal field conditions as water stress.

1.3 Aims and objectives

The study's main aim is to gain insights into the response of primed soybean seeds to storage conditions and duration.

Specific objectives

1.3.1 To determine the effect of seed priming on germination of low vigour soybean seeds.

1.3.2 To investigate the effect of storage duration and conditions on the viability, germination, vigour, and seedling establishment of primed soybean seeds.

1.4 Justification

More soybean producers are relying on farm-saved seeds in South Africa (AgriOrbit, 2019). It has been reported that about 80% of annual plantings are through farm-saved seeds. National Agricultural Marketing Council (NAMC, 2011) reported that about 75% of commercial farmers recycled their farm seeds. The quality of these seeds is not always guaranteed, as these seeds are not produced under proper production practices (Hlatshwayo, 2018). Farm-saved seeds are usually packaged in different packaging materials and stored under uncontrolled conditions, characterized by fluctuating storage conditions which may affect seed quality. Low seed quality may lead to poor crop yields (Pradhan & Badola, 2012). It has been established that seed priming is one of the pre-sowing techniques that can be applied to improve seed quality after storage (Arif et al., 2008; Mielezrski et al., 2016). The knowledge may be helpful in developing on-farm seed storage and handling protocols to improve seed quality.

1.5 Dissertation structure

Chapter 1

This introductory chapter provides a general introduction and background information summary. It also outlines the problem statement, aims and objectives, and justification of the study.

Chapter 2

This literature review chapter covers the following topics: origin and distribution, production, seed quality aspects, factors affecting seed quality, seed storage and deterioration, priming mechanism, methods, and storage potential of primed soybean seeds.

Chapter 3

This experimental chapter reports on the effect of seed priming on the germination of low vigour soybean seeds.

Chapter 4

This chapter reports on the effect of storage duration and conditions on the viability, germination, vigour, and seedling establishment of primed soybean seeds.

Chapter 5

This chapter gives the general overview, discussion, and recommendations for future research.

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CHAPTER 2: LITERATURE REVIEW

2.1 Origin and distribution

Soybean is a native of China. Although the exact site of origin is not clear, Southern China, North-Eastern China, and other regions like Korea and Japan are regarded as possible sites of origin (Kim et al., 2012). The cultivated soybean originates from its wild progenitor, *Glycine Soja*, 6000-9000 years ago (Dupare et al., 2008; Qiu & Chang, 2010; Sedivy et al., 2017). *G. soja* is found in most of East Asia, including China, Korea, Japan, and Russia (Jeong et al., 2019). Around the first century, soybean was introduced into neighboring nations (Japan, India, Nepal, and Russia) (Dupare et al., 2008). Traders who traveled to and from East Asia introduced soybean into Europe and America in the eighteenth century (Barnes et al., 2006). The first reported cultivation of soybean in Africa took place around 1858 in Egypt (Shurtleff & Aoyagi, 2009). In the nineteenth century, Chinese traders introduce Soybean to Sub-Saharan Africa (SSA) region (Khojely et al., 2018). It is suggested that South Africa was the first country in the SSA region to plant soybean in 1903 (Diers & Scaboo, 2019; Dlamini et al., 2014).

2.2 Production

From 1900 to 1930, soybean production was confined mainly to the Orient (China, Indonesia, Japan, and Korea). However, in the 1940s, the U.S.A. overtook the entire Orient (Scurek, 2009). To date, soybean has been produced throughout the world. On average, the world annually produced 28.6 million tonnes of soybean in 1961-1965 (Masuda & Goldsmith, 2009) and reached 304.9 million in 2015-2019 (Food & Agriculture Organization, 2020).

Recently, soybean production occupied approximately 6% of the world's arable land (Goldsmith, 2008). The United States of America, Brazil, Argentina, China, and India are the top soybean producers (Figure 2.1). These countries contribute approximately 84% of the total world production (Simpson, 2020). While all other countries, including South Africa, contribute to the remaining portion (16%). South Africa, Nigeria, Egypt, Uganda, Zambia, and Zimbabwe are the primary soybean-producing countries in Africa (Figure 2.2). Despite observed yield improvements (de Beer, 2016), South Africa's average yield remains low compared to the top soybean-producing countries. For instance, the U.S.A.'s average yield is around 3.1 t ha⁻¹, while in South Africa, it is 1.8 t ha⁻¹ (Figure 2.3). According to (Schulze et al., 2007), South Africa contributes less than 1% of global soybean production.

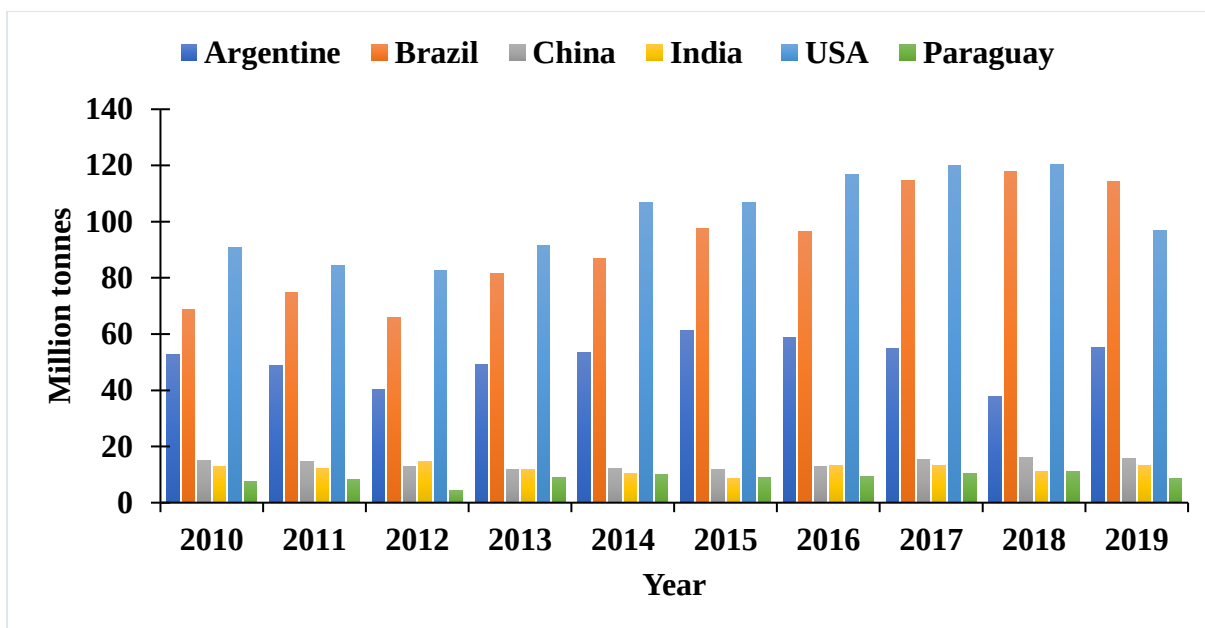


Figure 2.1 Top six soybean-producing countries globally (Food & Agriculture Organization, 2020).

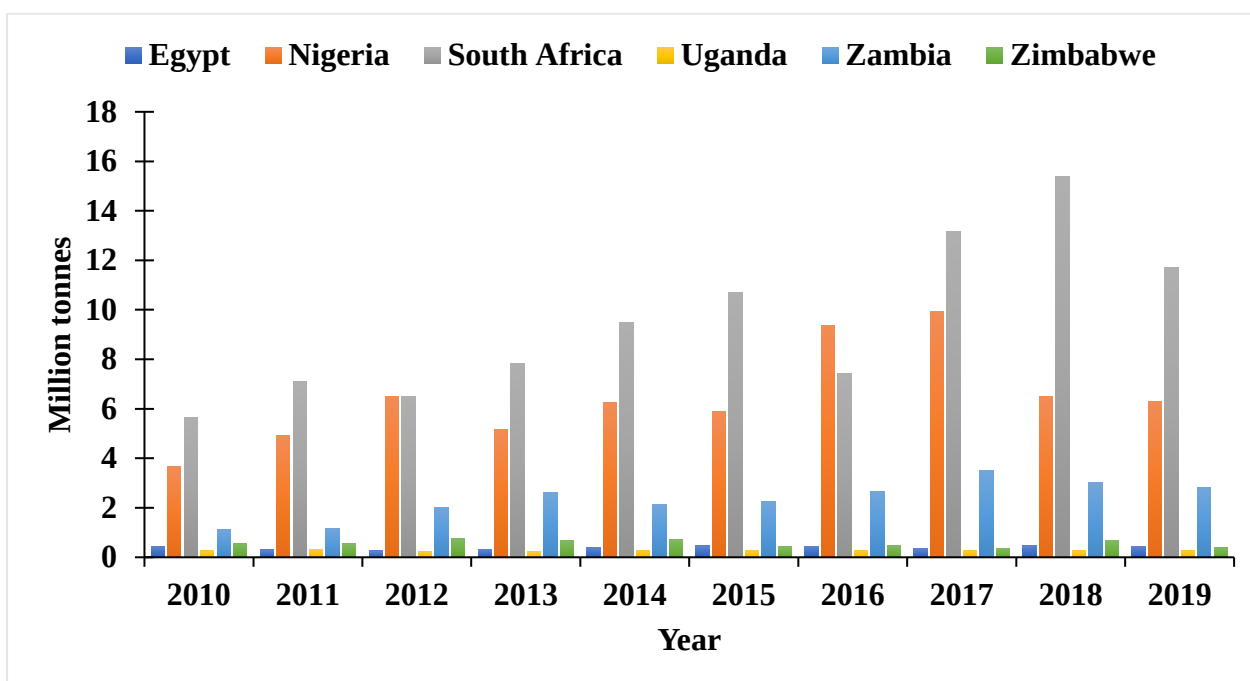


Figure 2.2 Top six soybean-producing countries in Africa (FAOSTAT; 2020).

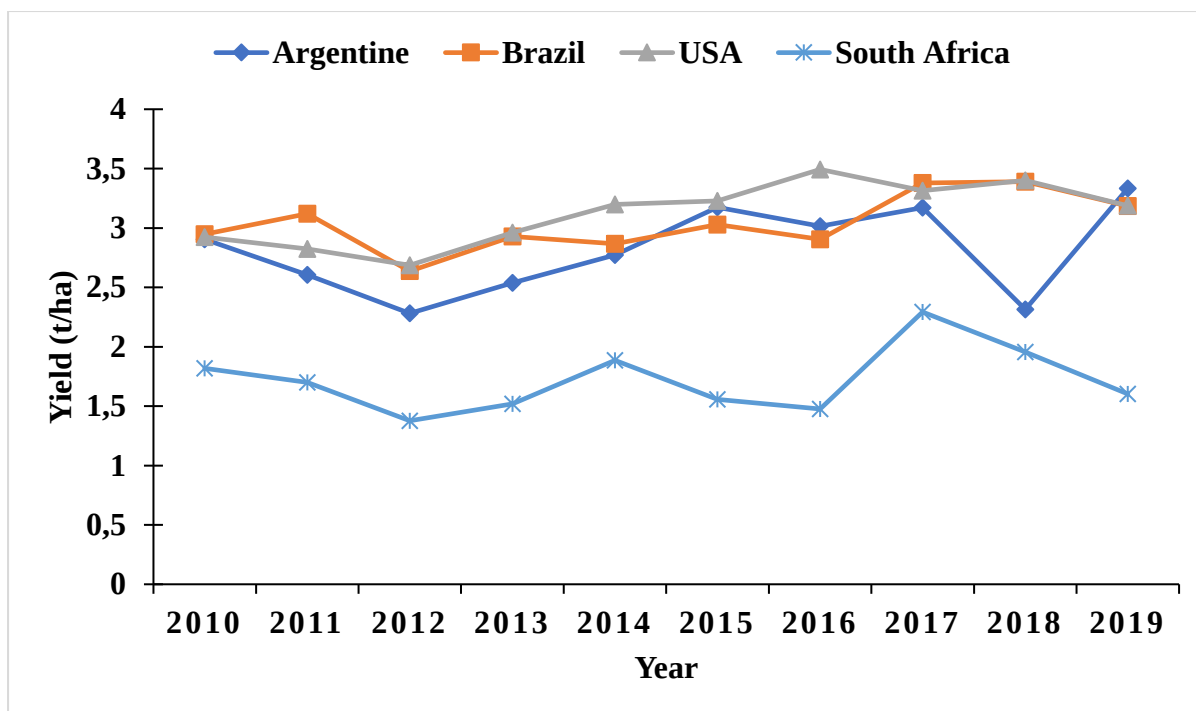


Figure 2.3: Average soybean yield (t/ha) for major producing countries (Brazil, USA, Argentina) and South Africa (FAOSTAT, 2020).

Several biotic and abiotic factors affect soybean yield, leading to yield gaps (Sentelhas et al., 2015). In general, biotic factors are pathogens that attack plants as parasites, while abiotic factors are the environmental (drought, flooding, salinity, cold, and heat) and physical conditions to which the plant or seed is exposed at the time of planting (Gupta et al., 2016; Orzolek, 1991). The lack of access to improved and well-adapted varieties, incorrect fertilizer application, and the use of farm-saved seeds also limit soybean production (Diers & Scaboo, 2019; Khojely et al., 2018; Mapuwei, 2014).

2.3 Farm-saved seeds and soybean production

South Africa's soybean industry is characterized by the use of farm-saved seeds, which are often of low germination and vigour (Scholtemeijer, 2017). Soybean farmers often save and store seeds on the farm for the next plant planting season (Wambugu et al., 2009). PANNAR (2013) estimates that 85% of annual plantings in South Africa are through farm-saved seeds. According to National Agricultural Marketing Council (NAMC, 2011), 75% of commercial farmers use recycled soybean seed. There are various reasons why farmers retain grain as seeds. The primary goal of seed storage on a farm has traditionally been to maintain seed stocks for sowing or to plant next season. (FAO, 2018). In general, farmers save their seeds for the next planting season due to financial constraints (Mahlangu et al., 2018). Whenever costs of

production increase, farmers search for ways to decrease costs. One strategy is to save and clean seed from a current harvest to use for the following year's planting (Clayton et al., 2009). However, seed quality may be compromised during storage (El-Abady et al., 2012).

Like any other crop, soybean cultivation is dependent on many factors (Diers & Scaboo, 2019; Khojely et al., 2018; Mapuwei, 2014; Sentelhas et al., 2015). Seed quality is one of the main factors that play a critical role in soybean production (Dlamini et al., 2014; Garoma et al., 2017; Wimalasekera, 2015). The concept of seed quality is further discussed below.

2.4 Seed quality

Seed quality refers to several seed characteristics that might have varying levels of practical significance in agriculture (Scott & Hampton, 1985). Seed quality is defined differently depending on the end-user. A high-quality seed produces rapid uniform plants under optimal and suboptimal conditions to a farmer. A stable fatty acid profile can be utilized to measure sound quality for an oilseed crop producer (Sabry, 2018). In general, seed quality encompasses features of genetic purity as well as physical and physiological factors such as the seed's physical purity, moisture content, viability, germination, seed vigor, and seed health (Bekele et al., 2019; McDonald & Copeland, 2012). A seed of an adapted variety with high genetic varietal, species, and physical purity; high germination and vigor; free of seed-borne pests; and appropriately cleaned, treated, tested, and labeled is considered high-quality seed (Bishaw et al., 2007).

To enhance agricultural productivity, guarantee food security, and improve farmers' lives, quality seed of adaptive crop varieties must be available, accessible, and used (Bishaw et al., 2007). Hence, seed quality is a crucial factor in crop yield and quality, particularly during increased weather uncertainties due to climate change (Wimalasekera, 2015).

The foundation for profitable soybean crop production and expansion is the high-quality seed that delivers healthy plant stands (Shelar et al., 2008). The seed must have a high germination capacity and be free from seed-borne diseases and foreign materials such as weed seed (Baek et al., 2019; Barnard et al., 2011). High-quality seeds should germinate 90 % or better (Delouche, 2021). Ambika et al. (2014) reported that the use of good quality seeds might increase crop yield by 15-20 %. The use of poor-quality seed lots may result in poor stand establishment, increased sensitivity to environmental stresses, and seedling abnormalities (Elias & Copeland, 1994a). Therefore, it is essential to discuss some critical attributes when dealing with seed quality. The three critical parameters (seed vigor, viability, and germination) will be discussed below.

2.4.1 Seed vigour

By definition, seed vigour is the sum of the seed's properties that determine the seed's activity and performance during germination and seedling emergence (Finch-Savage & Bassel, 2016; Gupta, 1993). However, this does not necessarily define seed vigour but describes the practical consequences (van de Venter, 2000). According to Sadeghi et al. (2011) and Yari et al. (2010), rapid and even field emergence is essential to achieve a high yield with having good quality and quantity in crops.

The aspects of performance linked to seed vigour are (i) seed germination and seedling growth rate and uniformity, (ii) field performance, including the extent, rate, and uniformity of seedling emergence, and (iii) performance after storage, particularly the retention of germination capacity (Hampton, 2000). Slow germination frequently exposes crop plants to severe environmental conditions; hence a crop's success is highly dependent on quick and synchronous seedling emergence (Dutta, 2018). A study by Caverzan et al. (2018) evaluated the effect of seed vigour on seed yield components. The authors found that seeds with high vigour had better shoot and root dry weight, leaf area, stem diameter, and plant height. The authors also reported an increased production variability among plants for low vigor seeds. While higher yields due to greater plant population density were reported for high vigour seeds. Seed vigour is a critical quality attribute that needs to be assessed in addition to germination and viability tests to gain insight into the seed performance in the field or storage (Gupta, 1993). The primary goal of seed vigour testing is to discover critical differences in physiological potential between seed lots, to identify lots with a higher likelihood of performing well following sowing or storage. Vigour testing is more important for seed stored under ambient storage conditions (Tatić et al., 2012). A widely used strategy measures specific aspects of seed deterioration that are indirectly proportional to seed vigour (Elias & Copeland, 1997; Marcos, 2015).

2.4.1.1 Accelerated aging test

Accelerated aging (AA) is a good indicator of vigour and storability since vigor loss and viability could be assessed at regular intervals (Rao et al., 2005). AA is one of the widely used tests to assess seed vigour due to the possibility of standard methodology and reproducibility and its efficiency in providing a good relationship with field emergence (Ghaderi-Far et al., 2010). The environmental parameters usually related to seed deterioration, such as storage temperature and relative humidity, are used in this test (TeKrony, 1993). Seeds are exposed to both high temperatures (41°C) and relative humidity (100 %), which triggers quick deterioration. High vigour seed lots withstand these stressful conditions, deteriorate at a slower

rate, and have higher germination following aging than low vigour seed lots (TeKrony, 2005). Seeds are exposed to the temperature of 41 °C because this is the highest temperature that hydrated proteins can withstand. Higher temperatures can cause protein denaturation and seed death, especially on less vigorous seeds (Marcos, 2015).

2.4.1.2 Electrical conductivity test

The conductivity test is used to determine the amount of electrolyte leakage from the seed coat based on the seed coat's age, storage life, and other factors such as drought stress. (Sadeghi et al., 2011). The rationale behind this test is that less vigorous seeds have a slower rate of cell membrane repair during imbibition, allowing them to discharge more solutes into the environment (Marcos, 2015).

2.4.1.3. Seedling growth test

Vigorous and uniform seedling emergence is also a vital component of seed vigour. Therefore, evaluating seedling length or dry seedling weight constitutes important vigour parameters (Marcos, 2015).

2.4.2 Seed viability

Seed viability is one of seed quality's most critical physiological traits (Baek et al., 2019). Seed viability means that the seed can germinate and produce normal seedlings. Though environmental factors on the apparent plant may prevent germination, viability is most likely maximum during the period of physiological maturity (Ghive et al., 2007). Seed viability steadily decreases after physiological maturity (Bradbeer, 1988; Copeland & McDonald, 2001; Ghive et al., 2007). Both seed viability and vigour play critical roles in seedling emergence, crop stand establishment, and yield potential (Rajala et al., 2011).

2.4.2.1 Tetrazolium test

The tetrazolium test (TZ) determines the percentage of viable seeds within a sample. It represents the number of viable seeds that can produce normal seedlings under ideal conditions (Elias & Garay, 2004). It provides valuable information about vigour, enabling the diagnosis of problems that affect seed quality (França-Neto & Krzyzanowski, 2019). It is based on dehydrogenase activity that catalyses mitochondrial respiration (Grzybowski et al., 2012; Souza et al., 2010). Enzyme dehydrogenase reacts with substrates and releases hydrogen ions to the oxidized, colourless TZ solution, which is changed into red formazan as ions are reduced. Therefore, living tissues of seeds immersed in the TZ solution turn red, while dead tissues remain unstained (Copeland & McDonald, 2012).

2.4.3 Seed germination

Seed germination is usually the most crucial stage of seedling establishment, as it determines whether a crop will be successful or not (Farhoudi & Tafti, 2012; Mohammadkhani & Heidari, 2008). Factors affecting an embryo's ability to germinate include temperature, light, oxygen, water, and species type (Genes & Nyomora, 2018). There must be sufficient oxygen to allow aerobic respiration and a suitable temperature to permit various processes to proceed at an adequate rate (Arteca, 1996; Bewley & Black, 2014). Germination is characterized by three distinct phases (Figure 2.4).

Phase I (Imbibition)

Seed imbibition is the first step in germination (Hershey, 1998). Seed imbibition includes two processes coinciding: the entry of water into the seed and the swelling of the seed (Leopold, 1983). During this phase, water movement is through apoplastic spaces, synthesized proteins from existing mRNAs, and repaired DNA and mitochondria (Dutta, 2018).

The water uptake by dry seed is three-phased, with a rapid initial (phase I) followed by lag phase (phase II). A further increase in water uptake occurs only after germination, as the embryonic axes elongate and break its covering phase III (Bennett, 2004; Bewley, 1997; Finch-Savage & Leubner-Metzger, 2006; Paul et al., 2010). A too rapid water uptake may cause water injury (imbibitional injury) (Woodstock, 1988). When water uptake is too rapid, the seed coat may be damaged, resulting in disruption of cell walls, blistering of the cotyledon surface, and extrusion of cellular contents. Cellular membranes may also leak ions and solutes during hydration (Bewley & Black, 2013).

Phase II and III

Phase II involves the activation and repair processes and the synthesis of proteins by translating new mRNAs and synthesizing new mitochondria (Dutta, 2018). Even though water uptake is minimal during this phase, major metabolic events occur in dormant and non-dormant seeds (Bewley & Black, 2013). Phase III includes completing the germination process and seedling growth, together with a significant increase in water absorption (Dutta, 2018; Miladinov et al., 2018).

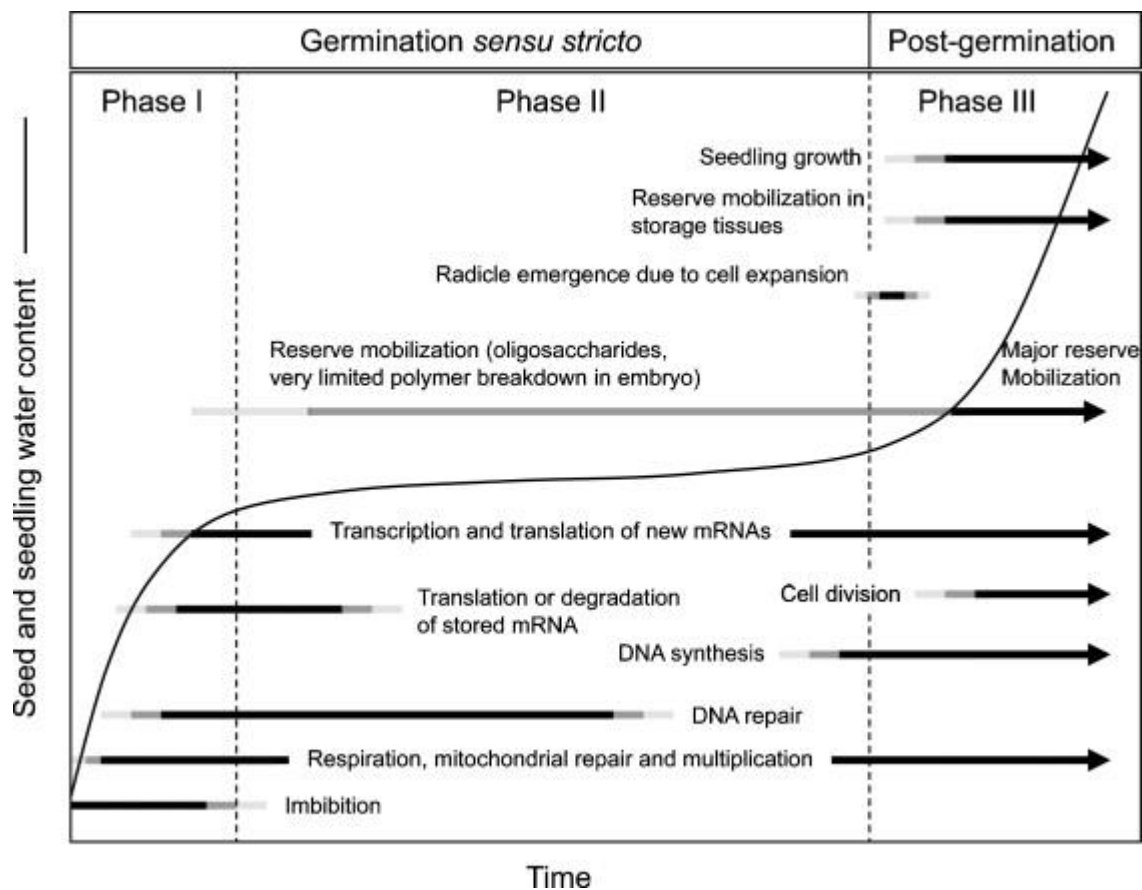


Figure 2.4: The time course of major events associated with germination and subsequent post-germinative growth (Bewley, 1997; Nonogaki et al., 2010).

2.5 Seed storage and deterioration

The quality of the seeds is determined by the interaction between genetic and environmental factors. The genetic factors include genetic makeup, age, nutritional status of the mother plant. Environmental features that contribute to seed quality include temperature, water status, photoperiod and light quality, soil nutrition, mechanical damage and injury, storage duration, and conditions (Wimalasekera, 2015).

The primary objective of seed storage is to maintain the seed in good physical and physiological status from harvest to sowing time (Delouche, 1992). Seed storage begins when the seed reaches physiological maturity before harvest and usually ends at planting time (Delouche, 2021). It includes seed protection and preservation. Proper and safe storage conditions are those that allow quality to be preserved without any loss for at least three years (Govender et al., 2008).

According to Elias and Copeland (1994b) and Singh et al. (2016), seed quality is affected by factors such as environmental conditions during seed production, seed type, initial seed quality,

pests and diseases, seed oil and moisture content, mechanical injuries during seed processing, packaging materials, storage temperature and relative humidity during storage. Deterioration refers to the physiological changes in the seed that cause it to lose viability. According to Kapoor et al. (2010), deterioration is characterized as the loss of quality, vitality, and vigour due to aging or unfavorable environmental factors. Seed deterioration begins at physiological maturity and proceeds during harvesting, processing, and storage. Genetic, production and environmental factors influence seed deterioration (Hampton, 2000). Seed viability gradually decreases after physiological maturity (Ghive et al., 2007). Traditionally, deterioration is associated with storage (Delouche, 2021).

One of the primary causes of decreased soybean productivity is deterioration during storage (Kandil et al., 2013). Seeds degrade, lose vigour, become sensitive to stressors during germination, and eventually die during storage (Nguyen et al., 2012). Deterioration can progress to the extent that seeds are unacceptable for planting (Byrd & Delouche, 1971). Seed deterioration under uncontrolled storage conditions is the major limitation to soybean production in the tropics and subtropics (Isaac et al., 2016). The X and Y points demonstrate the increasing variation between viability and vigour as seed deterioration increases over time (Figure 2.5).

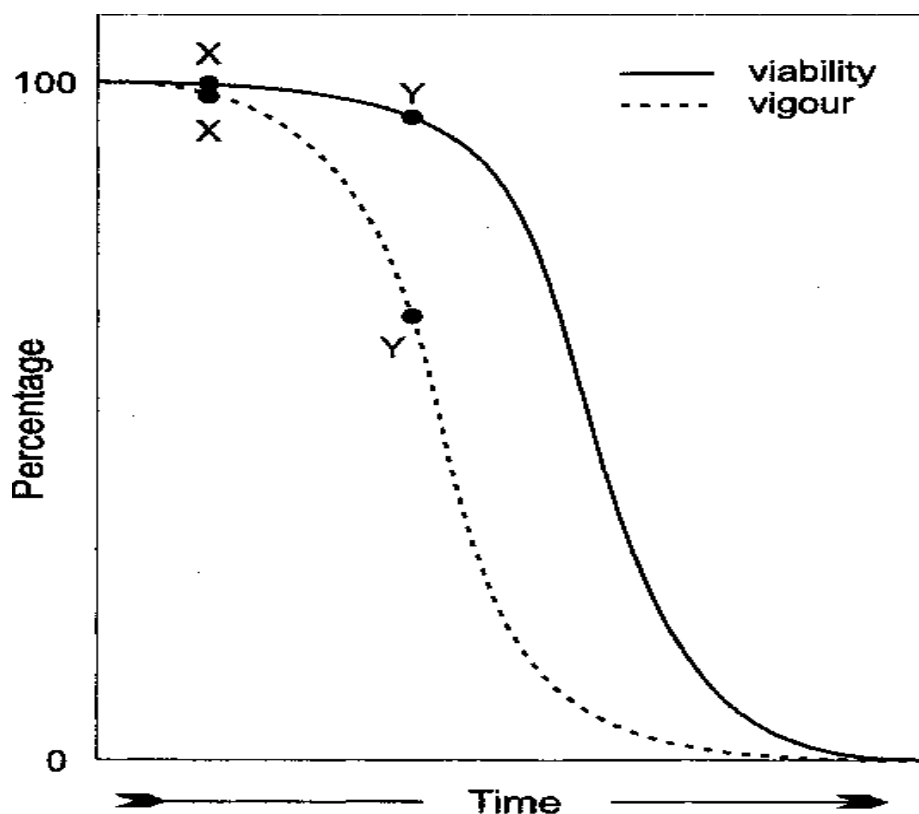


Figure 2.5: Relationship between seed viability and vigour over time (Hampton, 2000).

2.6 Factors influencing seed deterioration during storage

2.6.1 Temperature and relative humidity

Fluctuating air temperature, relative humidity, and storage period are critical factors affecting soybean quality (Kandil et al., 2013; Pradhan & Badola, 2012). Regardless of initial seed quality, improper storage conditions (air temperature and relative humidity) accelerate seed deterioration during storage (Singh et al., 2016). Hendges et al. (2017) reported that storage temperature of 10 °C provides better seed conservation, whereas the temperature above 30 °C promotes higher deterioration and reduced vigour. Miah et al. (2006) also observed a decline in germination and vigour with increased storage relative humidity (R.H). The maximum germination occurred under 50% storage R.H., while at 80% R.H., no germination was observed after just two months of storage.

2.6.2 Storage duration

Singh et al. (2016) assessed the effect of the storage period on the germination of soybean seeds. The findings revealed that there was a decline in germination over time. Seeds from short-term (1-3 years) storage had 40-70 % germination, while seeds with mid-term (4-6 years) had 0-17 %. Kandil et al. (2013) also made a similar observation. Malaker et al. (2008) reported a 20 % decline in germination after 10 months of storage. In addition to germination and vigor, the effect of the storage period on seed moisture has been reported. Seeds require proper packaging in order to maintain their storage stability (Patel et al., 2018). Autade and Ghuge (2018) studied the impact of various packaging materials on seed quality. According to the authors, soybean seed packed in polyethylene bags had the best seed germination, seedling length, dry weight, and vigour index. Another study by Nataraj and Gowda (2017) results indicated the highest germination (73%) in seeds stored in tin followed by seeds stored in polyethylene (72 %), with the lowest seed germination observed in seeds packed in cloth bags (68 %) at the end of storage period.

2.6.3 Genetic

The degree of quality loss on seed preserved using various storage procedures varies between plant species and within plant species (Bortey et al., 2016). A wide variation has also been observed in seed quality loss among different soybean cultivars during storage (Wien & Kueneman, 1981). Tatić et al. (2012) also observed differences in genotypes' sensitivity to storage conditions and duration. Hamed (2021) reported that the response to storage conditions varied among wheat cultivars and concluded that genotypes and storage methods significantly

impact seed vigour and other related traits. The observed differences among cultivars may be due to genetic factors (El-Abady et al., 2012).

2.6.4 Seed moisture content

The seed moisture content is the most influential factor affecting longevity during storage. The primary factor regulating loss of germinability during storage is high seed moisture content (Shelar et al., 2008). Ali et al. (2018) found that an increase in seed moisture content decreased seed vigour index and seedling's dry matter. Sheidaei et al. (2016) reported that increasing moisture content up to 14% could reduce seed quality. The authors concluded that 12% SMC is the optimum moisture for seed storage.

Although quality losses during storage are inevitable Hendges et al. (2017), but seed priming can enhance the germination rate of many plant species (Argerich et al., 1989). Priming may reverse the deleterious effects of seed aging through nucleic acids repair and build-up, increased protein synthesis, as well as membrane repair (Ghassemi-Golezani & Hosseinzadeh-Mahootchi, 2013).

2.7 Seed priming

Seed priming was first utilized in ancient Greece in the 4th century BC by Theophrastus, who soaked cucumber seeds in water to stimulate early germination and improve seed vigour (Miladinov et al., 2018). It is an essential modern technique that boosts emergence speed and uniformity, vigor, and yields (Uddin et al., 2021). Seed priming has been shown to promote germination and emergence in various crops, including vegetables and small-seeded grasses (Arif et al., 2008; Mehri, 2015; Ogbuehi et al., 2013; Sadeghi et al., 2011; Singh et al., 2014). It is a farmer-friendly strategy for boosting crop stand establishment and growth in optimal and suboptimal conditions (Langeroodi & Noora, 2017). Pirmani et al. (2013) and Mohammadi (2009) found that priming sunflower and soybean seeds can tremendously improve seed germination, germination rate, seed vigour index, shoot length, root length, and dry seedling weights, and reduce mean germination time and electrical conductivity of seed leachates. According to Arif et al. (2010) and Arif et al. (2008), priming hastens and improves emergence and enhances soybean grain yield. Through the accumulation of latent defensive proteins, it strengthens the cellular defense response and tolerance to both biotic and abiotic stress (Marthandan et al., 2020). It is reported that priming improves physiological, biochemical, yield, and yield parameters under both drought and salinity stress (Ahmadvand et al., 2012; Langeroodi & Noora, 2017). Reduced lag time of water uptake, enzyme activation, build-up of germination-enhancing compounds, metabolic repair during imbibition, and osmotic

adjustment all contribute to faster and more synchronized germination of primed seeds (Hussain et al., 2016).

However, Rouhi et al. (2011) observed that the germination rate dropped when seeds were primed with water. During hydropriming, the poor germination rate could be due to a varying degree of seed hydration, resulting in a lack of synchronous metabolic activation (Lutts et al., 2016).

2.7.1 Mechanism of seed priming

Priming involves controlled hydration or soaking of seeds in water or a solution of low osmotic potential to initiate the pre-germinative metabolism without radicle protrusion during phase II of germination (Dutta, 2018; Marthandan et al., 2020; Sher et al., 2019). For routine handling, seeds are re-dried to approximately their original weight after soaking (Farooq et al., 2019). After priming, the seed must be dried back to allow seed storage. Seeds are then rinsed with water and dried back to levels suitable for proper storage. This ensures that the priming's beneficial effect is maintained without losing quality caused by quick deterioration (Ibrahim, 2019). The rehydration of primed seeds (Figure 2.6) activates significant cellular changes such as nucleic acids and proteins, ATP synthesis, activation of sterols and phospholipids, and repairing DNA (Marthandan et al., 2020). The triggering of biochemical mechanisms of cell repair is associated with seed priming's advantages on seed performance: the restoration of metabolic activity can restore cellular integrity through nucleic acids build-up and synthesis of proteins, as well as the antioxidant defense system (Dawood, 2018).

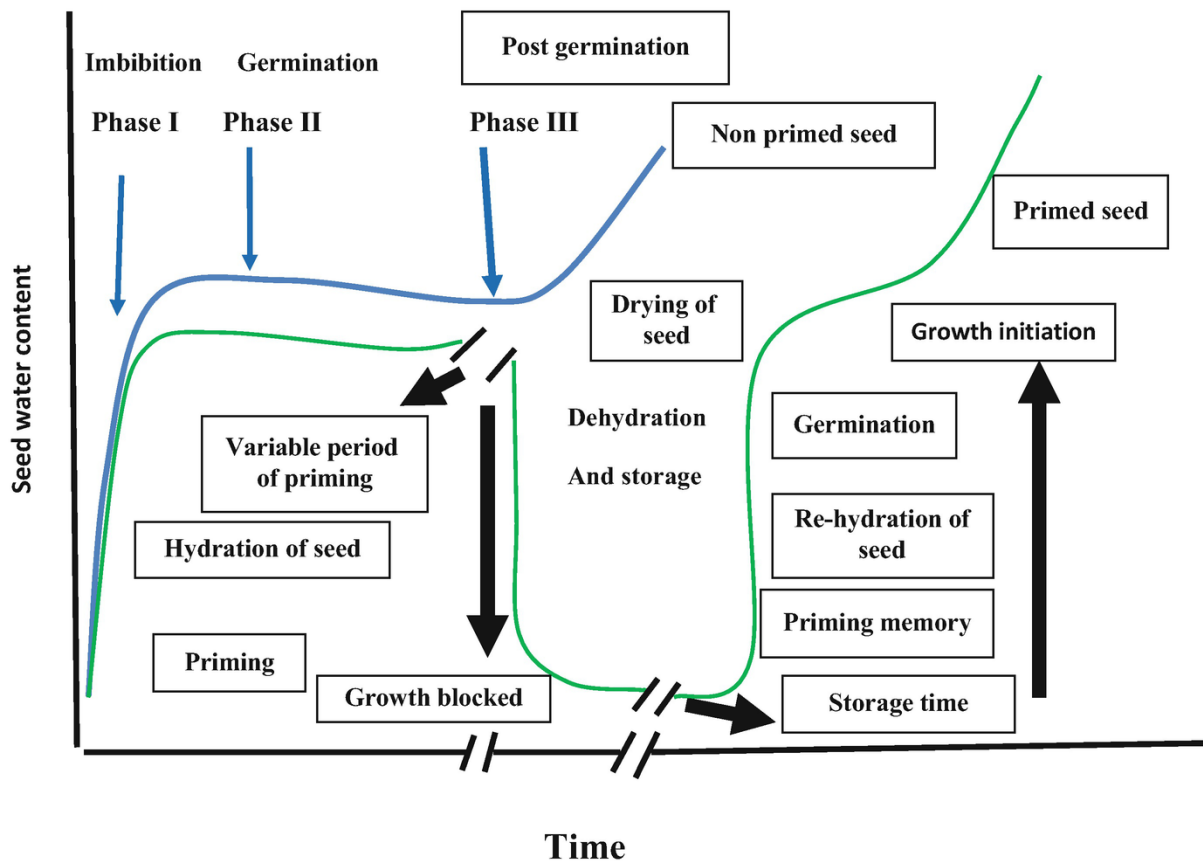


Figure 2.6 Schematic representation of normal germination and seed priming process (Bose et al., 2018) (Phase I; imbibition phase, phase II; germination phase, phase III; post-germination phase).

2.7.2 Priming methods

Different priming methods depend on how seed hydration is controlled (Castañares & Bouzo, 2018). Standard priming methods include hydro-priming and osmopriming (Lemmens et al., 2019; Tian et al., 2014).

2.7.2.1 Hydro-priming

Hydropriming is a cheap, easy-to-use, and environmentally-friendly technique for improving soybean output (Mehri, 2015). Seeds are soaked in water for a specific amount of time before sowing, based on the radicle protrusion time of each plant species (Sher et al., 2019). Hydro-priming can also refer to the steady addition of a small amount of water or short immersion in water (also referred to as steeping), including incubation in humid air (Mielezrski et al., 2016). The main disadvantage of hydro-priming is the uncontrolled, abrupt absorption of water, which may cause seed imbibition injury (Castañares & Bouzo, 2018). Another problem is that seeds are unevenly hydrated, resulting in non-uniform processes necessary to synchronise and improve germination (Dawood, 2018).

2.7.2.2 Osmopriming

Osmopriming is an easy and effective way of priming (Castañares & Bouzo, 2018). It entails the soaking of seeds in an aerated solution of sugars like sorbitol and mannitol, or polyethylene glycol (PEG), then drying them back nearly to their original weight (Sher et al., 2019). This process allows water to enter the seed while maintaining a low osmotic potential but delaying radicle protrusion (Arteca, 1996). During PEG priming, seed imbibes gradually, enabling membrane repair and re-organization due to prolonged priming and lower soaking speed (Pallaoro et al., 2016).

Osmopriming of seeds regulates physiological and biochemical activities. It also undertakes repair processes affecting germination, thus resulting in uniform, vigorous, and seedling emergence (Rao et al., 2005). Priming with PEG can improve seed germination, seedling emergence, and establishment, especially under stressful conditions (Zhang et al., 2015).

Uddin et al. (2021) reported that priming with PEG increased the seedling vigor index of mung beans under drought stress. The high viscosity of PEG solution, which compromises oxygen absorption, is the main drawback of this technique (Pallaoro et al., 2016). Another disadvantage of PEG priming is that it is not suitable for crops like sorghum, which have high tannin content because tannin can be removed with a PEG solution (Dawood, 2018). Tannin removal can lower seed germination (Waqas et al., 2019).

2.7.3 Factors affecting seed priming

Plant species, priming technique, length, temperature, seed quality, aeration, dehydration, and storage conditions for primed seeds all have a role in the success of seed priming (Dutta, 2018; Miladinov et al., 2018; Sadeghi et al., 2011).

Aeration, particularly in a PEG solution, is thought to be necessary to aid respiration, which is necessary for viability and emergence synchronization. This is due to the high viscosity of the PEG solution, which inhibits oxygen absorption, necessitating the aeration of the solution. Aeration has different effects according to the species: in onions, aeration of the PEG solution enhances germination capacity compared to non-aerated treatment (Dawood, 2018; Pallaoro et al., 2016).

Temperature is also critical since it influences the speed of chemical reactions and potential water value. Low priming temperature may lead to slower germination (Di Girolamo & Barbanti, 2012; Waqas et al., 2019). Priming at a temperature of 25 °C can increase germination percentage and decrease germination mean time in melon (Castañares & Bouzo, 2018).

Sadeghi et al. (2011) studied the effect of osmotic potential and priming duration on soybean seed quality traits. The researchers discovered that a -1.2 MPa osmotic potential and a 12-hour priming period boosted germination percentage, germination index, and seed vigour considerably. A decrease in mean germination time, the time to get 50% germination, and the electrical conductivity of seeds were also observed. Arif et al. (2010) observed a decrease in absolute growth rate and crop growth rate with increased priming duration. The relative growth rate was highest at 6-hour seed priming duration followed by 12 and 18 hours priming duration. Rouhi et al. (2011) concluded that the osmotic potential of -1.2 MPa and priming for 12 hours provides the best results. Soaking duration is also essential to ensure that radicle protrusion through seed coat does not occur (Cantliffe et al., 1984). Another important factor determining priming effects is seed quality. A healthy seed, free of pathogens, is required for optimum results (Dawood, 2018).

2.7.4 Storage potential of primed seeds

The major drawback of seed priming is the rapid loss of quality in primed seeds during storage. The loss of viability in primed seeds during storage is a significant limitation to widespread adoption and implementation of this approach (Wang et al., 2018). In general, primed seeds are stored for some time if not used immediately (Yan, 2017). An increased loss of longevity in primed seed has been associated with to loss of seed desiccation tolerance due to prolonged treatments (Dutta, 2018). The response of primed seeds to storage is also species and variety-dependent (Ozbay, 2018). Storage temperature, relative humidity, aeration, and seed moisture content are the main factors affecting seed longevity (Wang et al., 2018).

The effect of storage on primed seeds has been studied in several crops, and contrasting results have been reported. Abnavi and Ghobadi (2012) evaluated the effect of storage duration (0, 30, 45, 60 days) on primed wheat seeds. They discovered that keeping primed seeds for 30-60 days boosts shoot and radicle length, dry weight, germination percentage, and speed of germination. On the contrary, in a study conducted by Yan (2017), Chinese cabbage seeds were hydro-primed under different temperatures (4, 20, 30 °C) for 1, 3, 6, and 9 months. The researcher found a decrease in germination percentage, germination rate, and seedling vigour index of primed Chinese cabbage seeds stored at 30°C for 9 months compared to unprimed seeds. The author also observed no adverse effects when primed seeds were stored at 4°C and 20 °C for 9 months and 30 °c for 6 months. A similar study by Hussain et al. (2015) reported that storing primed seeds at 25°C significantly reduced the rice germination and seedling growth. However,

no adverse effects were observed at -4°C. The authors also reported that the beneficial effects of seed priming could be maintained only for 15 days of storage at 25°C. Farajollahi and Eivand (2016) reported that an 8-days delay in planting primed seeds stored at 25°C could significantly decrease the benefits of priming in wheat. When primed seeds are kept at 25°C, their germination and seedling growth are reduced due to a restriction in starch metabolism (Farooq et al., 2019). Therefore, further research is necessary to improve the storage of primed seeds.

2.8 Summary and conclusions

The present literature review has revealed that seed quality is one of the main factors critical for successful soybean cultivation. The literature has also shown that seed performance can be improved through priming in different crop species, and the positive effects of priming may be lost quickly during storage. However, there is very little or no information on the effect of priming on the performance of low vigour soybean seeds and how long primed seed can be stored without significant quality loss. As a result, more investigation is required on the effect of (i) seed priming on low vigour seed and (ii) storage duration and conditions on primed soybean seeds.

2.9 References

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CHAPTER 3 EFFECT OF SEED PRIMING ON GERMINATION OF LOW VIGOUR SOYBEAN (*GLYCINE MAX L.*) SEEDS

Abstract

Seed priming is a pre-sowing method involving hydrating seeds to the point where pre-germinative metabolic processes begin without germination. This study aimed to explore how seed priming affected low vigour soybean seed germination and seedling establishment. Seed ageing was used to simulate low vigour seeds. Seeds were aged at a temperature of 41°C and 100% relative humidity for 72 hours. The experiments were arranged using a Complete Randomized Design (CRD) with the following factors: Cultivars – 3 levels (LS6851R, DM5953RSF, PAN1521R); Seed ageing – 2 levels (Aged and Unaged seeds); Seed priming – 3 levels (Control-unprimed seeds, hydropriming, and osmopriming) giving a 3 x 2 x 3 factorial treatment structure with four replications totaling to 72 experimental units. The results showed that aging, cultivar, and priming significantly ($p < 0.001$) affected the measured germination and seedling growth traits. Aged seeds exhibited lower values (62 %, 2.73, 471, 7.04 cm, 5.07 cm, 12.11 cm, 0.72 g, 0.11 g, 976, and 7.79) in terms of final germination percentage (FGP), germination index (GI), coefficient of the velocity of germination (CVG), shoot length (SL), root length (RL), seedling length (SLL), fresh weight (FW), dry weight (DW), seedling vigour index (SVI) I and II compared to unaged seeds (93 %, 7.06, 805, 13.72 cm, 18.64 cm, 32.36 cm, 1.09 g, 0.13 g, 3000 and 12.08). On average, both FGP and SVI II of aged seeds were improved by 18% and 2.51 through osmopriming. Hydropriming had either negative or no effect on all measured traits of aged seeds. The priming effect was not significant on GI and CVG of aged seeds. Irrespective of priming treatment and cultivar, priming aged seeds also decreased RL and SLL. In conclusion, this study provided evidence that (i) aging results in loss of germinability and vigour, (ii) Priming with PEG can be used to improve FGP and SVI II of aged seeds, and (iii) hydropriming negatively affect FGP, DW, and SVI II of aged seeds.

Keywords: aging, priming, germination, cultivars, vigour

3.1 Introduction

Soybean (*Glycine max* L.) is one of the major legume crops globally (Arif et al., 2010). It serves as a good source of dietary protein and oil for animal feed and a staple crop for human consumption (Chen et al., 2012; Langeroodi & Noora, 2017; Sedivy et al., 2017). It contains 38-45% protein and 20% oil (Murungu et al., 2005; Sibande et al., 2015; Singh et al., 2016). According to Qiu and Chang (2010), soybean has the highest protein content and gross vegetable oil among cultivated crops globally. In addition to protein and oil content, soybean

seed contains calcium, iron, carotene, thiamine, and ascorbic acid (Engelbrecht et al., 2020; Rouhi et al., 2011). This crop also plays a significant role in atmospheric nitrogen fixation (Schmutz et al., 2010). Its ability to undertake symbiotic nitrogen fixation minimizes the need to apply many nitrogen fertilizers (Sinclair et al., 2014). Therefore, biological nitrogen fixation in soybean is economically and ecologically beneficial when most soils are deficient in nitrogen and nitrogen fertilizers are not affordable to farmers, owing to their financial constraints (Khojely et al., 2018). Due to a wide range of geographical adaptation, unique chemical composition, nutritional and health benefits, and industrial applications, soybean is an important crop globally (Ali & Singh, 2010; Hosken, 1999).

Seed quality is one of the primary factors that play a critical role in soybean cultivation (Dlamini et al., 2014; Garoma et al., 2017; Wimalasekera, 2015). It encompasses genetic purity and aspects of physical and physiological parameters such as seed purity, moisture content, viability, germination, seed vigor, and seed health (Bekele et al., 2019). From small-scale to large-scale farming, good seed quality is essential for sustainable and profitable production and is regarded as an essential agronomic trait (Finch-Savage & Bassel, 2016). Good quality seed is superior to other standard seeds in genetic and physiological purity and is free from seed-borne diseases and disorders (Wimalasekera, 2015). Seed germination is the most critical stage determining successful crop production (Mohammadkhani & Heidari, 2008). Soybeans' ability to germinate and develop rapidly is a significant quality attribute for seed production and food and commercial use (Paulsen, 2008).

In general, seeds are stored for varying times after harvest (Singh et al., 2016). According to El-Abady et al. (2012), soybean seed can lose quality during harvesting, processing, and storage. The loss of seed quality during ageing is defined as seed deterioration (Nazari et al., 2020). When the seed deteriorates and loses vigour during storage, it becomes predisposed to environmental stresses during germination and ultimately die (Nguyen et al., 2012; Paulsen, 2008). Seed priming has been identified as one of the techniques that can be applied to lessen the problems associated with seed deterioration (Langeroodi & Noora, 2017; Sibande et al., 2015).

Seed priming is applied prior to sowing in which seeds are moistened to the point where pre-germination metabolic processes are triggered without actual germination. Seeds are then re-dried to a weight close to their original weight for normal handling. Seeds can be immersed in tap water (hydropriming) or aerated polyethylene glycol solutions with low water potential (Farooq et al., 2019). Priming initiates metabolic activities such as protein, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, and DNA replication (Farhoudi & Tafti,

2012). A significantly higher final germination percentage (87 %) of primed seeds over untreated seeds (83 %) was reported by Agawane and Parhe (2015). Several authors have also reported a rapid and uniform seedling establishment for primed seeds (Langeroodi & Noora, 2017; Ruttanaruangboworn et al., 2017; Tian et al., 2014). Arif et al. (2008) concluded that seed priming hastens and improves the emergence soybean grain yield. Ghasemi et al. (2015) reported that the hydropriming of aged seeds improves FGP, GI, and decreased MGT. Park et al. (1999) also reported that osmopriming increased the FGP of aged soybean seeds by 37%. Comparing priming treatments, Rouhi et al. (2011) reported that osmopriming increased germination rate, whereas hydro-priming had the opposite effect. Miladinov et al. (2018) reported a 12% increase and 11% decrease in FGP depending on the cultivar and priming method. These contradicting results necessitate further research on soybean response to different priming techniques. Moreover, although much research has been done on seed priming in different crops, there is very little information about the effect of priming on the germination of seeds with low vigour. Therefore, this study was carried out to determine the effect of different seed priming treatments on germination and seedling establishment of low vigour soybean seeds.

3.2 Methods and Materials

3.2.1 Experimental site

The experiments were carried out at the Seed Science Laboratories, School of Agricultural, Earth & Environmental Sciences, University of KwaZulu-Natal.

3.2.2 Experimental material

This study used the seed of three soybean cultivars (LS6851R, DM5953RSF, PAN1521R). The seeds were sourced from Agricultural Research Council – Small Grains Institute (ARC-GI), Potchefstroom, North West Province, South Africa.

3.2.3 Experimental design and treatment structure

The experiments were arranged using a complete randomized design (CRD) with the following factors: Cultivars – 3 levels (LS6851R, DM5953RSF, PAN1521R); Seed ageing – 2 levels (Aged and Unaged seeds); Seed priming – 3 levels (Control-unprimed seeds, hydropriming, and osmopriming) giving a 3 x 2 x 3 factorial treatment structure with four replications totaling to 72 experimental units.

3.2.4 Experimental set-up

3.2.4.1 Accelerated seed ageing

To induce low vigour, seeds were subjected to the accelerated aging procedure (Pandey et al., 2017). Accelerated ageing exposes seeds for a shorter period to two environmental variables; high temperature and relative humidity, which cause rapid deterioration (TeKrony, 1993; Woltz & TeKrony, 2001). Seeds were aged at a temperature of 41°C and 100% relative humidity for 72 hours (TeKrony, 2005). A plastic box (11 x 11 x 3.5 cm) containing a mesh tray (10 x 10 x 3 cm) was used as an aging inner chamber (Mandizvo & Odindo, 2019a). A hundred ml of 10 % (w/v) sodium chloride (NaCl) solution was added to each plastic box. The mesh tray was carefully inserted to avoid water splash (Vilakazi, 2018). Seeds were weighed and placed on a mesh tray, one layer deep, to ensure even moisture uptake. The boxes were then placed in an aging chamber, as illustrated in (Figure 3.1). To assure proper air circulation and temperature uniformity inside the chamber, an air space of 2.5 cm was allowed between plastic boxes (Pandey et al., 2017; Rouhi et al., 2011).

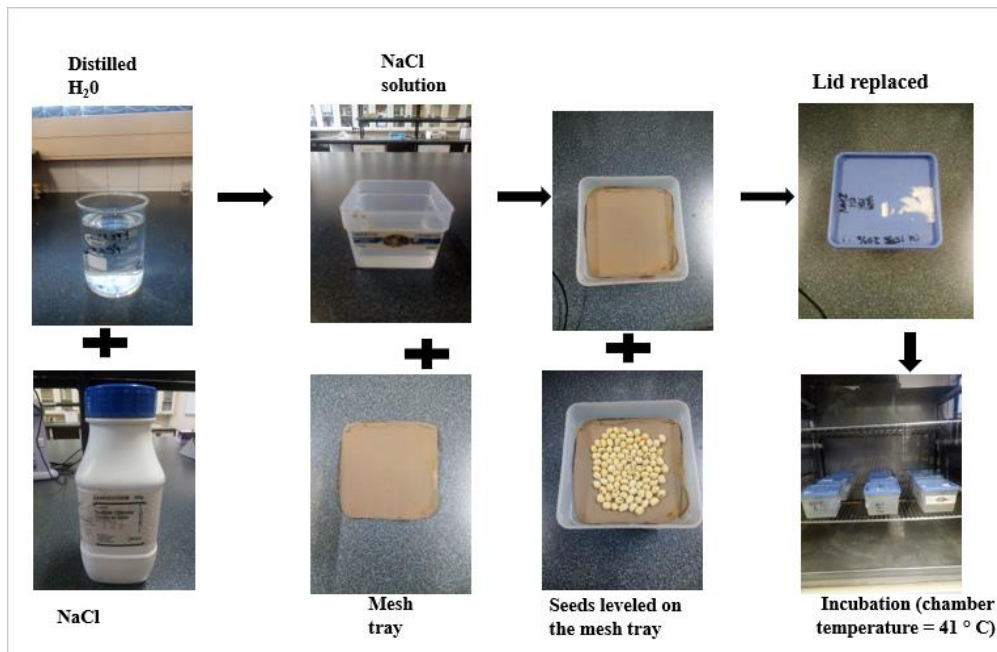


Figure 3.1 Schematic illustration for the accelerated aging experiment set-up.

3.2.4.2 Priming

Before priming, the initial seed mass was recorded. The two selected priming treatments were applied as described below.

(a) Hydro-priming:

Seeds were soaked in a beaker with distilled water (Hosseini et al., 2007; Murungu et al., 2005; Tilden & West, 1985). Seeds were then allowed to imbibe until a constant seed mass was achieved. Seed mass was determined at a 2-hour interval until a constant seed mass was reached (Chimonyo & Modi, 2013). After soaking, seeds will be placed on paper towels to dry back to the initial seed mass (Rouhi et al., 2011).

(b) Osmo-priming:

Seeds were immersed in a PEG solution with a -0.15 MPa osmotic potential. The solution was prepared by adding 100g of PEG (6000) salt into 1 liter of water. The solution was then incubated in a chamber (Labcon, L.T.I.E, South Africa) set at a temperature of 25 °C to attain a desired osmotic potential. After priming, seeds were rinsed and dried to return to their original seed mass. The solution's osmotic potential was calculated as described by (Michel & Kaufmann, 1973) (equation 1).

$$\Psi_s = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T \quad (1)$$

Where C is the concentration of PEG-6000 in g/L H₂O, and T is the temperature in degrees Celsius.

3.2.4.3 Germination test

Germination test was carried out using a roller paper towel method (Mandizvo & Odindo, 2019a). For each treatment, ten seeds were used for this test. Using distilled water, three paper towels were moistened. Seeds were then placed on two moistened paper towels and covered using the remaining paper towel. The paper towels were rolled then sealed in zip-lock bags with elastic bands at the opposite ends. Zip-lock bags were then be placed in a germination chamber set at a temperature of 25°C. Germination was assessed by counting the seeds with at least 2 mm root protrusion daily for 7 days after sowing. The final germination percentage (FGP) was calculated using equation 1 (Sibande et al., 2015). The mean germination time (MGT) and germination index (GI) were calculated according to (Sadeghi et al., 2011).

$$FGP = \left(\frac{\text{seeds germinated}}{\text{total seeds}} \right) \times 100\% \quad (2)$$

$$GI = \frac{\text{no of germinated seed}}{\text{days of first count}} + \dots + \frac{\text{no of germinated seed}}{\text{days of final count}} \quad (3)$$

$$MGT = \left(\frac{\sum D.N_i}{\sum N_i} \right) \quad (4)$$

Where D was the number of days from the start of the germination test, and N was the number of newly germinated seeds on the day. The coefficient of the velocity of germination was calculated using equation 4 (Mandizvo & Odindo, 2019b).

$$CVG=100 \times \sum Ni / \sum NiTi \quad (5)$$

Where Ni is the number of germinated seeds per day, Ti is the number of days from the start of the experiment.

3.2.4.4 Seedling growth test

After the germination test, seedling vigour was assessed by measuring shoot length, root length, and seedling length and determining the seedling dry mass (Govender et al., 2008; Verma & Verma, 2015). Seedling vigour index I and II were according to equations 5 and 6 (Kumar et al., 2012), respectively.

$$SVI\ I = \text{Final germination percentage} \times \text{seedling length} \quad (5)$$

$$SVI\ II = \text{Final germination percentage} \times \text{seed dry mass} \quad (6)$$

3.2.5 Statistical analysis

The data was analyzed using GenStat®, 20.1 Edition (VSN International, Hemel Hempstead, UK, 2020) at the 5% level of significance. Tukey's test with GenStat® was used to separate the means of significantly different variables. The Pearson correlation coefficient (r) between measured traits was performed using Graphpad Prism®, 9.3 version (Graphpad Software, San Diego, CA, 2021).

3.3 Results

3.3.1 Final germination percentage

The effect of aging, cultivar, and priming on final germination percentage (FGP) was highly significant ($p < 0.001$). A significant effect ($p < 0.05$) between aging and priming was observed. No significant effect was observed for cultivar x priming interaction (Table 3.1). Unaged seeds exhibited the highest final germination percentage (92%) compared to aged seeds (62%). The cultivar PAN1521R had the highest FGP (94%), followed by LS6851R (75%) and DM (63%). Osmopriming recorded the highest FGP (88%) among the priming treatments, followed by the control-unprimed (78%) and hydropriming (67%). Hydropriming of aged seeds exhibited the lowest FGP (48%) compared to osmopriming (78%) and control (60%). Aged seeds of cv. DM5953RSF and LS6851R recorded the lowest FGP under hydropriming (25%) compared to osmopriming (60 and 75%) control (30 and 58%) (Table 3.2).

Table 3.1 Analysis of variance showing mean squares and significance test for FGP, GI, MGT, and CVG of three cultivars subjected to aging and priming

Source of variation	d.f	FGP	GI	MGT	CVG
Aging (A)	1	16501.4***	336.70***	25.84***	2002418***
Cultivar (C)	2	6084.7***	49.04***	2.55***	228080***
Priming (P)	2	2709.7***	6.53***	0.46*	8494 ^{ns}
A×C	2	5093.1***	16.55***	1.17***	20925 ^{ns}
A×P	2	559.7*	1.36 ^{ns}	0.71**	90891**
C×P	4	305.6 ^{ns}	0.93 ^{ns}	0.52**	80941***
A×C×P	4	513.9**	9.61***	0.24 ^{ns}	88746***
Residual	54	135.6	0.5617	0.12	14112.

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ns, Not Significant; d.f=degrees of freedom; FGP= Final germination percentage; GI=Germination index; MGT=Mean germination time; CVG=Coefficient of velocity of germination.

Table 3.2 Effect of aging, cultivar, and priming on FGP, GI, MGT and CVG

Cultivar	Aging	Priming	FGP	GI	MGT	CVG
DM5953RSF	Aged	Unprimed	30 ^d	1,25 ^e	2,62 ^{bcd}	525,1 ^{defgh}
		Hydropriming	25 ^d	0,72 ^e	2,96 ^{abc}	422,9 ^{fgh}
		Osmopriming	60 ^{bc}	1,60 ^e	3,63 ^a	299,0 ^{gh}
	Unaged	Unprimed	87,50 ^{ab}	5,96 ^{cd}	1,62 ^{ef}	723,4 ^{bcdef}
		Hydropriming	75,00 ^{ab}	5,91 ^{cd}	1,53 ^{ef}	785,7 ^{bcde}
		Osmopriming	97,5 ^a	6,60 ^{bc}	1,89 ^{ef}	604,7 ^{cdefg}
LS6851R	Aged	Unprimed	57,50 ^{bc}	2,31 ^e	2,52 ^{cd}	508,1 ^{efgh}
		Hydropriming	25,00 ^d	0,72 ^e	2,96 ^{abc}	422,9 ^{fgh}
		Osmopriming	75,00 ^{ab}	2,22 ^e	3,43 ^{ab}	288,9 ^h
	Unaged	Unprimed	97,50 ^a	5,94 ^{cd}	1,60 ^{ef}	736,6 ^{bcde}
		Hydropriming	97,50 ^a	8,12 ^{ab}	1,38 ^{ef}	862,2 ^{abc}
		Osmopriming	100 ^a	7,91 ^{ab}	1,39 ^{ef}	822,6 ^{bcd}
PAN1521R	Aged	Unprimed	92,50 ^a	4,57 ^d	2,15 ^{cde}	535,3 ^{defgh}
		Hydropriming	82,50 ^{ab}	5,89 ^{cd}	2,09 ^{cde}	676,6 ^{bcdef}
		Osmopriming	100 ^a	5,27 ^{cd}	2,05 ^{de}	563,7 ^{cdefgh}
	Unaged	Unprimed	100 ^a	8,50 ^{ab}	1,30 ^{ef}	923,0 ^{ab}
		Hydropriming	82,50 ^{bc}	5,35 ^{cd}	1,83 ^{def}	635,7 ^{bcdef}
		Osmopriming	95,00 ^a	9,21 ^a	1,05 ^f	1150,3 ^a
LSD			16,51	1,06	0,49	168,4
CV%			15,1	15,3	16,3	18,6

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance. FGP= Final germination percentage; GI=Germination index; MGT=Mean germination time; CVG=Coefficient of velocity of germination.

3.3.2 Germination index

There was a highly significant interaction ($p < 0.001$) between cultivar, aging, and priming with respect to germination index (GI). The interactions between ageing and priming and cultivar and priming were both not significant regarding on GI ($P > 0.05$) (Table 3.1). The unaged seeds recorded the highest GI (7.1), while aged seeds recorded the lowest (2.7). Cultivar PAN1521R had the highest GI (6.5), followed by cv. LS6851R (4.5). Cultivar DM5953RSF recorded the lowest GI (3.7). Regarding priming, the highest GI (5.5) was recorded from osmopriming.

Hydropriming and unprimed recorded the lowest GI of 4.4 and 4.8, respectively. For aged seeds, the highest GI (5.2) was recorded from cv. PAN1521R, followed by LS6851R (1.7) and DM5953RSF (1.2) (Table 3.2).

3.3.3 Mean Germination Time

The effect of aging and cultivar on mean germination time (MGT) was highly significant ($p < 0.001$). Highly significant ($p < 0.01$) effects of A×P and C×P were also recorded. The effect of priming of MGT was significant ($p < 0.05$). No significant difference was recorded for A×C×P interaction (Table 3.1). Aged seeds had the highest MGT (2.7 days), while unaged seeds exhibited the lowest MGT (1.5 days). Cultivar DM5953RSF and LS6851R recorded the highest MGT of 2.4 and 2.2 days. The lowest MGT (1.4 days) was recorded from cv. PAN1521R. When comparing priming treatments, osmoprimed seeds had the highest MGT (2.2 days), while the unprimed seeds exhibited the lowest (2.0 days). For A×P interaction, osmopriming of aged seeds exhibited the highest MGT (3.0 days) compared to hydropriming (2.67 days) and unprimed (2.43 days) (Table 3.2).

3.3.4 Coefficient of the velocity of germination

Highly significant ($p < 0.001$) difference in coefficient of germination (CVG) was observed among aging, cultivar, C×P, and A×C×P. A highly significant ($p < 0.01$) difference for A×P was also observed. No significant difference was recorded for priming and A×P (Table 3.1). Aged seeds recorded the lowest CVG (471) compared to unaged seeds (805). Cultivar PAN1521R recorded the highest CVG (747), followed by LS6851R (607) and DM5953RSF (560). Osmopriming of unaged seeds of cv. PAN1521R recorded the highest CVG (1150.3), while the lowest CVG (288.9) was exhibited by aged cv. LS6851R under osmopriming treatment (Table 3.2).

3.3.5 Shoot, root, and seedling length

A highly significant difference ($p < 0.001$) in the shoot, root, and seedling length was observed among aging, cultivar, priming, A×C, and A×C×P treatments. The C×P interaction had no significant effect on shoot length (Table 3.3). The highest shoot length (SL), root length (RL), and seedling length (SL) were recorded from unaged seeds (13.7, 18.64, and 32.36 cm), while the lowest was recorded from aged seeds (7, 5.07, and 12.11 cm). The control treatment (unprimed) recorded higher SL, RL, and SLL (12.23, 14.56, and 26.80 cm) than hydropriming (10.08, 10.36, and 20.44 cm) and osmopriming (8.82, 10.65, and 19.47 cm). Hydropriming of aged seeds recorded the lowest SL, RL, and SLL (4.95, 2.80, and 7.75 cm) compared to unprimed (8.39, 7.99, and 16.38 cm), and osmopriming (7.77, 4.43, and 12.20 cm) (Table 3.4).

Table 3.3 Analysis of variance showing mean squares and significance test for shoot length, and seedling length of three cultivars subjected to aging and priming

Source of variation	d.f.	SL	RL	SLL
Aging	1	803.649***	3315.422***	7383.690***
Cultivar	2	184.604***	215.194***	786.336***
Priming	2	71.446***	132.404***	379.961***
Aging×Cultivar	2	78.949***	163.004***	465.909***
Aging×Priming	2	104.529**	11.532**	177.679***
Cultivar×Priming	4	7.963ns	89.459***	148.027***
Aging×Cultivar×Priming	4	29.510***	81.838***	196.381***
Residual	54	3.531	2.215	8.082
Total	71			

*Significant at $p<0.05$; **Significant at $p<0.01$; ***Significant at $p<0.001$; ns, Not Significant;
d.f=degrees of freedom; SL=Shoot length; RL=Root length, SLL=Seedling length

Table 3.4 Effect of aging, cultivar, and priming on shoot length, root length, and seedling length

Cultivar	Aging	Priming	SL	RL	SLL
DM5953RSF	Aged	Unprimed	4,08 ^{efg}	2,10 ^h	6,18 ^f
		Hydropriming	1,54 ^g	0,87 ^h	2,41 ^f
		Osmopriming	5,66 ^{efg}	1,33 ^h	6,99 ^{ef}
	Unaged	Unprimed	14,63 ^{abc}	18,45 ^{bc}	33,08 ^a
		Hydropriming	14,18 ^{abc}	19,66 ^{abc}	33,84 ^a
		Osmopriming	8,10 ^{de}	17,42 ^c	25,52 ^b
LS6851R	Aged	Unprimed	6,17 ^{defg}	2,61 ^h	8,78 ^{ef}
		Hydropriming	2,50 ^{fg}	0,50 ^h	3,00 ^f
		Osmopriming	6,83 ^{def}	3,68 ^{gh}	10,51 ^{de}
	Unaged	Unprimed	16,17 ^{ab}	22,15 ^{ab}	38,32 ^a
		Hydropriming	18,21 ^a	21,66 ^{ab}	39,87 ^a
		Osmopriming	8,12 ^{de}	11,16 ^{de}	19,28 ^{bc}
PAN1521R	Aged	Unprimed	14,91 ^{abc}	19,27 ^{abc}	34,18 ^a
		Hydropriming	10,83 ^{cd}	7,01 ^{fg}	17,84 ^{cd}
		Osmopriming	10,83 ^{cd}	8,28 ^{ef}	19,10 ^{bc}
	Unaged	Unprimed	17,43 ^{ab}	22,82 ^a	40,25 ^a
		Hydropriming	13,25 ^{bc}	12,44 ^d	25,69 ^b
		Osmopriming	13,38 ^{abc}	22,05 ^{ab}	35,44 ^a
	LSD		2.664	2,11	4.03
	CV%		18,1	12,6	12.8

*According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance. SL=Shoot length; RL=Root length, SLL=Seedling length

3.3.6 Seedling Fresh and Dry Weight

The highly significant ($p < 0.001$) difference in fresh seedling weight (FW) was observed among aging, cultivar, priming, A×C, A×C×P treatments. A significant ($p < 0.05$) difference was also observed among the A×P treatment. No significant difference was observed for C×P interaction (Table 3.5). The least FW was recorded from aged seeds (0.72 g), while the greater FW was exhibited by unaged seeds (1.08 g). Osmopriming and hydropriming recorded lower FW (0.86 and 0.78 g) than the control-unprimed (1.06 g). Hydropriming of aged seeds scored the lowest

FW (0.56 g) compared to osmopriming and control (0.73 and 0.87 g) (Table 3.6). For dry seedling weight, highly significant ($p<0.001$) was observed among cultivar and $A\times C\times P$ treatment. The highly significant ($p<0.01$) effect of aging on dry weight was also evident. Significant ($p<0.05$) difference was observed among priming, $A\times C$, and $C\times P$. The aging and priming interaction had no significant effect (Table 3.5). When comparing priming treatments, osmopriming recorded more dry weight (0.13 g) than control (0.12 g) hydropriming (0.11 g) (Table 3.6)

Table 3.5 Analysis of variance showing mean squares and significance test for FW, DW, SVI I, and SVI II of three cultivars subjected to aging and priming

Source of variation	d.f.	FW	DW	SVI I	SVI II
Aging (A)	1	2.38456***	0.0042936**	73737761***	331.145***
Cultivar (C)	2	1.04290***	0.0072091***	10464701***	155.674***
Priming (P)	2	0.51212***	0.0022047**	3498767***	70.915***
A×C	2	0.34155***	0.0016757*	5580072***	107.700***
A×P	2	0.05309*	0.0005598ns	893156***	4.414ns
C×P	4	0.02701ns	0.0011792*	1895200***	7.565ns
A×C×P	4	0.12404***	0.0034570***	1825125***	27.688***
Residual	54	0.01580	0.0004443	100007.	3.405
Total	71				

*Significant at $p<0.05$; **Significant at $p<0.01$; ***Significant at $p<0.001$; ns, Not Significant; d.f.=degrees of freedom, FW=Fresh weight, DW=Dry weight, SVI=Seedling vigour index

Table 3.6 Effect of aging, cultivar, and priming on FW, DW, SVI I, and SVI II

Cultivar	Aging	Priming	FW	DW	SVI I	SVI II
DM5953RSF	Aged	Unprimed	0,60 ^{fgh}	0,10 ^a	196 ^h	3,30 ^{ef}
		Hydropriming	0,50 ^{hi}	0,13 ^a	64 ^h	3,13 ^{ef}
		Osmopriming	0,59 ^{gh}	0,13 ^a	357 ^h	7,85 ^{cde}
	Unaged	Unprimed	1,15 ^{abc}	0,13 ^a	2891 ^{cde}	11,47 ^{abc}
		Hydropriming	0,90 ^{bcdefg}	0,13 ^a	2545 ^{def}	10,00 ^{abcd}
		Osmopriming	0,86 ^{cd} efg	0,15 ^a	2488 ^{defg}	14,52 ^a
LS6851R	Aged	Unprimed	0,69 ^{defgh}	0,10 ^a	538 ^h	6,15 ^d e
		Hydropriming	0,18 ⁱ	0,03 ^b	89 ^h	0,97 ^f
		Osmopriming	0,65 ^{efgh}	0,12 ^a	783 ^h	9,25 ^{bcd}
	Unaged	Unprimed	1,21 ^{ab}	0,11 ^a	3738 ^{ab}	10,91 ^{abcd}
		Hydropriming	1,12 ^{abc}	0,12 ^a	3887 ^{ab}	12,52 ^{abc}
		Osmopriming	0,93 ^{bcdef}	0,11 ^a	1928 ^{fg}	11,55 ^{abc}
PAN1521R	Aged	Unprimed	1,33 ^a	0,14 ^a	3176 ^{bcd}	13,16 ^{ab}
		Hydropriming	0,99 ^{bcd}	0,14 ^a	1674 ^g	13,27 ^{ab}
		Osmopriming	0,94 ^{bcde}	0,13 ^a	1910 ^{fg}	13,05 ^{ab}
	Unaged	Unprimed	1,40 ^a	0,14 ^a	4024 ^a	13,90 ^{ab}
		Hydropriming	0,99 ^{bcd}	0,12 ^a	2132 ^{efg}	9,81 ^{abcd}
		Osmopriming	1,19 ^{ab}	0,15 ^a	3370 ^{abc}	14,07 ^a
LSD			0.19	0.03	448.3	2,62
CV%			13.9	17.2	15,9	18,6

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance, FW=Fresh weight, DW=Dry weight, SVI=Seedling vigour index

3.3.7 Seedling vigour index

Highly significant ($p < 0.01$) difference in seedling vigour index I (SVI I) was evident among all treatments. SVI II was also significantly ($p < 0.001$) affected by aging, cultivar, priming, and $A \times C \times P$. However, $A \times P$ and $C \times P$ had no significant effect on SVI II (Table 3.5). The lowest SVI I value (976) was recorded from aged seeds, while unaged seeds recorded the highest (3000). Unprimed seeds had the highest SVI I (2427), followed by osmoprimed (1806) and hydroprimed (1732). SVI II was the highest in osmoprimed seeds (11.72) compared to

unprimed (9.82) and hydroprimed (8.29) seeds. Hydropriming of aged seeds exhibited the lowest SVI I (609) compared to osmopriming and control (1017 and 1303). The highest SVI II was observed from aged seeds under osmopriming (10.05). The lowest SVI II was recorded from unprimed (7.54) and hydropriming (5.79) (Table 3.6).

3.3.8 Correlation of seed germination and seedling growth parameters

Pearson correlation coefficients (r) showing the level of associations for studied parameters among soybean cultivars subjected ageing and priming are presented in (Figure 3.2). FGP was strongly and positively correlated with GI ($r=0.87^{***}$), SL ($r=0.80^{***}$), SLL ($r=0.80^{***}$), FW ($r=0.85^{***}$), DW ($r=0.59$; $p=0.01$), SVI I ($r=0.84^{***}$), and SVI II ($r=0.96^{***}$). The FGP was also positively correlated with CVG ($r=0.60^{**}$) and DW ($r=0.59^{**}$). The mean germination time was strongly and negatively correlated with FGP ($r=-0.71^{***}$), GI ($r=-0.92^{***}$), CVG ($r=-0.94^{***}$), SL ($r=-0.77^{***}$), RL ($r=-0.86^{***}$), SLL ($r=-0.84^{***}$), FW ($r=-0.78^{***}$), SVI I ($r=-0.85^{***}$), and SVI II ($r=-0.67^{**}$). The MGT was weakly and negatively correlated with DW ($r=-0.36^{ns}$). The germination index was strongly and positively correlated with CVG ($r=0.90^{***}$), SL ($r=0.80^{***}$), RL, SLL, and SVI II ($r=0.86^{***}$), FW ($r=0.82^{***}$), DW ($r=0.52^{*}$), and SVI ($r=0.88^{***}$). The coefficient of velocity of germination was strongly and positively correlated with SL ($r=0.70^{**}$), RL ($r=0.79^{***}$), SLL ($r=0.77^{***}$), FW ($r=0.71^{***}$), SVI ($r=0.78^{***}$), and SVI II ($r=0.60^{**}$). The positive correlation ($r=0.35$) between CVG and DW was not significant. The shoot length was strongly and positively correlated with RL ($r=0.91^{***}$), SLL ($r=0.96^{***}$), FW ($r=0.91^{***}$), SVI I ($r=0.95^{***}$), and SVI II ($r=0.76^{***}$). The correlation of SL with DW was weak and positive ($r=0.46^{ns}$). The root length was positively correlated with SLL ($r=0.99^{***}$), FW ($r=0.87^{***}$), DW ($r=0.49^{*}$), SVI I ($r=0.98^{***}$). The seedling length was significantly and positively correlated with FW ($r=0.91^{***}$), DW ($r=0.49^{*}$), SVI I ($r=0.99^{***}$), and SVI II ($r=0.78^{***}$). The fresh weight was positively correlated with DW ($r=0.65^{***}$), SVI ($r=0.92^{***}$), and SVI II ($r=0.85^{***}$). There was positive correlation between DW and SVI ($r=0.49^{*}$) as well as SVI II ($r=0.75^{***}$). The seedling vigour index I was strongly and positively correlated with SVI II ($r=0.81^{***}$).

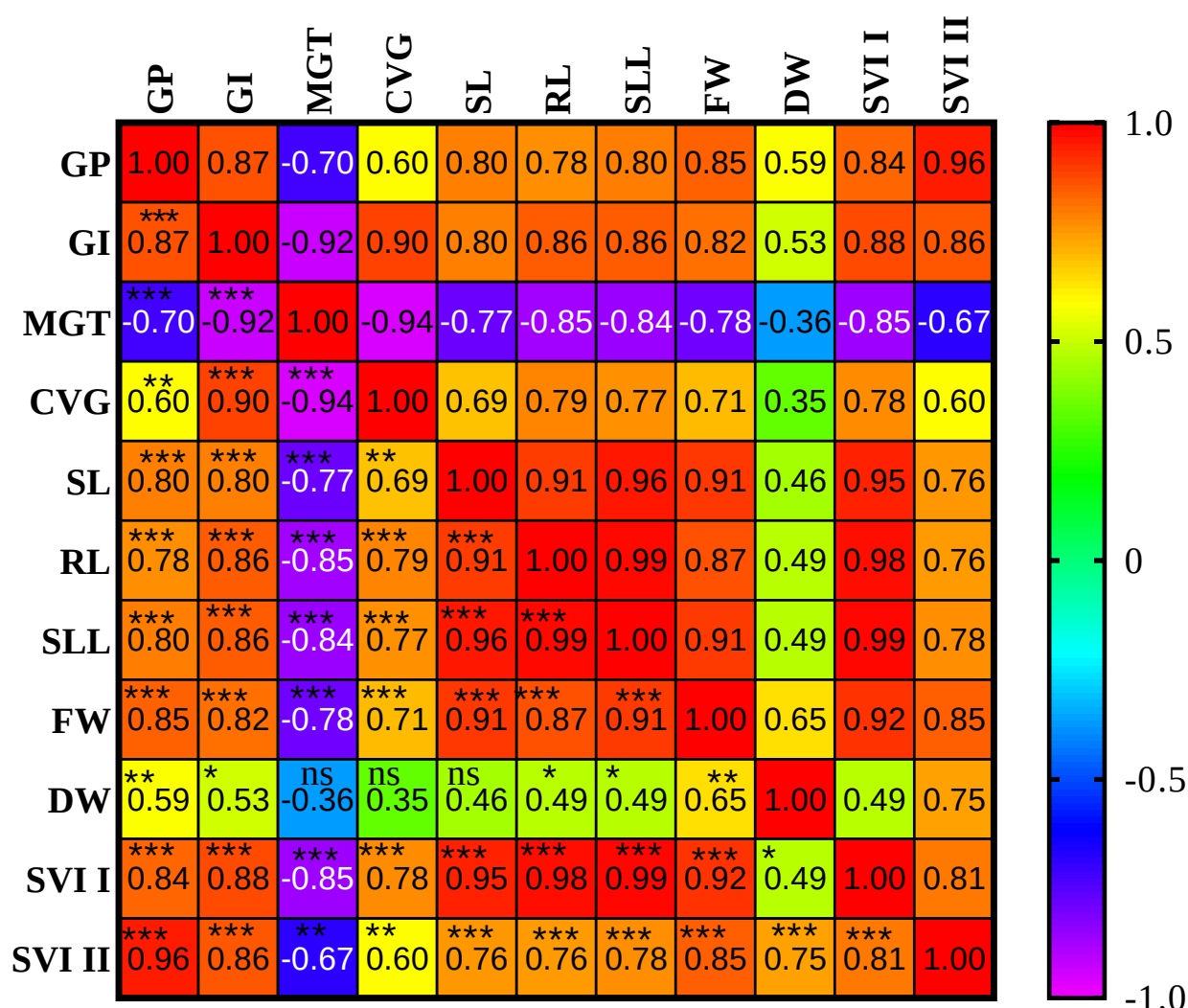


Figure 3.2 Pearson correlation coefficients (r) for seed germination and seedling growth parameters. **GP=final germination percentage, GI=germination index, MGT=mean germination time, CVG=coefficient of the velocity of germination. SL=Shoot length; RL=Root length, SLL=Seedling length, FW=Fresh weight; Dry weight; SVI I= Seedling vigour index I; SVI II=Seedling vigour index II

3.4 Discussion

3.4.1 Seed germination

The present study assessed the effect of priming on the physiological quality of soybean seeds with low vigour. Reduction in vigour and viability usually marks the beginning of seed aging (Ghassemi-Golezani & Hosseinzadeh-Mahootchi, 2013). Therefore, natural seed aging was assimilated by subjecting seeds to accelerated aging to induce low vigour. Results revealed that aging significantly affected all measured germination traits. Subjecting seed to accelerated aging caused a reduction of 31% in germination. Unaged seeds had the highest FGP (93%),

with the lowest FGP (62%) recorded from aged seeds. Other researchers reported similar results. Rastegar et al. (2011) reported a significant decline in germination after subjecting seeds to accelerated aging. Ghasemi et al. (2015) found that seed aging reduced FGP, GI, SL, DW, and increased MGT. Poor performance of aged seeds may be due to lipid peroxidation, disruption of membrane integrity, mitochondrial dysfunction, and insufficient ATP production (Mohammadi et al., 2011; Weerasekara et al., 2021). Reduction in germination capacity and dry seedling weight after seed ageing is associated with poor reserves utilization and conversion efficiency of mobilized reserves (Ghassemi-Golezani & Hosseinzadeh-Mahootchi, 2013).

However, the effect of aging also varied among cultivars. Cultivar DM5953RSF recorded the highest FGP decrease (49 %), followed by cv. LS6851R (45%). However, only a 3% difference in FGP was observed for cv. PAN1521R. These results show that cv. PAN1521R performed better than other cultivars. This variation across cultivars could be attributed to genetic and chemical makeup differences, which can affect seed deterioration and vigor loss rates (Tatić et al., 2012). A similar observation was made by El-Abady et al. (2012), who reported that cv. G-21 outperformed other cultivars in terms of final germination percentage after accelerated aging.

The effect of priming on FGP was significant. Osmopriming significantly improved FGP by 10%, while hydropriming decreased FGP by 12% regardless of aging and cultivar treatment. Osmopriming of aged seeds improved FGP by 18%. These results agree with (Rouhi et al., 2011; Sadeghi et al., 2011), who reported that osmopriming improves FGP. Park et al. (1999) reported that osmopriming increased the FGP of aged soybean seeds by 37%. The positive effects of osmopriming are due to improved RNA and DNA synthesis (Salehzade et al., 2009). Priming can undo the adverse effects of seed ageing by repairing and building up nucleic acids, increasing the synthesis of proteins, and repairing membranes (Ghassemi-Golezani & Hosseinzadeh-Mahootchi, 2013).

In contrast, hydropriming of aged seeds resulted in a 10% and 12 % decline in FGP of unaged and aged seeds, respectively. During hydropriming, uncontrolled water uptake causes testa weakening and electrolyte leakage, thus interfering with germination (Rouhi et al., 2011). According to Singh et al. (2010), immersing soybean seeds in water for 1-8 hours might result in imbibition damage, which reduces germination. These results also align with Miladinov et al. (2018), who reported a 12% increase and 11% decrease in FGP depending on the cultivar and priming method.

Priming of aged seeds did not affect GI and CVG. Osmoconditioning increased MGT, while hydropriming had no significant effect on the MGT of aged seed. However, the MGT of unaged seeds decreased with osmopriming. These findings disagree with Chirchil (2015), who reported that MGT decreased under osmopriming. The authors also observed an increase with hydropriming. Sadeghi et al. (2011) also reported an increase in FGP, GI, and MGT decrease under osmopriming. The seed lot having the greater GI is considered to be vigorous (Gupta, 1993). Ghasemi et al. (2015) reported that the hydropriming of aged seeds improves FGP, GI, and decreased MGT.

3.4.2 Seedling growth

Seedling growth reflects the ability of vigorous seeds to efficiently shift food reserves from storage tissues to the embryo axis. Uniform seedling emergence is also an essential part of seed vigour. Therefore, evaluating seedling length or dry weight constitutes important vigour parameters (Marcos, 2015). The present study revealed that aging, cultivar, priming, and an interaction $A \times C \times P$ significantly affected all measured seedling growth parameters. Seed ageing reduced SL, RL, SLL, FW, DW, SVI I, and SVI II by 6.7 cm, 13.57 cm, 20.25 cm, 0.36 g, 0.02 g, 2024, and 4.29, respectively. Seed priming negatively affected the RL and SLL of aged seeds regardless of the priming method. Osmopriming of aged seeds improved SVI II by 2.51 but did not affect SVI I, SL, and FW. Hydropriming decreased SL, FW, SVI I and did not affect SVI II. To some extent, these findings agree with (Ghassemi-Golezani et al., 2011), who observed no beneficial effect of priming on soybean performance. It is also evident that the hydropriming of aged seeds negatively affected many seedling growth traits. Regardless of the priming treatment, priming of aged seeds of cv. DM5953RSF and LS6851R significantly decreased RL, SVI I, FW, DW. Hydropriming of unaged seeds improved SL and SLL for cv. LS6851R but had the opposite effect on aged seeds of cv. DM5953RSF. The detrimental effect of hydropriming on seed performance may be due to imbibition injury (Rouhi et al., 2011; Singh et al., 2010). These results disagree with those who reported hydropriming improved germination, root length, and aged seed lots (Ghasemi et al., 2015; Kalsa et al., 2011). This study further revealed that osmopriming of fresh seeds reduced SL and SLL. Osmopriming of fresh seeds of cv. DM5953RSF also improved SVI II, while hydropriming of aged seeds had the opposite effect. Priming had no positive effect on SVI I, RL for both aged and unaged seeds. As expected osmoprimed fresh seeds had a better performance than hydropriming of aged seeds in dry seedling weight. Singh et al. (2014) reported a similar observation. This may be due to improved DNA and RNA synthesis under osmopriming (Salehzade et al., 2009).

According to Weerasekara et al. (2021), who compared primed and unprimed seeds, priming improves seed and seedling performance by improving enzyme activity and protein synthesis, repairing cell membranes, and increasing antioxidant defense mechanisms.

3.5 Conclusion

When comparing aged seeds and unaged seeds, aged seeds exhibited lower scores as expected. The response of aged seeds varied with priming method, cultivar, and measured parameter. Priming improved some parameters, while others were unaffected or adversely affected. The FGP, MGT, and SVI II were increased, while SL, FW, and SVI I were not affected by osmopriming. Hydropriming decreased both FGP and SVI I but did not affect MGT and SVI II. The RL and SLL were negatively affected regardless of the priming method, while GI and CVG remained unchanged. Based on the results of this study, it is concluded that the germination capacity of low vigour soybean seeds can be improved through osmopriming. Therefore, choosing the correct priming method is key to the successful priming of aged soybean seeds.

3.6 References

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CHAPTER 4 EFFECT OF STORAGE DURATION AND CONDITIONS ON SEED GERMINATION, VIABILITY, VIGOUR, AND SEEDLING ESTABLISHMENT OF PRIMED SOYBEAN SEEDS

Abstract

Seed priming has successfully improved seed performance in many crops under both optimal and suboptimal conditions. However, the poor longevity of primed seeds during storage has hindered the adoption of this technique. This study investigated the effect of storage duration and ambient conditions on viability, germination, vigour, and seedling establishment of primed soybean seeds. A three-factor experiment was undertaken using a completely randomized design (CRD) with the following factors: Cultivars – 3 levels (DM5953RSF, LS6851R, PAN1521R); Seed priming – 3 levels (Control, hydropriming, osmopriming); Storage duration – 8 levels (0, 1, 3, 7, 30, 60, 90, 120 days) giving a 3×3×8 factorial treatment structure with three replications totaling to 216 experimental units. The results indicated a highly significant interaction effect ($p < 0.001$) between storage duration, cultivar, and priming treatments. Osmoprimed seeds maintained the highest final germination percentage FGP (93 %) for up to 30 days after priming (DAP) compared to hydroprimed seeds, which maintained the highest GP (90 %) for 7 DAP. Hydroprimed seeds at the storage of 120 days recorded the lowest seed viability (68 %), SLL (5.69 cm), compared to osmoprimed (16.26 cm) and unprimed seeds (15.37 cm). The EC of primed seeds remained lower for most storage (0-90 DAP) than for unprimed seeds. However, an increase in EC was evident after 60 days. As a result, EC of all treatments was similar [osmopriming ($14 \mu\text{S cm}^{-1} \text{ g}^{-1}$), hydropriming ($16 \mu\text{S cm}^{-1} \text{ g}^{-1}$), control ($15 \mu\text{S cm}^{-1} \text{ g}^{-1}$)] after 120 days. Based on these findings, it is concluded that (i) hydroprimed and osmoprimed seeds can be stored for 30 days, without any significant germination and vigour loss, (ii) increase in storage duration negatively affect the germination, viability, vigour, and seedling establishment of primed soybean seeds, regardless of a priming and cultivar treatment.

Keywords: priming, storage potential, viability, vigour

4.1 Introduction

Seed priming is a strategy used prior sowing for improving seed germination and vigour (Varier et al., 2010). It involves controlled hydration or soaking of seeds in water or a solution of low osmotic potential to initiate the pre-germinative metabolism without radicle protrusion during phase II of germination (Dutta, 2018; Marthandan et al., 2020; Sher et al., 2019). After soaking, seeds are rinsed with water and dried back to approximately their actual weight for routine

handling (Farooq et al., 2019a). Drying-back ensures that the positive effect of priming is maintained without the quality loss during handling and storage (Ibrahim, 2019). Widely used priming methods are hydropriming and osmopriming. Seeds are immersed in water during hydropriming before sowing for a specific duration. In osmopriming, seeds are soaked in an aerated solution of sugars or polyethylene glycol, then dried back to their original weight (Sher et al., 2019).

Seed priming has successfully been demonstrated to improve seed germination and seedling emergence in various crops, particularly vegetables and small-seeded grasses (Arif et al., 2008; Mehri, 2015; Ogbuehi et al., 2013; Sadeghi et al., 2011; Singh et al., 2014). Under both optimal and suboptimal conditions, primed seeds typically demonstrate uniform germination and emerge as vigorous seedlings (Yan, 2017). Several reports have proved that this technique is effective in promoting seed germination and seedling growth under salinity (Ahmadvand et al., 2012), drought (Langeroodi and Noora, 2017; Mangena, 2020), chilling (Hussain et al., 2016a), and waterlogging (Hussain et al., 2016b) stress.

Although many positive effects of priming have been reported, the main drawback of this technique is the rapid loss of quality in primed seeds during storage. The viability loss in primed seeds during storage is a major constraint to this technique's broad adoption and implementation (Wang et al., 2018). In general, primed seeds are kept for some time if not used immediately (Yan, 2017). However, storage conditions and duration may reverse the benefits of priming (Parera and Cantliffe, 1994). According to Dutta (2018), the loss of seed desiccation tolerance as a result of prolonged treatments, results in decreased seed longevity. The response of primed seeds to storage is also influenced by species and variety (Ozbay, 2018).

The effect of storage on primed seeds has been studied in several crops, and contrasting results have been reported. Abnavi and Ghobadi (2012) evaluated the effect of storage duration (0, 30, 45, 60 days) on primed wheat seeds. They discovered that keeping primed seeds for 30-60 days boosts shoot and radicle length, dry weight, germination percentage, and speed of germination. On the contrary, in a study conducted by (Yan, 2017), Chinese cabbage seeds were hydroprimed under different temperatures (4, 20, 30 °C) for 1, 3, 6, and 9 months. The researcher observed a decline in germination percentage, rate, and seedling vigour index of primed Chinese cabbage seeds stored at 30°C for 9 months compared to unprimed seeds. The author also observed no adverse effects when primed seeds were kept at 4°C and 20 °C for 9 months and 30 °C for 6 months. A similar study by Hussain et al. (2015) reported that storing primed rice seeds at 25°C tremendously lowered germination and seedling growth. However, no adverse effects were observed at -4°C. The authors also reported that the positive effects of

seed priming could be maintained only for 15 days of storage at 25°C. According to Wang et al. (2018), storing of primed seed under high RH for more than 15 days is deteriorative. Farajollahi and Eisvand (2016) reported that an 8-days delay in planting primed seeds stored at 25°C could significantly decrease the benefits of priming in wheat. When primed seeds are held at 25°C, their germination and seedling growth are reduced due to a restriction in starch metabolism (Farooq et al., 2019b). These contrasting reports trigger the need for further research on the storability of primed seeds. In addition to these contradictory findings, further research is necessary because there has been very little focus on the storage potential of primed soybean seeds under uncontrolled storage conditions. Therefore, this study aimed to investigate the effect of storage duration and conditions on the viability, germination, vigour, and seedling establishment of primed soybean seeds.

4.2 Methods and materials

4.2.1 Experimental site

The experiments were undertaken at the Seed Science Laboratories, School of Agricultural, Earth & Environmental Sciences, University of KwaZulu-Natal.

4.2.2 Experimental material

The study used seeds of three soybean cultivars (LS6851R, DM5953RSF, PAN1521R). The seeds were sourced from Agricultural Research Council – Grain Institute (ARC-GI), Potchefstroom, North West Province, South Africa.

4.2.3 Experimental design

The applied experimental design was a three-factorial experiment that was arranged in a completely randomized design (CRD) with three replications. The first factor was a cultivar with three levels (DM5953RSF, LS6851R, PAN1521R). The second factor was priming with three levels (unprimed, hydropriming, osmopriming). The third factor was the storage period with eight levels (0, 1, 3, 7, 30, 60, 90, 120 days).

4.2.4 Experimental set-up

4.2.4.1 Priming

Before priming, the initial seed mass was recorded. The two selected priming treatments (hydropriming, osmopriming) were applied as described below.

(a) Hydro-priming:

Seeds were soaked in a beaker with distilled water (Hosseini et al., 2007; Murungu et al., 2005; Tilden and West, 1985). Seeds were then allowed to imbibe until a constant seed mass was

achieved. Seed mass was determined at a 2-hour interval until a constant seed mass was reached (Chimonyo and Modi, 2013). After soaking, seeds were placed on paper towels to dry back to the initial seed mass (Rouhi et al., 2011a).

(b) Osmo-priming:

Seeds were immersed in a Polyethylene Glycol (PEG) solution with an osmotic potential of -15 MPa. The solution was prepared by adding 100g of PEG (6000) salt into 1 liter of water. The solution was then incubated in a chamber (Labcon, LTIE, South Africa) set at a temperature of 25 °C to attain a desired osmotic potential. After priming, seeds were rinsed and dried back to return to their initial seed mass. The solution's osmotic potential was calculated as described by (Michel and Kaufmann, 1973) (equation 1)

$$\Psi_s = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T \quad (1)$$

Where C is the concentration of PEG-6000 in g/L H₂O, and T is the temperature in degrees Celsius.

4.2.4.2 Storage period

Primed seeds were kept at room temperature (22/28 °C) for up to six months. Seeds were sampled initially at frequent intervals 0, 1, 3, 7 and after that at longer intervals, 30, 60, 90, and 120 days for evaluation of tetrazolium, conductivity, and seed moisture content. The germination, seedling growth, and accelerated ageing tests were carried out at 0, 7, 30, 60, 90, and 120 days intervals.

4.2.4.3 Germination test

Germination test was carried out using a roller paper towel method (Mandizvo and Odindo, 2019). For each treatment, ten seeds were used for this test. Using distilled water, three paper towels were moistened. Seeds were then placed on two moistened paper towels, and the last paper towel was used to cover them. The paper towels were rolled, then sealed with the elastic bands at the opposite ends and sealed in the zip-lock bags. Zip-lock bags were then kept in a germination chamber set at a temperature of 25°C. Germination will be assessed by counting the number of seeds with at least 2 mm root protrusion daily for 7 days after sowing. The final germination percentage (FGP) was calculated using equation 1 (Sibande et al., 2015), whereas the mean germination time (MGT) was calculated according to equation 2 (Sadeghi et al., 2011).

$$FGP = \left(\frac{\text{seeds germinated}}{\text{total seeds}} \right) \times 100\% \quad (2)$$

$$MGT = \left(\frac{\sum D.N.}{\sum N} \right) \quad (3)$$

Where D was the number of days from the beginning of the germination test, and N will be the number of newly germinated seeds on the day

4.2.4.4 Seedling growth test

After the germination test, seedling vigour was assessed by measuring shoot length, root length, and seedling length and determining the seedling fresh and dry weight (Govender et al., 2008; Verma and Verma, 2015).

4.2.4.5 Accelerated seed aging

To induce low vigour, seeds were subjected to the accelerated ageing procedure (Pandey et al., 2017). Accelerated aging exposes seeds for a shorter period to two environmental variables, high temperature, and relative humidity, which cause rapid deterioration (TeKrony, 1993; Woltz and TeKrony, 2001). Seeds were aged at a temperature of 41°C and 100% relative humidity for 72 hours (TeKrony, 2005). A plastic box (11 x 11 x 3.5 cm) containing a mesh tray (10 x 10 x 3 cm) was used as an aging inner chamber (Mandizvo and Odindo, 2019). A 100 ml of 10 % (w/v) sodium chloride solution was added to each plastic box. The mesh tray was carefully inserted to avoid water splash (Vilakazi, 2018). Seeds were weighed and placed on a mesh tray, one layer deep, to ensure even moisture uptake. The boxes were then placed in an aging chamber. To ensure proper and uniform air circulation and temperature inside the chamber, an air space of 2.5 cm was allowed between plastic boxes (Pandey et al., 2017; Rouhi et al., 2011a). After ageing, seeds were subjected to a paper towel germination method described under the germination test above.

4.2.4.6 Electrical Conductivity test

Ten grams per replicate were weighed and kept in a glass beaker filled with 100ml of distilled water for 24 hours to release cytoplasmic solute in the imbibing medium (Copeland and McDonald, 2001; Mandizvo and Odindo, 2019). After this period, the EC of the solute was determined using the pH/E.C. water tester. The tester probe was rinsed with distilled water after each measurement to prevent cross-contamination. The EC was then computed using equation 4 (Vilakazi, 2018).

$$EC (\mu S \text{ cm}^{-1} \text{ g}^{-1}) = \frac{\text{conductivity reading} - \text{background reading}}{\text{weight of replicate}} \quad (\text{equation 4})$$

Where background reading was a control/blank.

4.2.4.7 Tetrazolium (TZ) test

A solution of 2,3,5-triphenyl tetrazolium chloride (1.0%) was prepared by adding 1 g of tetrazolium salt in 100 ml of water. Water-soaked seeds used for the conductivity test were used for the TZ test (Govender et al., 2008). Seeds were preconditioned to (i) ensure complete

hydration of all the tissues and proper penetration of tetrazolium solution, (ii) prevent damage to cotyledons and embryo axis while cutting seeds, and (iii) activate the germination process (Patil and Dadlani, 2009). Preconditioned seeds were then cut longitudinally using a scalpel blade to expose the embryo. The prepared seeds were then placed in Petri dishes containing TZ solution for 2 hours at 25 °C. The preparation room was kept dark during this experiment because of TZ sensitivity to light (Vilakazi, 2018). The number of stained and non-stained embryos was then determined. The percentage of viable seeds was computed as indicated by equation 3 (Mangena and Mokwala, 2019).

$$\text{Seed viability}\% = \left(\frac{\text{number of stained seeds}}{\text{total number of seeds tested}} \right) \times 100 \% \quad (\text{equation 3})$$

4.2.4.8 Seed moisture content

Seed moisture content was evaluated using a grain moisture meter (YH, Lds-1g, China).

4.2.5 Statistical analysis

The analysis of variance (ANOVA) was performed on data collected using GenStat®, 20.1 Edition (VSN International, Hamel Hampstead, UK, 2020) at the 5% level of significance. The means of significantly different variables were also separated using Tukey's test with GenStat® at the 5% significance level. The Pearson correlation coefficient (r) between measured traits was performed using GraphPad Prism®, 9.3 version (GraphPad Software, San Diego, CA, 2021).

4.3 Results

4.3.1 Final germination percentage

Highly significant ($p < 0.001$) difference was observed among cultivars, priming, storage, and P×S treatments in final germination percentage. Nevertheless, no significant difference was recorded from C×P, C×S, and C×P×S treatment (Table 4.1).

Table 4.1 Analysis of variance showing mean squares and significance test for FGP, MGT, and AA of three cultivars subjected to priming and storage treatments

Treatment	d.f	FGP	MGT	AA
Cultivar (C)	2	3750.6***	2.54***	10430.2***
Priming (P)	2	3719.1***	0.40**	23143.2***
Storage (S)	5	1072.1***	1.29***	3132.8***
C×P	4	177.5 ^{ns}	0.11 ^{ns}	1546.9***
C×S	10	198.8 ^{ns}	0.16*	417.7*
P×S	10	727.3***	0.41***	439.5*
C×P×S	20	4356.8 ^{ns}	0.09 ^{ns}	402.1*
Residual	108	163.0	0.07	218.5

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ns, Not Significant; d.f=degrees of freedom; FGP= Germination percentage; MGT=Mean germination time; AA=Seed vigour based on accelerated aging test

On average, the highest final germination percentage (FGP) was exhibited by cv. PAN1521R (92 %), followed by LS6851R (84 %), with the lowest recorded by cv. DM5953RSF (76 %). Cultivar LS6851R and DM5953RSF recorded the highest MGT of 1.8, and 1.7, respectively, compared to PAN1521R (1.34) (Figure 4.1).

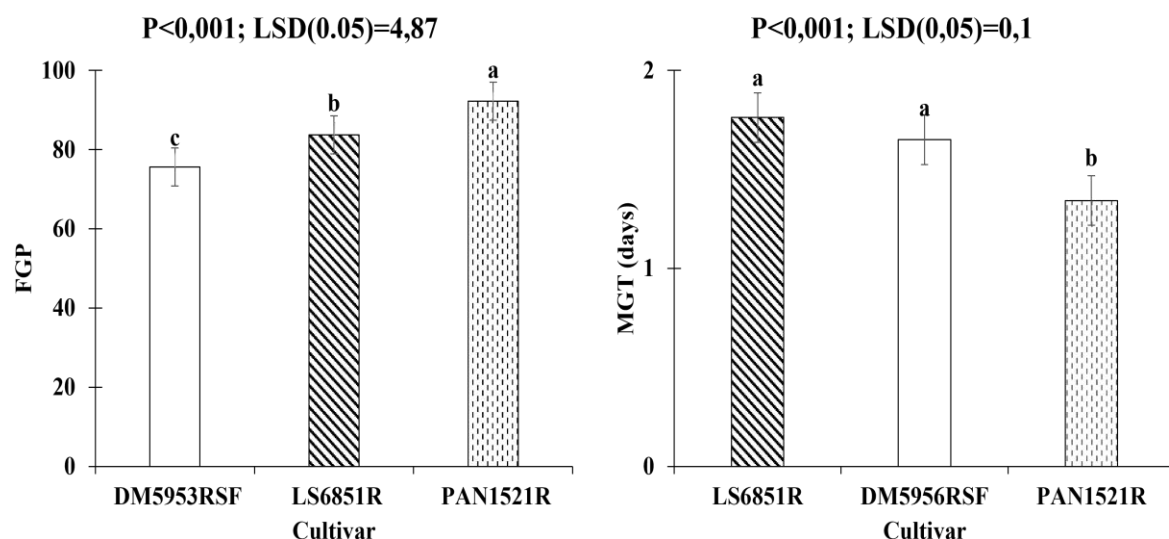


Figure 4.1 Effect of cultivar on final germination percentage (FGP) and mean germination time (MGT).

When comparing priming treatments effect, the highest FGP of 89 and 88 % was recorded from osmopriming and control (unprimed) treatment. Hydropriming exhibited the lowest FGP (74 %) (Figure 4.2).

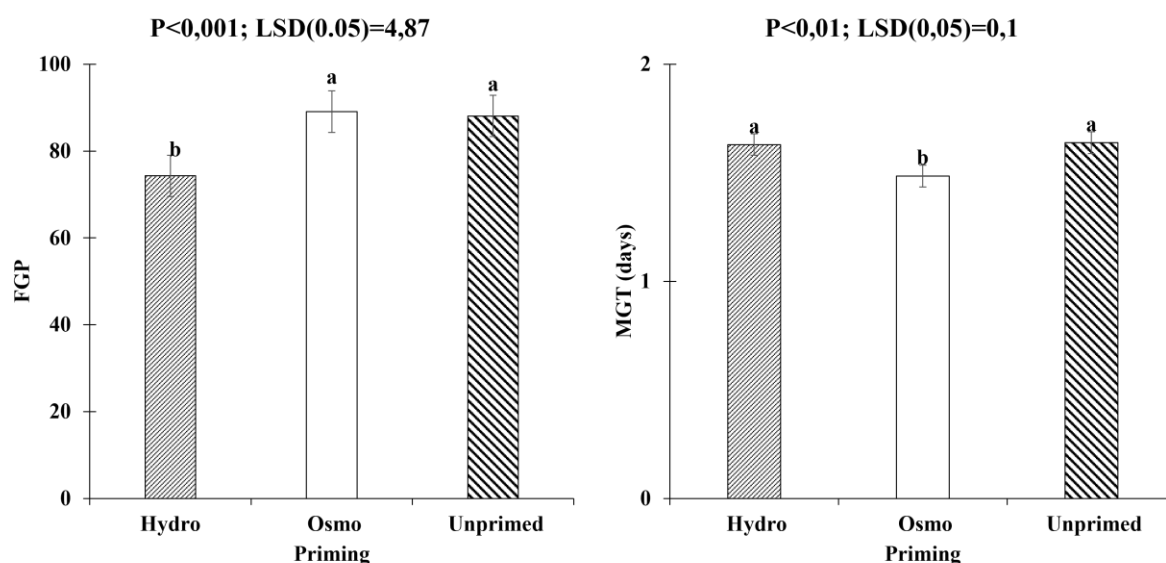


Figure 4.2 Effect of priming on final germination percentage (FGP) and mean germination time (MGT).

For storage duration, the highest FGP (92-90 %) was obtained at 0-7 days after priming (DAP), whereas the lowest value of 77% was recorded at 60 and 120 DAP (Figure 4.3).

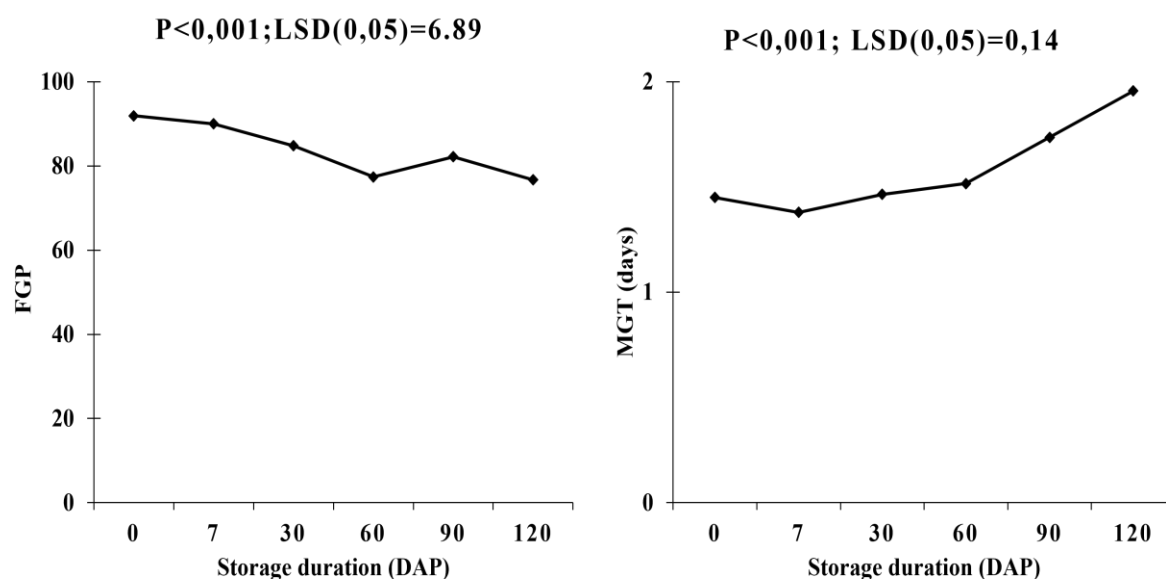


Figure 4.3 Effect of storage duration on germination percentage and mean germination time (MGT). DAP = days after priming.

For P×S, both hydropriming and osmopriming recorded their respective highest FGP of 92 and 94% at 0 DAP, while exhibiting their lowest FGP of 50 and 80 % at 120 and 60 DAP, respectively. Interestingly, unprimed seeds recorded the highest FGP (94 %) at the end of storage (120 DAP) and the lowest FGP (80 %) at 60 DAP (Figure 4.4).

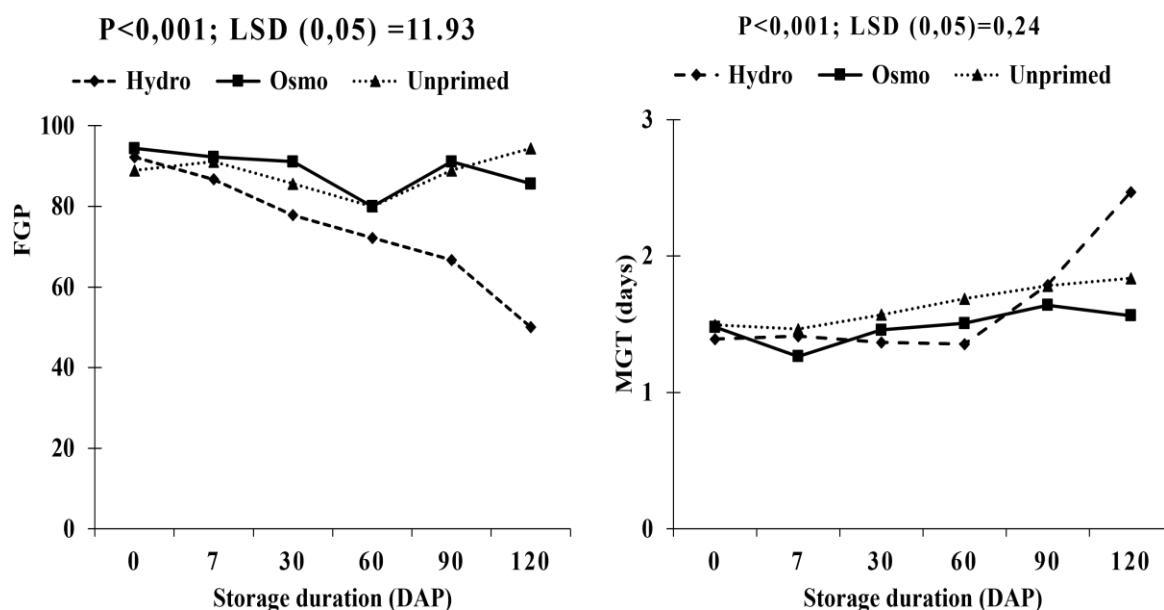


Figure 4.4 Effect of storage duration and priming on germination percentage and mean germination time (MGT). DAP = days after priming.

4.3.2 Mean germination time

A highly significant difference ($p < 0.001$) in mean germination time (MGT) was evident among cultivars, priming, and storage duration. A significant difference ($p < 0.05$) was also observed from the C×S interaction. No significant effect was observed for other treatment combinations (Table 4.1). Cultivar LS6851R and DM5953RSF recorded the highest MGT of 1.8, and 1.7, respectively, compared to PAN1521R (1.34) (Figure 4.1). Both unprimed and hydroprimed seeds recorded the highest values of 1.63, while osmopriming recorded the lowest MGT (1.48) (Figure 4.2). In terms of storage duration, the highest MGT (1.96) was recorded at 120 DAS, while the lowest values (1.38 -1.52) were recorded between 0 and 60 DAS (Figure 4.3). Cultivar PAN1521R, LS6851R, and DM5953RSF recorded the highest MGT (1.87, 2.04, and 1.95) at 120 DAS, while the lowest values (1.04, 1.57, and 1.42) were recorded at 7, 60, and 30 DAS, respectively. For P×S interaction, primed seeds recorded the highest MGT (1.64-2.45) at 90-120 DAS (Figure 4.4).

4.3.3 Shoot, root, and seedling length

Highly significant ($p < 0.001$) difference in the shoot, root, and seedling length was observed among cultivar, priming, storage duration, and their interactions (Table 4.2). Cultivar PAN1521R exhibited greater shoot length (SL) (Table 4.3), root length (RL) (Table 4.4), and seedling length (SLL) (Table 4.5) with an average of 11.77, 15.58, and 27.35 cm, followed by LS6851R (10.29, 12.43, and 22.72 cm) and DM6963RSF (9.23, 12.53, and 21.76cm).

Comparing priming treatments, unprimed and osmoprimed seeds recorded the highest SL (11.61 and 10.97 cm), with the lowest SL (8.71 cm) recorded from hydroprimed seeds. Unprimed seeds also recorded greater RL (16.10 cm), followed by osmopriming (13.75 cm) and hydropriming (10.69 cm). Concerning storage duration, the highest SL (14.99 cm), RL (21.38 cm), and SLL (35.72 cm) were recorded at 0-7 days after storage (DAP), with the lowest values of SL (6.85 cm), RL (6.35 cm), and SLL (12.34 cm) being recorded at 60-120 DAP. For P×S interaction, the control (unprimed), hydropriming, and osmopriming produced the highest SL (17.70, 12.97, and 14.93 cm), RL (23.23, 20.28, and 23.24 cm), and SLL (40.93, 33.25, and 38.18 cm) during 0-7 DAP. The lowest values of SL (4.29, 3.51, and 5.55 cm), RL (5.57, 2.18, and 4.63 cm), and SLL (10.04, 5.69, and 10.18 cm) recorded at 90, 120, 60 DAP.

Table 4.2 Analysis of variance showing mean squares and significance test for SL, RL, and SLL of three cultivars subjected to priming and storage treatments

Treatment	d.f	SL	RL	SLL
Cultivar (C)	2	125.77***	397.97***	958.95***
Priming (P)	2	87.83***	173.17***	482.28***
Storage (S)	5	431.27***	1440.55***	3430.46***
C×P	4	31.93***	91.73***	214.07***
C×S	10	40.53***	61.39***	184.38***
P×S	10	12.77***	16.03***	42.65***
C×P×S	20	8.70***	17.02***	41.49***
Residual	108	2.23	4.41	10.75

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ns, Not Significant; d.f=degrees of freedom; SL=Shoot length; RL=Root length; SLL=Seedling length.

Table 4.3 Effect of cultivar, priming, and storage duration on the shoot length

Priming	DAP	Shoot Length (cm)		
		DM5953RSF	LS6851R	PAN1521R
Unprimed	0	15,45 ^{bcdef}	21,43 ^a	16,23 ^{bc}
	7	11,02 ^{defghijklmn}	13,28 ^{bcdefghi}	15,28 ^{bcdef}
	30	16,60 ^{abc}	15,35 ^{bcdef}	16,71 ^{abc}
	60	8,14 ^{ijklmnopqrstu}	12,56 ^{bcdefghij}	10,66 ^{efghijklmnop}
	90	3,91 ^{stuvw}	4,43 ^{rstuvw}	4,52 ^{rstuvw}
	120	6,95 ^{lmnopqrstuv}	10,80 ^{efghijklmn}	5,73 ^{pqrstuvw}
Hydro	0	11,07 ^{defghijklm}	11,95 ^{cdefghijkl}	15,88 ^{bcd}
	7	11,77 ^{cdefghijkl}	9,67 ^{hijklmnopq}	17,18 ^{ab}
	30	9,42 ^{hijklmnopqr}	9,64 ^{hijklmnopq}	15,59 ^{bcde}
	60	3,67 ^{tuvw}	6,09 ^{mnopqrstuvw}	9,10 ^{ijklmnopqr}
	90	3,50 ^{uvw}	5,77 ^{opqrstuvw}	5,89 ^{opqrstuvw}
	120	3,07 ^{uvw}	5,17 ^{qrstuvw}	2,29 ^{vw}
Osmo	0	13,33 ^{bcdefghi}	13,04 ^{bcdefghij}	16,52 ^{abc}
	7	14,34 ^{bcdefgh}	14,93 ^{bcdefg}	15,53 ^{bcde}
	30	12,13 ^{bcdefghijk}	10,40 ^{fghijklmnop}	15,05 ^{bcdefg}
	60	5,99 ^{nopqrstuvw}	1,64 ^w	9,02 ^{ijklmnopqr}
	90	8,61 ^{ijklmnopqrst}	10,21 ^{ghijklmnopq}	9,60 ^{hijklmnopq}
	120	7,15 ^{klmnopqrstuv}	8,84 ^{ijklmnopqrs}	11,06 ^{defghijklm}
LSD		2,42		
CV%		14,3		

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance.

Table 4.4 Effect of cultivar, priming, and storage duration on the root length

Priming	DAP	Root Length (cm)		
		DM5953RSF	LS6851R	PAN1521R
Unprimed	0	25,60 ^{ab}	21,43 ^{abcdefg}	22,66 ^{abcdef}
	7	17,02 ^{cdefghijk}	21,10 ^{abcdefgh}	25,49 ^{ab}
	30	25,18 ^{ab}	23,44 ^{abcde}	16,71 ^{defghijkl}
	60	13,61 ^{ijklmn}	15,95 ^{fghijkl}	14,68 ^{ghijklm}
	90	5,28 ^{opqrs}	5,95 ^{opqrs}	6,03 ^{opqrs}
	120	10,77 ^{jklmnopq}	11,47 ^{jklmno}	7,52 ^{nopqrs}
Hydro	0	16,55 ^{efghijkl}	17,39 ^{cdefghij}	26,92 ^a
	7	16,39 ^{efghijkl}	13,55 ^{ijklmn}	26,01 ^{ab}
	30	11,05 ^{jklmnop}	10,23 ^{klmnopq}	19,22 ^{bcdefghi}
	60	3,74 ^{qrs}	4,14 ^{pqrs}	9,65 ^{ijklmnopqr}
	90	1,16 ^s	2,27 ^s	7,63 ^{mnopqrs}
	120	1,63 ^s	2,62 ^{rs}	2,27 ^s
Osmo	0	21,64 ^{abcdefg}	19,43 ^{bcdefghi}	24,05 ^{abc}
	7	22,05 ^{abcdef}	23,79 ^{abcd}	23,89 ^{abc}
	30	15,78 ^{fghijkl}	14,15 ^{hijklmn}	19,17 ^{bcdefghi}
	60	4,82 ^{opqrs}	1,61 ^s	7,47 ^{nopqrs}
	90	7,77 ^{mnopqrs}	8,15 ^{mnopqrs}	10,72 ^{jklmnopq}
	120	5,28 ^{opqrs}	7,05 ^{nopqrs}	10,39 ^{jklmnopq}
LSD		3,4		
CV%		15,5		

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance.

Table 4.5 Effect of cultivar, priming, and storage duration on seedling length

Priming	Storage duration (DAP)	Seedling Length (cm)		
		DM5953RSF	LS6851R	PAN1521R
Unprimed	0	41,05 ^a	42,87 ^a	38,89 ^{abc}
	7	28,04 ^{cdefghij}	34,38 ^{abcdef}	40,77 ^a
	30	41,78 ^a	38,79 ^{abc}	33,43 ^{abcdefg}
	60	21,75 ^{hijklmn}	28,51 ^{bcdefghij}	25,34 ^{efghijk}
	90	9,19 ^{qrst}	10,37 ^{opqrst}	10,55 ^{opqrst}
	120	17,72 ^{klmnopqrs}	22,27 ^{hijklm}	13,25 ^{mnopqrst}
Hydro	0	27,61 ^{defghij}	29,34 ^{bcdefghi}	42,80 ^a
	7	28,16 ^{cdefghij}	23,23 ^{ghijklm}	43,19 ^a
	30	20,47 ^{ijklmnop}	19,87 ^{ijklmnopqrs}	34,81 ^{abcdef}
	60	7,41 st	10,23 ^{pqrst}	18,75 ^{ijklmnopqr}
	90	4,71 ^t	8,04 ^{rst}	13,52 ^{ijklmnopqrst}
	120	4,66 ^t	7,79 ^{rst}	4,56 ^t
Osmo	0	34,97 ^{abcdef}	32,47 ^{abcdefgh}	40,57 ^a
	7	36,39 ^{abcde}	38,71 ^{abcd}	39,42 ^{ab}
	30	27,91 ^{cdefghij}	24,55 ^{fghijkl}	34,22 ^{abcdefg}
	60	10,81 ^{nopqrst}	3,25 ^t	16,49 ^{klmnopqrs}
	90	16,39 ^{klmnopqrs}	18,37 ^{ijklmnopqrs}	20,32 ^{ijklmnop}
	120	12,73 ^{mnopqrst}	15,89 ^{klmnopqrs}	21,45 ^{hijklmno}
LSD		5,31		
CV%		13,7		

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance. DAP = days after priming.

4.3.4 Seedling fresh and dry weight

Analysis of variance results shows that both seedling dry (DW) and fresh weight (FW) was significantly affected by cultivar, priming, and storage duration and their interactions (Table 4.6). Treatment means for fresh weight are presented in Table 4.7, whereas Table 4.8 represents means for dry weight. Cultivar PAN1521R exhibited higher FW and DW (1.07 and 0.14 g) than DM5953RSF (0.86 and 0.12 g) and LS6851R (0.84 and 0.12 g). Unprimed seeds recorded the highest FW and DW (1.03 and 0.13 g), followed by osmoprimed (0.97 and 0.12g) and hydroprimed seeds (0.77 and 0.12 g). In terms of storage duration, the highest FW with an

average of 1.09 g was recorded at 0-30 DAP compared to the lowest FW (0.76 g) recorded at 60-120 DAP. Unprimed, hydroprimed, and osmoprimed seeds had their respective highest FW (1.28, 1.05, and 1.13 g) at 0-30 DAP. Interestingly, unprimed seeds exhibited more DW (0.14 g) at 90-120 DAP, while both hydropriming and osmopriming exhibited uniform values with an average of 0.13 g throughout the storage period except at 60 DAP. The lowest DW values were recorded at 60 DAP by the control (0.12 g), hydropriming (0.10 g), and osmopriming (0.08 g).

Table 4.6 Analysis of variance showing mean squares and significance test for FW and DW of three cultivars subjected to priming and storage treatments

Treatment	d.f	FW	DW
Cultivar (C)	2	1.00***	0.014***
Priming (P)	2	0.93***	0.003***
Storage (S)	5	0.82***	0.002***
C×P	4	0.11***	0.001**
C×S	10	0.16***	0.001**
P×S	10	0.07***	0.001*
C×P×S	20	0.04***	0.001***
Residual	108	0.01	0.00025

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ns, Not Significant; d.f=degrees of freedom; FW=Fresh weight; DW=Dry Weight

Table 4.7 Effect of cultivar, priming, and storage duration on fresh weight

Priming	Storage duration	Fresh Weight (g)		
		DM5953RSF	LS6851R	PAN1521R
Unprimed	0	1,21 ^{abcde} gh	0,99 ^{bcde} ghijk	1,25 ^{abcde}
	7	0,89 ^{efgh} ijklm	0,93 ^{defgh} ijkl	1,19 ^{abcde} gh
	30	1,26 ^{abcde}	1,19 ^{abcde} gh	1,39 ^a
	60	0,71 ^{ijklm} nop	1,12 ^{abcde} ghi	1,13 ^{abcde} ghi
	90	0,79 ^{ijklm} no	0,88 ^{fgh} ijklmn	0,74 ^{ijklm} nop
	120	0,98 ^{bcde} ghijkl	1,02 ^{abcde} ghij	0,92 ^{defgh} ijkl
Hydro	0	0,86 ^{gh} ijklmn	0,84 ^{hij} klmn	1,23 ^{abcde} fg
	7	0,95 ^{cde} ghijkl	0,84 ^{hij} klmn	1,35 ^{ab}
	30	0,85 ^{hij} klmn	0,80 ^{ijklm} no	1,04 ^{abcde} ghij
	60	0,40 ^{pq}	0,61 ^{lmnop}	0,99 ^{bcde} ghijk
	90	0,41 ^{pq}	0,54 ^{mno} pq	0,71 ^{ijklm} nop
	120	0,46 ^{opq}	0,52 ^{nop} q	0,47 ^{opq}
Osmo	0	1,05 ^{abcde} ghij	1,07 ^{abcde} ghij	1,32 ^{abc}
	7	1,05 ^{abcde} ghij	1,05 ^{abcde} ghij	1,28 ^{abcd}
	30	1,07 ^{abcde} ghij	0,98 ^{bcde} ghijkl	1,30 ^{abcd}
	60	0,65 ^{klm} nop	0,14 ^q	1,00 ^{bcde} ghik
	90	1,02 ^{abcde} ghijk	0,80 ^{ijklm} no	1,07 ^{abcde} ghij
	120	0,80 ^{ijklm} no	0,80 ^{ijklm} no	0,97 ^{cde} ghijkl
LSD		0,18		
CV%		11,9		

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance. DAP = days after priming.

Table 4.8 Effect of cultivar, priming, and storage duration on seedling weight

Priming	Storage duration (DAP)	Dry Weight (g)		
		DM5953RSF	LS6851R	PAN1521R
Unprimed	0	0,13 ^{ab}	0,11 ^{abc}	0,14 ^{ab}
	7	0,13 ^{ab}	0,11 ^{abc}	0,14 ^{ab}
	30	0,13 ^{abc}	0,11 ^{abc}	0,15 ^{ab}
	60	0,10 ^{bc}	0,12 ^{abc}	0,14 ^{ab}
	90	0,15 ^b	0,12 ^{abc}	0,15 ^{ab}
	120	0,16 ^a	0,14 ^{ab}	0,13 ^{ab}
Hydro	0	0,12 ^{abc}	0,11 ^{abc}	0,13 ^{ab}
	7	0,12 ^{abc}	0,11 ^{abc}	0,14 ^{ab}
	30	0,12 ^{abc}	0,10 ^{bc}	0,13 ^{ab}
	60	0,07 ^c	0,10 ^{bc}	0,14 ^{ab}
	90	0,11 ^{abc}	0,10 ^{bc}	0,14 ^{ab}
	120	0,14 ^{ab}	0,10 ^{bc}	0,15 ^{ab}
Osmo	0	0,12 ^{abc}	0,11 ^{abc}	0,13 ^{ab}
	7	0,13 ^{ab}	0,12 ^{abc}	0,14 ^{ab}
	30	0,13 ^{ab}	0,11 ^{abc}	0,13 ^{ab}
	60	0,10 ^{bc}	0,02 ^d	0,13 ^{ab}
	90	0,14 ^{ab}	0,10 ^{bc}	0,14 ^{ab}
	120	0,14 ^{ab}	0,10 ^{bc}	0,13 ^{ab}
LSD	0,18	0,02		
CV%	11,9	13.0		

According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance. DAP = days after priming.

4.3.5 Accelerated ageing test

The analysis of variance shows that the effect of cultivar, priming, storage, and P×S interaction on seed vigour based on the accelerated ageing test was highly significant ($p < 0.001$). Highly significant ($p < 0.01$) effect was also observed from the C×P×S interaction (Table 4.1). Cultivar PAN1521R recorded the higher FGP (38.15 %) compared to DM5953RSF (17.59 %) and LS6851R (11.6 %). Comparing priming treatments, the highest value was observed from

osmopriming (46.30 %), with the lowest observed from hydropriming (12.22) and control (8.89 %). Higher values (25.56-34.07 %) for storage duration were recorded at 0-30 DAP. Sixty DAP recorded the lowest FGP (8.15 %). For P×S, osmopriming and the control recorded the highest FGP (60 and 15.56 %) at 0-7 DAP. Hydroprimed seeds exhibited the highest vigour only at 0 DAP. Hydropriming recorded the lowest vigour (3.66 %) at 60-120 DAP, while both osmopriming and control exhibited their lowest values at 60 DAP (Figure 4.5).

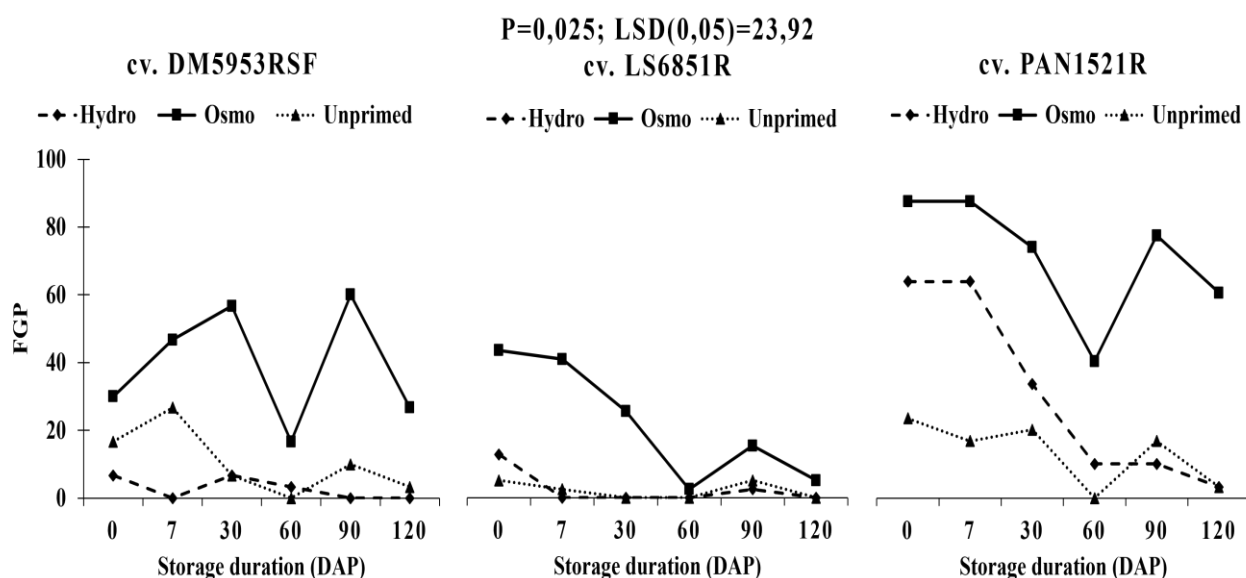


Figure 4.5 Germination percentage after the accelerated aging test. DAP= days after priming

4.3.6 Tetrazolium Test

Highly significant ($p<0.001$) difference in viability was observed among priming, storage, and P×S treatments. Highly significant ($p<0.01$) difference was also observed among the C×P×S interaction. There was no significant difference among cultivar and C×P treatments (Table 4.9). Both unprimed seeds and osmoprimed seeds recorded the highest viability of 95 %, while hydroprimed seeds recorded the lowest viability of 90 %. In terms of storage duration, the highest viability was evident from 0-60 DAP, with an average of 97%. The lowest viability (78%) was recorded after DAP. Unprimed seeds recorded the highest viability (100 %) during 1, 30, and DAP. Hydroprimed seeds showed higher viability 97 % at 1-7 DAP, while hydropriming had the highest viability at 7 DAP. All priming treatments (control, hydropriming, and osmopriming) had the lowest viability (80, 68, and 86 %) at 120 DAP (Figure 4.6).

Table 4.9 Analysis of variance showing mean squares and significance test for electrical conductivity, viability (TZ), and moisture content for the seed of three cultivars subjected to different priming and storage duration treatments

Treatment	d.f.	EC	TZ	SMC
Cultivar (C)	2	312.23***	29.17 ^{ns}	22.01***
Priming (P)	2	876.66***	459.72***	25.94***
Storage (S)	7	156.40***	1368.19***	12.58***
C×P	4	27.52 ^{ns}	105.56 ^{ns}	5.55***
C×S	14	24.62 ^{ns}	42.39 ^{ns}	1.68***
P×S	14	74.32***	130.09***	2.21***
C×P×S	28	11.60 ^{ns}	96.56**	0.61 ^{ns}
Residual	144	18.20	43.06	0.51

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$; ns, Not Significant; d.f.=degrees of freedom; EC=Electrical conductivity; SMC=Seed moisture content; TZ=Tetrazolium test

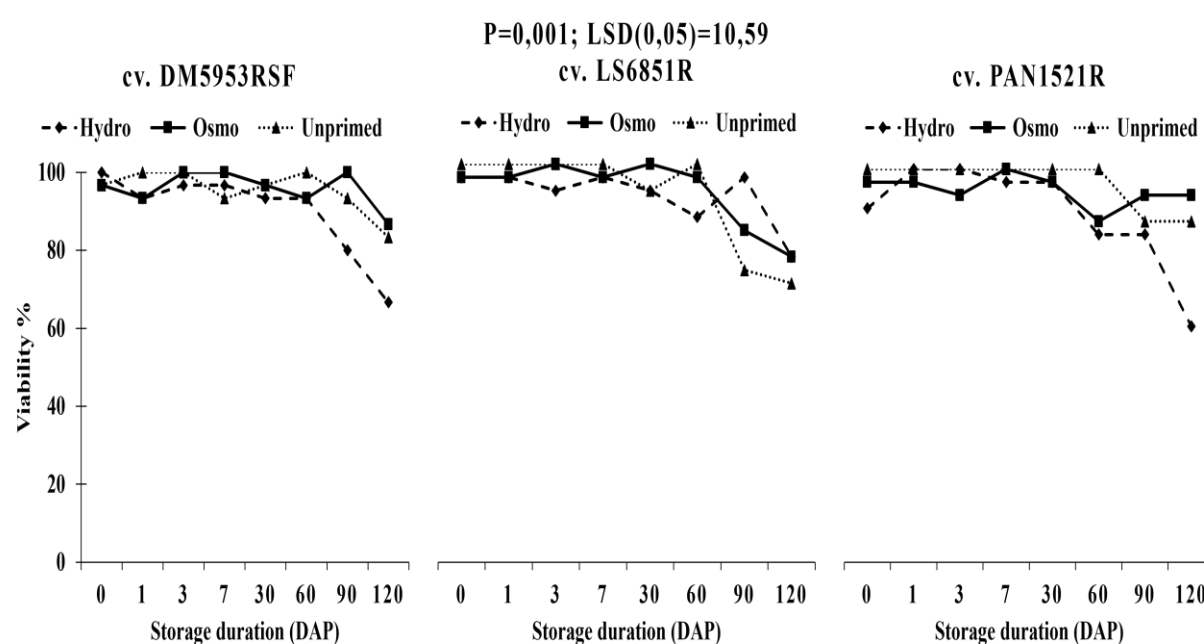


Figure 4.6 Effect of priming and storage duration on the viability of soybean cultivars.
DAP=days after priming

4.3.7 Electrical Conductivity

Highly significant ($p < 0.001$) difference in electrical conductivity (EC) of leachate was observed among cultivar, priming, storage, and P×S treatments (Table 4.9). Both cv.

DM5953RSF and LS6851R exhibited a high EC of $13 \mu\text{S cm}^{-1} \text{g}^{-1}$. The lowest EC ($10 \mu\text{S cm}^{-1} \text{g}^{-1}$) was exhibited by cv. PAN1521R. Unprimed seeds recorded the highest EC ($16.02 \mu\text{S cm}^{-1} \text{g}^{-1}$), while hydroprimed and osmoprimed seeds recorded the lowest EC of 9.55 and $10.51 \mu\text{S cm}^{-1} \text{g}^{-1}$ (Figure 4.7). In terms of storage duration, the highest EC ($15.15 \mu\text{S cm}^{-1} \text{g}^{-1}$) was recorded at 120 DAP. The lowest EC ($7.22 \mu\text{S cm}^{-1} \text{g}^{-1}$) was recorded at 0 DAP. For P×S interaction, control exhibited the greater EC ($21.23 \mu\text{S cm}^{-1} \text{g}^{-1}$) at 3 DAP, while hydropriming and osmopriming exhibited the greater EC (16.26 and $13.82 \mu\text{S cm}^{-1} \text{g}^{-1}$) at 120 DAP (Figure 4.8).

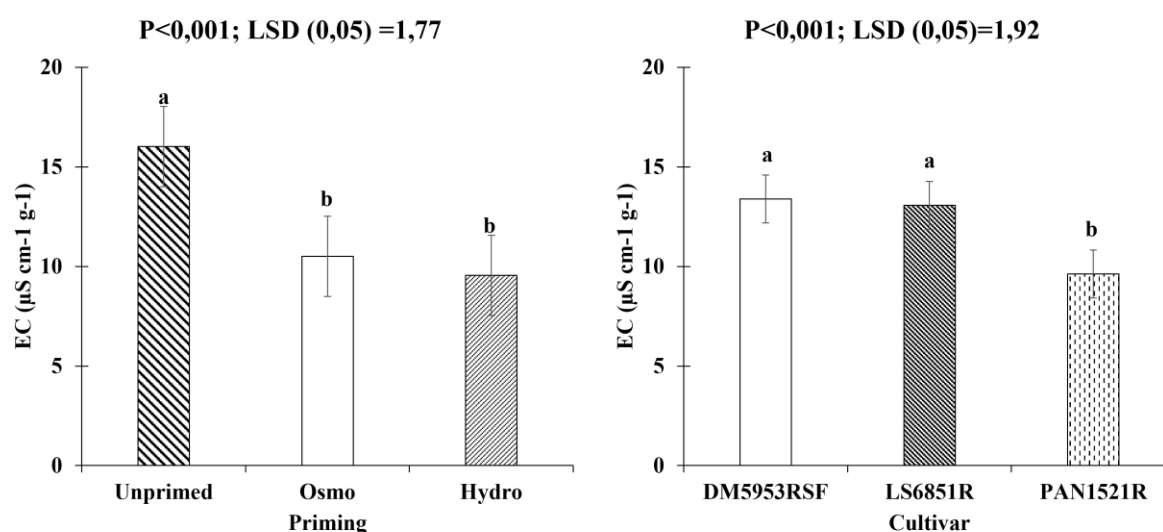


Figure 4.7 Effect of priming and cultivar on the electrical conductivity of seed leachate

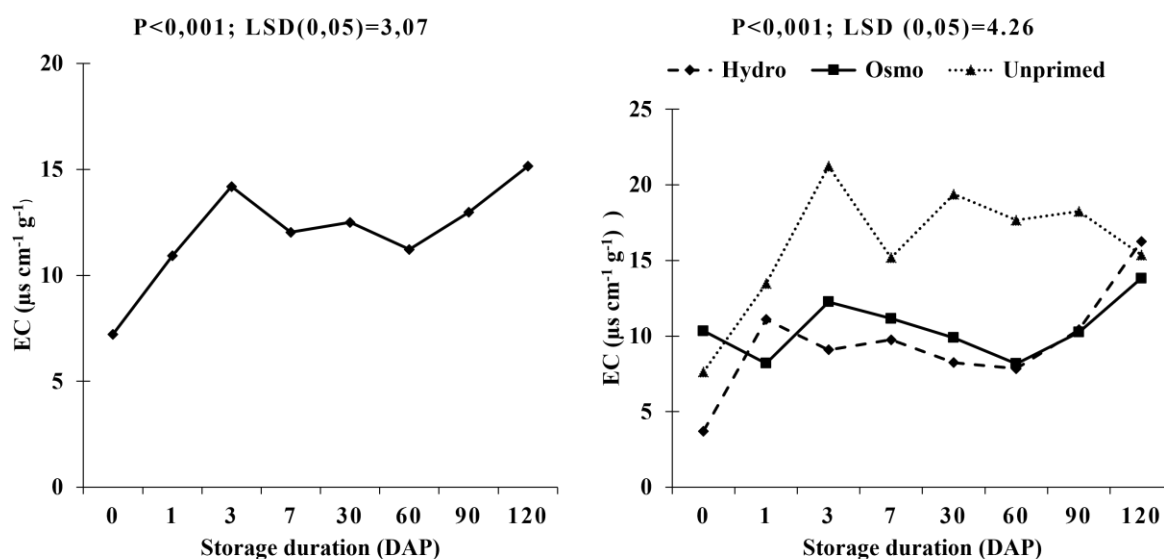


Figure 4.8 Effect of storage duration and its interaction with priming on the electrical conductivity of seed leachate. DAP = days after priming.

4.3.8 Seed moisture content

The analysis of variance results shows a highly significant ($p < 0.001$) difference in seed moisture content (SMC) was observed among cultivar, priming, storage, $C \times P$, $C \times S$, and $P \times S$. No significant difference was observed from $C \times P \times S$ (Table 4.9). Cultivar PAN1521R had the highest SMC (11.33 %) compared to DM5953RSF (10.95 %) and LS6851R (10.24 %). Hydropriming exhibited the highest SMC (11.52 %) compared to the control (10.64 %) and osmopriming (10.37 %). Regarding storage duration, 0 DAP exhibited the highest SMC (11.70 %), while the lowest SMC (9.83 %) exhibited by 120 DAP. For $P \times S$ interaction, unprimed seeds recorded the lowest and highest SMC (9.42 and 11.32 %) at 120 and 0-3 DAP. Hydroprimed seeds exhibited the lowest and highest SMC (9.91 and 12.38 %) at 90 and 0 DAP. Osmopriming recorded the lowest and highest SMC (9 and 11.26 %) at 120 and 0-1 DAP (Table 4.10).

Table 4.10 Effect of cultivar, priming, storage duration on seed moisture content

		Seed Moisture Content %		
Priming	Storage duration (DAP)	DM5953RSF	LS6851R	PAN1521R
Unprimed	0	11,47 ^{bcdefghijk}	10,97 ^{bcdefghijk}	11,93 ^{bcdefghi}
	1	11,27 ^{bcdefghijk}	10,80 ^{cdefghijk}	11,73 ^{bcdefghij}
	3	11,10 ^{bcdefghijk}	11,13 ^{bcdefghijk}	11,47 ^{bcdefghijk}
	7	11,23 ^{bcdefghijk}	10,63 ^{defghijk}	11,30 ^{bcdefghijk}
	30	10,30 ^{cdefghijk}	10,20 ^{efghijk}	10,73 ^{cdefghijk}
	60	10,27 ^{efghijk}	10,00 ^{ghijkl}	10,40 ^{efghijk}
	90	10,03 ^{fghijkl}	9,70 ^{ijkl}	10,00 ^{ghijkl}
	120	9,42 ^{ijkl}	9,12 ^{kl}	9,71 ^{ijkl}
Hydro	0	12,57 ^{abcde}	11,17 ^{bcdefghijk}	13,40 ^{ab}
	1	12,37 ^{abcdefg}	11,03 ^{bcdefghijk}	13,17 ^{ab}
	3	12,07 ^{bcdefghi}	10,53 ^{defghijk}	12,93 ^{abcd}
	7	11,67 ^{bcdefghij}	10,40 ^{efghijk}	12,50 ^{abcdef}
	30	11,63 ^{bcdefghij}	10,73 ^{cdefghijk}	12,30 ^{bcdefgh}
	60	10,57 ^{defghijk}	9,77 ^{ijkl}	11,33 ^{bcdefghijk}
	90	9,93 ^{ghijkl}	9,40 ^{ijkl}	10,40 ^{efghijk}
	120	14,83 ^a	9,25 ^{ijkl}	12,42 ^{abcdefg}
Osmo	0	11,07 ^{bcdefghijk}	10,97 ^{bcdefghijk}	11,73 ^{bcdefghij}
	1	10,90 ^{cdefghijk}	10,93 ^{bcdefghijk}	11,73 ^{bcdefghij}
	3	10,60 ^{defghijk}	10,60 ^{defghijk}	11,27 ^{bcdefghijk}
	7	10,20 ^{efghijk}	10,43 ^{efghijk}	10,97 ^{bcdefghijk}
	30	9,87 ^{hijkl}	10,43 ^{efghijk}	11,10 ^{bcdefghijk}
	60	9,60 ^{ijkl}	10,17 ^{efghijk}	10,17 ^{efghijk}
	90	9,67 ^{ijkl}	9,77 ^{ijkl}	9,60 ^{ijkl}
	120	9,78 ^{ijkl}	7,62 ⁱ	9,61 ^{ijkl}
LSD (0.05)	1,16			
CV%	6,6			

*According to Tukey's test, the means under the same column superscripted with the same letters are not significantly different at $\alpha = 0.05$ level of significance. DAP=days after priming.

4.3.9 Correlation of seed germination, viability, and vigour parameters measured

The germination percentage (GP) was significantly ($p < 0.001$) and positively correlated with AA ($r = 0.53$), SL (0.58), RL($r = 0.57$), SLL($r = 0.59$), FW (0.72), TZ ($r = 0.43$), and DW ($r = 0.24$; $p < 0.05$). The negative correlation of GP with EC ($r = -0.24$) and SMC ($r = -0.13$) was not significant. The mean germination time (MGT) was significantly and ($p < 0.001$) negatively correlated with AA ($r = -0.53$), SL and SLL($r = -0.55$), RL and TZ ($r = -0.54$), FW ($r = -0.58$), EC ($r = -0.50$). The negative correlation of MGT with DW ($r = -0.14$) and SMC ($r = -0.17$) was insignificant. The AA was significantly ($p \leq 0.001$) and positively correlated with SL and

SLL($r=0.45$), RL ($r=0.45$), FW ($r=0.54$), TZ ($r=0.32$; $p<0.05$), but negatively correlated with EC ($r=-0.41$). The correlation of AA with SMC ($r=0.14$) was not significant. The shoot length was significantly ($0.05>p\leq 0.001$) and positively correlated with RL, SLL, FW, TZ, DW, SMC ($r=0.28$). The correlation between of SL with DW ($r=0.23$), and EC ($r=-0.22$) was not significant). The root length was significantly ($P<0.001$) and positively correlated with SLL ($r=0.99$), FW ($r=0.83$), TZ ($r=0.60$), SMC ($r=0.38$). The seedling length showed a significant ($p<0.001$) and positive correlation with FW ($r=0.85$), TZ (0.61), SMC ($r=0.35$; $p<0.01$). However, SL correlation with DW ($r=0.24$) and EC ($r=-0.19$) was insignificant. The fresh weight was significantly and positively correlated with DW($r=0.56$) and TZ ($r=0.46$). The DW correlations with TZ ($r=-0.15$), EC($r=0.18$), and SMC($r=0.21$) with were insignificant. TZ was negatively correlated with EC ($r=-0.23$) and positively correlated with SMC ($r=0.05$). However, the correlation of TZ with SMC and EC was insignificant. The correlation of EC with SMC was significant ($p<0.05$) and negative ($r=-0.30$) (Figure 4.9).

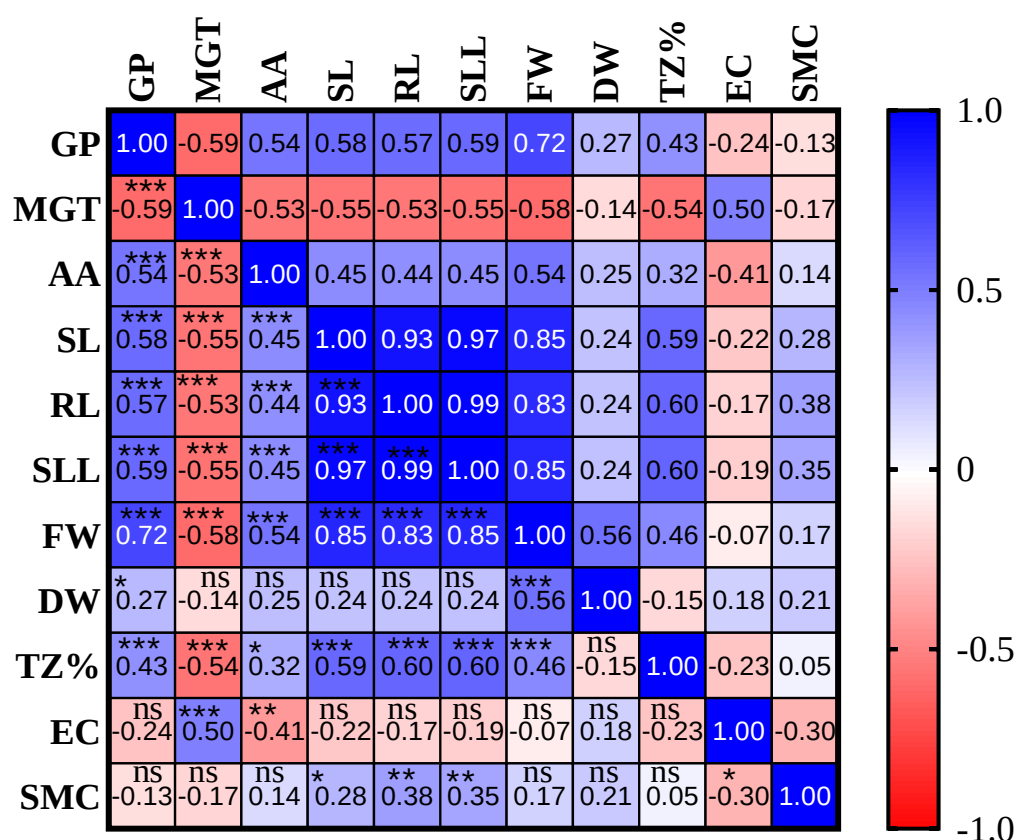


Figure 4.9 Pearson correlation coefficients (r) for seed germination and seedling growth parameters. *Significant at $p<0.05$; **Significant at $p<0.01$; ***Significant at $p<0.001$; ns, Not Significant. GP= final germination percentage; MGT=mean germination time; AA=seed vigour based on accelerated ageing test; SL=Shoot length; RL=root length; SLL=seedling

length; FW=fresh weight; DW=dry weight; TZ%=seed viability percentage based on tetrazolium test; EC=electrical conductivity of seed leachate, SMC=seed moisture content.

4.4 Discussion

The rapid loss of quality in primed seeds during storage is the main drawback to the adoption of seed priming (Wang et al., 2018). The present study investigated the storage potential of primed soybean seeds under uncontrolled conditions. This study revealed that quality traits of primed soybean seeds were affected significantly by the cultivar, priming method, and storage duration in days after priming (DAP). Cultivar PAN1521R showed better performance for all measured traits than the other two cultivars. The difference among cultivars can be attributed to genetic and chemical composition differences that regulate the expression of seed deterioration and vigour decline (Tatić et al., 2012). This study also shows that hydropriming significantly reduced FGP by 14 %, while osmopriming had no effect. Rouhi et al. (2011b) also reported that hydropriming had an adverse effect on soybean germination capacity. The results also show that the mean germination time was only improved through osmopriming (Figure 4.2). Sadeghi et al. (2011) reported similar findings. As expected, a decline in FGP and an increase in MGT with increased storage duration were evident. At the beginning of storage (0-7 DAP), FGP was 92 %, but it was 77 % at the end of storage (120 DAP). The response of primed seeds to storage duration also varied with the priming method. Osmoprimed seeds maintained their highest germination capacity (93 %) without any significant loss for up to 30 DAP. After that, the germination percentage declined. However, an instant decline in FGP just after 0 DAP was evident under hydropriming. By the end of the storage period (120 DAP), hydroprimed seeds had lost 42 % of germination capacity. A sharp increase in MGT after 60 days was also evident under hydroprimed seeds. This increase means that seed germination was delayed as DAP increased. Similar results were reported by Hussain et al. (2015) in rice. The authors established that the positive effects of priming could only be maintained for only 15 days. The decline in germination with increased storage duration under ambient conditions may be due to the aging phenomenon accompanied by food reserves depletion (Beedi et al., 2018). Osmopriming significantly reduced shoot length at 60 DAP. Hydroprimed seeds were able to maintain the highest root length for 7 DAP. Storing of hydroprimed seeds for 120 days significantly reduced root length. A similar observation was made for seedling length. Priming did not improve seedling length but reduced seedling length with increased storage duration. Priming had no positive effect on fresh seedling weight since the highest fresh weight was

recorded from unprimed seeds. Storing of osmoprimed seeds for 60 days significantly reduced fresh weight. Unprimed seeds maintained better performance throughout the storage, while osmopriming reduced seedling weight within 60 days. These results disagree with Abnavi and Ghobadi (2012), who reported that storing primed seeds for 60 days could improve germination, shoot, and radicle length.

An increase in storage duration also resulted in a decline in viability. The viability of hydroprimed seeds was significantly reduced with increased storage duration. These results support the findings of this study in germination test, in which germination percentage was reduced by hydropriming and 120 days storage. Osmoprimed seeds were able to maintain their highest viability for 30 DAP.

AA test results showed that seed vigour could be improved through osmopriming, but a decline in seed vigour became evident after 7 days of storage. These results agree with Farajollahi and Eisvand (2016), who reported that an 8-days delay in planting primed seeds stored at 25°C could significantly decrease the benefits of priming in wheat. Unprimed seeds recorded the highest EC values while priming significantly reduced the EC irrespective of a priming method. The increased electrolytes based on the EC test indicate membrane damage due to the loss of ability to quickly and thoroughly reorganize cellular membranes (Castañares and Bouzo, 2020; Mir-Mahmoodi et al., 2011). The lower electrolytes leakage for primed seeds may result from improved plasma membrane structure due to slow hydration in the priming treatments (Brar et al., 2020). This means priming triggered some membrane repair, thus reducing the EC. These results agree with Sadeghi et al. (2011), who reported that the highest EC was associated with unprimed seeds (control). Although priming significantly reduced the EC, a sharp increase was evident between 60 and 120 DAP. Beedi et al. (2018) also reported an increase in the EC with an increase in storage period in chickpea. These results suggest that storing primed seeds beyond 60 days may reverse the beneficial effects of priming with respect to electrolyte leachates. Unexpectedly, SMC was reduced with prolonged storage irrespective of priming method. A similar trend was also reported by Kandil et al. (2013). The authors reported the highest average moisture percentage before storage and the lowest moisture percentage after 12 months. According to Ali et al. (2018), a decline in SMC during storage may be due to the storage material which may have allowed moisture loss.

4.5 Conclusion

The loss of quality on primed seeds during storage is the main drawback of the priming technique. The findings of this study show that the response of primed soybean seeds varied with cultivar, priming method, and storage duration. Based on these findings, it is concluded that (i) hydroprimed and osmoprimed seeds can be stored for 0 and 30 days, respectively, without any significant germination and vigour loss, and (ii) increase in storage duration negatively affect the germination, viability, vigour, and seedling establishment of primed soybean seeds, regardless of a priming and cultivar treatment.

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CHAPTER 5 GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 General overview and discussion

Soybean farmers in South Africa and elsewhere globally often save their seeds for the next planting season due to financial constraints (Mahlangu et al., 2018). Retained seeds begin to lose viability and vigour when harvested, processed, or stored (El-Abady et al., 2012). Fluctuating air temperature, relative humidity, and storage period are critical factors affecting soybean seed quality during storage (Kandil et al., 2013; Pradhan et al., 2012). Low seed quality may lead to poor crop yields (Pradhan et al., 2012). Seed priming is the pre-sowing strategy that could be applied to improve seed quality after storage (Arif et al., 2008; Mielezrski et al., 2016). On the contrary, the loss of quality on primed seeds during storage is the main drawback of the priming technique (Wang et al., 2018). In this study, the application of priming on seed germination and vigour of stored soybean cultivars was investigated. The study also looked how long primed soybean seeds can be stored, especially under an uncontrolled environment.

A critical step in the study was to conduct a literature review (chapter 2). The review established that seed quality plays a critical role in the success of soybean production. It also established that the use of farm-saved was a common practice among soybean producing farmers. The quality of farm-saved seeds may be compromised during storage. It further revealed seed priming has the potential of improving seed quality. However, there was little information on the application of seed priming on germination of low vigour soybean cultivar. It was not clear how long primed soybean seeds could be stored with any significant loss in quality.

The study revealed that the effect of ageing, cultivar, priming and storage treatment on all measured variables (final germination percentage, mean germination time, germination index, coefficient of velocity of germination, shoot-, root-, and seedling-length, and seedling vigour index, was significant ($p < 0.001$) (chapter 3). These results agree with Park et al. (1999), who reported an improvement in the germination capacity of aged soybean through osmopriming. However, hydropriming had either negative or no effect on all measured traits of aged seeds. The priming effect was not significant on GI and CVG of aged seeds. Irrespective of priming treatment and cultivar, priming aged seeds also decreased RL and SLL.

The work done on storage duration and conditions revealed that the effect of cultivar, priming and storage treatment on all measured variables (final germination percentage, mean

germination time, seedling mass, seedling length, seed moisture content, and viability) was significant ($p < 0.001$) (chapter 4). Osmoprimed seeds maintained the highest final germination percentage for 0- 30 days of storage compared to hydroprimed seeds, which maintained the highest final germination percentage for 0-7 days. Hydroprimed seeds at the storage of 120 days recorded the lowest seed viability and seedling length compared to osmoprimed and unprimed seeds. The EC of primed seeds remained lower for most storage (0-90 days) than for unprimed seeds. These results agree with Sadeghi et al. (2011), who reported that the highest EC was associated with unprimed seeds (control). However, an increase in EC was evident after 60 days. As a result, the EC of all priming treatments was similar after 120 days of storage. These results also agreed with Beedi et al. (2018), who reported an increase in the EC with an increase in storage period in chickpea.

5.2 Conclusion

When comparing aged seeds and unaged seeds, aged seeds exhibited lower scores as expected. The response of aged seeds varied with priming method, cultivar depending on a measured parameter. Priming improved some parameters, while others were unaffected or adversely affected. The FGP, MGT, and SVI II were increased, while SL, FW, and SVI I was not affected by osmopriming. Hydropriming decreased both FGP and SVI I but did not affect MGT and SVI II. The RL and SLL were negatively affected regardless of the priming method, while GI and CVG remained unchanged. Based on the results of this study, it is concluded that (i) the germination capacity of low vigour soybean seeds can be improved through osmopriming, (ii) hydroprimed and osmoprimed seeds can be stored for 0 and 30 days, respectively, without any significant germination and vigour loss, and (iii) increase in storage duration negatively affect the germination, viability, vigour, and seedling establishment of primed soybean seeds, regardless of a priming and cultivar treatment.

5.3 Recommendations for future research

It is recommended that soybean farmers can use osmopriming as a method to improve the seed performance of low vigour soybean seeds. Prolonged storage of primed soybean is not recommended irrespective of priming method or cultivar type. However, it is essential to note that the findings of this study were under a controlled environment (laboratory), using only two priming agents and three soybean cultivars. In addition, storage duration was limited to 120 days. Therefore, there is a need for further research to, (i) assess the performance of primed soybean seeds under an uncontrolled environment (field), (ii) explore the potential of other priming agents on germination of aged soybean seeds, (iii) evaluate the effect of osmopriming

under different PEG rates and priming duration, and (iv) determine the storage potential of primed soybean over a long storage period.

5.4 References

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APPENDICES

Appendix 1 Analysis of variance tables for chapter 3

Variate: FGP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	16501.4	16501.4	121.65	<.001
Cultivar	2	12169.4	6084.7	44.86	<.001
Priming	2	5419.4	2709.7	19.98	<.001
Aging.Cultivar	2	10186.1	5093.1	37.55	<.001
Aging.Priming	2	1119.4	559.7	4.13	0.021
Cultivar.Priming	4	1222.2	305.6	2.25	0.075
Aging.Cultivar. Priming	4	2055.6	513.9	3.79	0.009
Residual	54	7325.0	135.6		
Total	71	55998.6			

Variate: GI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	336.7012	336.7012	599.47	<.001
Cultivar	2	98.0915	49.0458	87.32	<.001
Priming	2	13.0530	6.5265	11.62	<.001
Aging.Cultivar	2	33.1084	16.5542	29.47	<.001
Aging.Priming	2	2.7139	1.3569	2.42	0.099
Cultivar.Priming	4	3.7090	0.9273	1.65	0.175
Aging.Cultivar.Priming	4	38.4619	9.6155	17.12	<.001
Residual	54	30.3297	0.5617		
Total	71	556.1687			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	25.8455	25.8455	218.25	<.001
Cultivar	2	5.1009	2.5504	21.54	<.001
Priming	2	0.9182	0.4591	3.88	0.027
Aging.Cultivar	2	2.3395	1.1698	9.88	<.001
Aging.Priming	2	1.4127	0.7063	5.96	0.005
Cultivar.Priming	4	2.0787	0.5197	4.39	0.004
Aging.Cultivar.Priming	4	0.9788	0.2447	2.07	0.098
Residual	54	6.3948	0.1184		
Total	71	45.0690			

Variate: CVG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	2002418.	2002418.	141.89	<.001
Cultivar	2	456161.	228080.	16.16	<.001
Priming	2	16988.	8494.	0.60	0.551
Aging.Cultivar	2	41849.	20925.	1.48	0.236
Aging.Priming	2	181782.	90891.	6.44	0.003
Cultivar.Priming	4	323762.	80941.	5.74	<.001
Aging.Cultivar.Priming	4	354984.	88746.	6.29	<.001
Residual	54	762048.	14112.		
Total	71	4139992.			

Variate: Shoot_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	803.649	803.649	227.60	<.001
Cultivar	2	369.208	184.604	52.28	<.001
Priming	2	142.892	71.446	20.23	<.001
Aging.Cultivar	2	157.899	78.949	22.36	<.001
Aging.Priming	2	209.059	104.529	29.60	<.001
Cultivar.Priming	4	31.852	7.963	2.26	0.075
Aging.Cultivar.Priming	4	118.042	29.510	8.36	<.001
Residual	54	190.675	3.531		
Total	71	2023.275			

Variate: Root_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	3315.422	3315.422	1496.67	<.001
Cultivar	2	430.388	215.194	97.14	<.001
Priming	2	264.808	132.404	59.77	<.001
Aging.Cultivar	2	326.008	163.004	73.58	<.001
Aging.Priming	2	23.065	11.532	5.21	0.009
Cultivar.Priming	4	357.836	89.459	40.38	<.001
Aging.Cultivar.Priming	4	327.350	81.838	36.94	<.001
Residual	54	119.621	2.215		
Total	71	5164.498			

Variate: Seedling_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	7383.690	7383.690	913.57	<.001
Cultivar	2	1572.672	786.336	97.29	<.001
Priming	2	759.922	379.961	47.01	<.001
Aging.Cultivar	2	931.818	465.909	57.65	<.001
Aging.Priming	2	355.359	177.679	21.98	<.001
Cultivar.Priming	4	592.109	148.027	18.32	<.001
Aging.Cultivar.Priming	4	785.526	196.381	24.30	<.001
Residual	54	436.441	8.082		
Total	71	12817.537			

Variate: Fresh_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	2.38456	2.38456	150.95	<.001
Cultivar	2	2.08581	1.04290	66.02	<.001
Priming	2	1.02424	0.51212	32.42	<.001
Aging.Cultivar	2	0.68309	0.34155	21.62	<.001
Aging.Priming	2	0.10619	0.05309	3.36	0.042
Cultivar.Priming	4	0.10804	0.02701	1.71	0.161
Aging.Cultivar.Priming	4	0.49615	0.12404	7.85	<.001
Residual	54	0.85304	0.01580		
Total	71	7.74113			

Variate: Dry_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	0.0042936	0.0042936	9.66	0.003
Cultivar	2	0.0144181	0.0072091	16.22	<.001
Priming	2	0.0044093	0.0022047	4.96	0.011
Aging.Cultivar	2	0.0033514	0.0016757	3.77	0.029
Aging.Priming	2	0.0011195	0.0005598	1.26	0.292
Cultivar.Priming	4	0.0047168	0.0011792	2.65	0.043
Aging.Cultivar.Priming	4	0.0138279	0.0034570	7.78	<.001
Residual	54	0.0239937	0.0004443		
Total	71	0.0701303			

Variate: SVI_I

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	73737761.	73737761.	737.33	<.001
Cultivar	2	20929403.	10464701.	104.64	<.001
Priming	2	6997534.	3498767.	34.99	<.001
Aging.Cultivar	2	11160144.	5580072.	55.80	<.001
Aging.Priming	2	1786311.	893156.	8.93	<.001
Cultivar.Priming	4	7580800.	1895200.	18.95	<.001
Aging.Cultivar.Priming	4	7300500.	1825125.	18.25	<.001
Residual	54	5400352.	100007.		
Total	71	134892804.			

Variate: SVI_II

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Aging	1	331.145	331.145	97.25	<.001
Cultivar	2	311.347	155.674	45.72	<.001
Priming	2	141.831	70.915	20.83	<.001
Aging.Cultivar	2	215.400	107.700	31.63	<.001
Aging.Priming	2	8.828	4.414	1.30	0.282
Cultivar.Priming	4	30.262	7.565	2.22	0.079
Aging.Cultivar.Priming	4	110.751	27.688	8.13	<.001
Residual	54	183.880	3.405		
Total	71	1333.443			

Appendix 2 Analysis of variance tables for chapter 4

Variate: FGP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	7501.2	3750.6	23.02	<.001
Priming	2	7438.3	3719.1	22.82	<.001
Storage	5	5360.5	1072.1	6.58	<.001
Cultivar.Priming	4	709.9	177.5	1.09	0.366
Cultivar.Storage	10	1987.7	198.8	1.22	0.287
Priming.Storage	10	7272.8	727.3	4.46	<.001
Cultivar.Priming.Storage	20	4356.8	217.8	1.34	0.172
Residual	108	17600.0	163.0		
Total	161	52227.2			

Variate: MGT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	5.08918	2.54459	38.44	<.001
Priming	2	0.80822	0.40411	6.10	0.003
Storage	5	6.44825	1.28965	19.48	<.001
Cultivar.Priming	4	0.45936	0.11484	1.73	0.148
Cultivar.Storage	10	1.61031	0.16103	2.43	0.012
Priming.Storage	10	4.13568	0.41357	6.25	<.001
Cultivar.Priming.Storage	20	1.90228	0.09511	1.44	0.121
Residual	108	7.14929	0.06620		
Total	161	27.60258			

Variate: AA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	7560.5	3780.2	17.30	<.001
Priming	2	9890.1	4945.1	22.63	<.001
Storage	5	22101.2	4420.2	20.23	<.001
Cultivar.Priming	4	2450.6	612.7	2.80	0.029
Cultivar.Storage	10	49172.8	4917.3	22.50	<.001
Priming.Storage	10	5509.9	551.0	2.52	0.009
Cultivar.Priming.Storage	20	8927.2	446.4	2.04	0.010
Residual	108	23600.0	218.5		
Total	161	129212.3			

Variate: Shoot_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	175.659	87.830	39.37	<.001
Priming	2	251.545	125.773	56.38	<.001
Storage	5	2156.329	431.266	193.32	<.001
Cultivar.Priming	4	127.735	31.934	14.31	<.001
Cultivar.Storage	10	127.658	12.766	5.72	<.001
Priming.Storage	10	405.292	40.529	18.17	<.001
Cultivar.Priming.Storage	20	173.960	8.698	3.90	<.001
Residual	108	240.927	2.231		
Total	161	3659.105			

Variate: Root_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	346.341	173.171	39.25	<.001
Priming	2	795.933	397.967	90.21	<.001
Storage	5	7202.758	1440.552	326.54	<.001
Cultivar.Priming	4	366.914	91.728	20.79	<.001
Cultivar.Storage	10	160.270	16.027	3.63	<.001
Priming.Storage	10	613.937	61.394	13.92	<.001
Cultivar.Priming.Storage	20	340.420	17.021	3.86	<.001
Residual	108	476.452	4.412		
Total	161	10303.025			

Variate: Seedling_L

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	964.56	482.28	44.86	<.001
Priming	2	1917.90	958.95	89.21	<.001
Storage	5	17152.31	3430.46	319.12	<.001
Cultivar.Priming	4	856.28	214.07	19.91	<.001
Cultivar.Storage	10	426.53	42.65	3.97	<.001
Priming.Storage	10	1843.76	184.38	17.15	<.001
Cultivar.Priming.Storage	20	829.83	41.49	3.86	<.001
Residual	108	1160.97	10.75		
Total	161	25152.14			

Variate: Fresh_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	1.85219	0.92609	76.13	<.001
Priming	2	2.01251	1.00626	82.72	<.001
Storage	5	4.11398	0.82280	67.64	<.001
Cultivar.Priming	4	0.45024	0.11256	9.25	<.001
Cultivar.Storage	10	0.73420	0.07342	6.04	<.001
Priming.Storage	10	1.61497	0.16150	13.28	<.001
Cultivar.Priming.Storage	20	0.87832	0.04392	3.61	<.001
Residual	108	1.31384	0.01217		
Total	161	12.97025			

Variate: Dry_W

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	0.0281009	0.0140504	55.63	<.001
Priming	2	0.0066079	0.0033039	13.08	<.001
Storage	5	0.0138876	0.0027775	11.00	<.001
Cultivar.Priming	4	0.0043449	0.0010862	4.30	0.003
Cultivar.Storage	10	0.0075770	0.0007577	3.00	0.002
Priming.Storage	10	0.0049658	0.0004966	1.97	0.044
Cultivar.Priming.Storage	20	0.0133701	0.0006685	2.65	<.001
Residual	108	0.0272782	0.0002526		
Total	161	0.1061323			

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	624.46	312.23	17.15	<.001
Priming	2	1753.32	876.66	48.16	<.001
Storage	7	1094.79	156.40	8.59	<.001
Cultivar.Priming	4	110.09	27.52	1.51	0.202
Cultivar.Storage	14	344.64	24.62	1.35	0.184
Priming.Storage	14	1040.42	74.32	4.08	<.001
Cultivar.Priming.Storage	28	324.72	11.60	0.64	0.918
Residual	144	2621.33	18.20		
Total	215	7913.77			

Variate: SMC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	44.0253	22.0127	42.84	<.001
Priming	2	51.8765	25.9383	50.48	<.001
Storage	7	88.0723	12.5818	24.49	<.001
Cultivar.Priming	4	22.1872	5.5468	10.80	<.001
Cultivar.Storage	14	23.5919	1.6851	3.28	<.001
Priming.Storage	14	30.9370	2.2098	4.30	<.001
Cultivar.Priming.Storage	28	17.2254	0.6152	1.20	0.244
Residual	144	73.9885	0.5138		
Total	215	351.9043			

Variate: Viability_ %

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	2	58.33	29.17	0.68	0.510
Priming	2	919.44	459.72	10.68	<.001
Storage	7	9577.31	1368.19	31.78	<.001
Cultivar.Priming	4	422.22	105.56	2.45	0.049
Cultivar.Storage	14	593.52	42.39	0.98	0.472
Priming.Storage	14	1821.30	130.09	3.02	<.001
Cultivar.Priming.Storage	28	2703.70	96.56	2.24	0.001
Residual	144	6200.00	43.06		
Total	215	22295.83			