EVALUATION OF BEST PRACTICES FOR LOCAL CHICORY PRODUCTION

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The research work reported in this thesis was as a result of experiments carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, from May 2014 to September 2015, under the supervision of Prof. Isa Bertling and Dr Alfred O. Odindo (School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal; South Africa).

By submitting this thesis electronically, I hereby declare that the entirety of the research was as a result of my own investigations. It therefore represents my original work except where otherwise stated and due acknowledgments are accorded.

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DEDICATION

This thesis is dedicated to my late father Sibonelo Abednigo Manyoni and my Uncle Thula Enoch Makhathini.

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ABSTRACT

Chicory roots obtained from *Cichorium intybus* are commonly used to produce a caffeine-free coffee substitute. Although the crop has been produced in South Africa for many decades, the country still relies on imported chicory roots to meet its chicory needs, as satisfactory yields are often not achieved. The low yields are associated with the use of poor quality seed, which often results in poor crop establishment. In addition, there are limited options for weed control in chicory since only one herbicide is currently registered for use with chicory in South Africa. Chicory seeds vary in seed coat colour and research has indicated that seed coat colour maybe associated with seed quality of chicory. Results by various authors showed dark chicory seeds to have better performance than light coloured seeds however, contrary findings showing poor performance of dark coloured seeds compared to light coloured seeds have also been reported. There is a need to gain a deeper understanding of the possible association between seed coat colour variation and seed performance in chicory so as to come up with best management practises in order to obtain maximum crop establishment and optimum yields. The aim of the study was to evaluate the use of the image analysis in determining seed coat colour differences in chicory and to gain a deeper understanding of possible associations between seed coat colour variation and seed quality with respect to germination and vigour. In addition, the study assessed the effect of seed coat colour on germination, seedling growth and development of chicory in response to different priming solutions and durations. Lastly, a field experiment was conducted to identify the optimal planting density of chicory with respect to seed coat colour and weed management strategies. Seeds (cv. Orchies) were obtained from Nestle®, KwaZulu-Natal. In the first experiment (chapter three) seeds were separated visually into eight seed colours and then separated and assigned to a certain group using an image analysis system. This analysis system indicated that two colour categories could be separated with respect to hue. These groups were categorized as light and dark coloured seeds. Results also showed significant interactions ($P < 0.05$) between seed colour and seed quality test with respect to germination percentage and mean germination time. There were highly significant interactions ($P < 0.001$) between seed coat colour and seed quality test as detected by the germination velocity index (GVI) and imbibition time. Electrolyte leakage from the seeds was not significantly different ($P > 0.05$) between the seed colour groups. Results from chapter four showed osmo- and hydro-priming to improve seed quality of chicory through improvements in germination velocity index (GVI) and mean germination time (MGT). Osmo-priming resulted in relatively high improvements in seed quality compared with hydro-priming. Priming improved seedling establishment (mean emergence time (MET), seedling length, shoot length,

root length, fresh mass and, root/ shoot ratio). Results from the field trial showed the interaction of planting density, seed coat colour, and weeding method to be significant for total plot yield. This suggested that, no optimal crop stand exists with regards to weeding methods and seed coat colour. On the other hand, if the agronomic parameter of interest is biomass plot yield, the optimal plant density would be 200 000 plants ha^{-1}. Herbicide application tended to reduce agronomic performance of dark coloured seeds.

CHAPTER 1

GENERAL INTRODUCTION AND RESEARCH AIM

1.1 Background

Root chicory (*Cichorium intybus* L. var. *sativum*) is an erect perennial herb belonging to the Asteraceae family (Barcaccia et al. 2003). It is an important root crop cultivated in many parts of the world for the preparation of a coffee powder substitute due to its colour, taste and aroma (Bais and Ravishankar 2001; Muvamengwana 2004). In addition, roots are also used for the commercial production of inulin, a polysaccharide used as a sugar substitute (Silva 1996; Toneli et al. 2008). South Africa produces 8 500 tons of chicory roots, representing 2% of the total world production (459 848 tons) (DAFF 2017; FAOSTAT 2017). Agronomic practices in the country's production environments show, that for higher yields, a plant population of 150 000 plants ha^{-1} is required (Stapelberg and Coertze 1995); however, this is often not achieved due to poor seed quality (Corbinaeu and Côme 1990; Pimpini et al. 2002). In addition, weeds have an equally yield-reducing effect on plant growth and development (Everaarts 1993).

Seed quality is a broad term which includes seed viability and vigour (Korkmaz et al. 2004). Shaban (2013) defined viability as the ability of the embryo to germinate. Meanwhile, seed vigour is defined as those properties that determine the potential for rapid, uniform emergence and development of normal seedling under a wide range of field conditions (AOSA 1983; Korkmaz et al. 2004). Several factors affect seed quality, such as environmental conditions during seed development, harvesting and handling procedures as well as conditions in which the seeds are stored (Corbinaeu and Côme 1990; Pimpini et al. 2002). Generally, seeds of poor quality have erratic emergence and fail to establish uniform crop stands, consequently resulting in low productivity (Ghassemi-Golezani et al. 2010).

Research indicates that seed colour can be associated with seed quality (Powell 1989; Peñaloza et al. 2005; Atak et al. 2008; Atis et al. 2011). Seed colour in *C. intybus* varies from dark brown, straw-coloured and mottled to pale brownish (Minnar 1984). Variation in seed colour in chicory may be influenced by the position of the inflorescence (capitulum), environmental conditions at maturity, seasonal variation and genetic characteristics of the mother plant (Minnar 1984). Contradictory reports exists with regards to seed colour and germination capacity in chicory. Dark chicory seed were reported to perform better than light-coloured seeds with regards to

seed germination (Adlakha and Chhibber 1963; Minnaar 1984; Pimpini et al. 2002). On the other hand, findings by Corbineau and Côme (1990) demonstrated poor germination of black chicory seeds compared with light-coloured seeds. Seed colour has been found to influence the rate of imbibition (Chachalis and Smith 2000; Lui et al. 2007; Ertenkin and Kirdar 2010) and solute leakage (Pekşen et al. 2004; Mavi 2010; Atis et al. 2011). Research by Srimathi and Malarkodi (2002), Zhang et al. (2006), Hosamani et al. (2013) and Durga et al. (2014), suggested that seed colour might be associated with aging of seeds. A relationship between seed colour and seedling emergence was established by Zhang et al. (2013), Yuyama and Silva Filho (2003) and Zondi (2012). Despite these reports, very little information exists on the relationship between seed colour and seed quality in chicory. Several studies have established a close relationship between seed colour and field emergence in *Cichorium intybus* L. var. *foliosum* (witloof chicory), *Brassica juncea* L. (mustard) *Brassica napus* (sesame), and *Vigna subterrarea* (L.) Verdc (Bambara) (Kruistum et al. 1994; Charjan et al. 2012; Zhang et al. 2013; Mabhaudhi et al. 2013). Research further indicates that differences in seed colour maybe associated with variation in seedling growth (Yuyama and Silva Filho 2003; Atak 2008; Charjan et al. (2012); Karivarhadaraaju et al. 2001. Reports have also suggested that seed colour may be associated with crop growth and yield. This was supported by Mabhaudhi et al. (2013) on Bambara, Ikhajiagbe and Mensha (2012) on *Splenostylis stenocarpa* (African yam) and Talukdar (2011) on *Lathyrus sativus L* (grasspea).

Seed priming methods are commonly employed to enhance seed quality of existing seed lots. It is a pre-sowing treatment which involves the hydration of the seeds to a point where germination is initiated, although radical protrusion does not takes place, it improves germination and early seedling growth (Bradford et al. 2007; Mabhaudhi and Modi 2011). Moreover, seed priming also enhances seed tolerance to unfavourable environmental conditions (Ghobadi et al. 2012). Seed priming methods include soaking in water (hydro-priming), soaking in low water potential solutions (osmo-priming) (Sharma et al. 2014) and incorporation of plant growth regulators (hormonal-priming) (Ghobadi et al. 2012). Soaking in salt solution (halo-priming), treating seeds with low or high temperatures (thermo-priming), treatment of seeds with solid matrices (solid matrix priming) (Tzortzakis 2009) are other priming methods used. Soaking seeds in solutions containing limiting nutrients (nutrient priming), soaking seeds in natural or synthetic chemical compounds (chemical priming), fungicide seed soaking (priming with fungicides), pre-treatment of seeds with thiol compounds (redox-priming), nutrient priming and hydration of seeds using biological compounds (bio-priming) (Jisha et al. 2013) have also been reported as successful methods. Hydro priming, osmo priming and hormonal

priming have been reported to improve germination and seedling properties in chicory (Sambo et al. 2004; Tzortzakis 2009; Mohammand et al. 2010 Dehkordie et al. 2012). Sinefu et al. (2011) associated a positive response to seed priming with seed colour in bambara. Seed priming methods have been used successfully in many other crops and the possibility of establishing a relationship between seed colour and priming will generate useful information to promote crop establishment.

One management practice that is of utmost importance to enhance root chicory production, is effective weed management. In the event of weed infestation, chicory can be regarded as a poor competitor for resources, due to its slow growth habit, particularly during the first two months after planting (Stapelberg and Coertze 1995). Chicory is also reported to be susceptible to most herbicides, mainly because it belongs to the *Asteraceae* family of plants and is therefore, related to many weed species (Wilson et al. 2004). Additionally, chicory is considered both a root crop and a leaf vegetable, because the plant has a variety of uses herbicide registration for chicory is a challenging exercise (Wilson et al. 2004). Weed competition in chicory is minimized by applying pre-emergence herbicide, benfluralin (Balan®), which is currently the only registered herbicide in South Africa and/or by hand weeding and hand hoeing (Stapelberg and Coertze 1995). An integrated approach of herbicide and hand-weeding can provide more effective weed control in root crops such as chicory.

In South Africa, one of the biggest challenges in chicory production is that seeds are commonly of unknown quality and, therefore, produce variable plant stands (Minnar 1984). Based on previous findings, seed quality can be considered as one of the major factor for determining optimal plant stands in chicory. The role of seed colour on growth and yield may possibly suggest that subsequent crop growth and yield in chicory could be related to seed colour as well. To improve South African yield levels of chicory, the impact of low seed quality on chicory production highlights the need to further investigate the connection between seed colour and seed quality as well as the need to limited weed competition.

1.2 Research aim

The aim of the study was to lay a foundation for improving chicory management practices; in particular the relationship between seed colour and seed quality and their effect on management practices evaluated.

1.3 Research objectives

Low quality seed obtained by South African chicory farmers has resulted in poor crop establishment and low yields. Chicory seeds differ in colour and seed colour is one of various factors influencing seed quality. Thus, it is important to determine if differences in seed colour can be aligned with seed quality characteristics of chicory. Seed priming has been shown to improve seed performance in chicory and perhaps a positive response to priming is correlated with seed colour. In order to come up with the best management practices for chicory, there is a need to establish the potential effects of seed colour on the growth and development of chicory as well as to determine the best weeding practices for chicory, since only one herbicide is registered for the crop in South Africa. Therefore, the study considered most of the significant limitations to improving chicory production in South Africa, trying to establish recommendations for weed management by determining the effects of seed colour in various aspects of chicory production. In particular, the following areas were addressed:

- The possible associations between seed colour and seed performance with respect to imbibition, germination capacity and vigour.
- The response of chicory seeds varying in seed colour on seed priming methods and duration with respect to germination, seedling emergence, seedling growth and development.
- Assessment of the effects of seed colour and plant population on canopy characteristics, chicory biomass and biomass plot yield and root yield related parameters.
- The effects of two weed management methods on canopy characteristics, chicory biomass and biomass plot yield and root yield related parameters.

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CHAPTER 2
LITERATURE REVIEW

2.1 Chicory history and importance

Chicory cultivation can be traced back to the ancient Egyptians, who cultivated chicory as a medicinal plant, as coffee substitute, as a vegetable crop and occasionally for use as animal feed (Street et al. 2013). Root chicory is today predominantly grown for production of coffee powder in Belgium, India and South Africa. The roots are cut into slices, dried and roasted and powdered in a form of granules (final product). The coffee powder may be used on its own as a coffee substitute or mixed with coffee beans (Bais and Ravishankar 2001). Chicory roots can also be grown for commercial production of inulin, a polysaccharide composed of a chain of fructose molecules. Due to its low-calorie value, inulin is used mainly by food industries as a sugar substitute (Silva 1996; Toneli et al. 2008). Chicory has also been reported to possess several medicinal properties (Kocsis et al. 2003; Pushparaj et al. 2007; Muthusamy et al. 2008; Mulabagal et al. 2009). In South Africa, roots are not only roasted to make a coffee substitute, but also used to make tea to treat jaundice and for the preparation of chicory syrup, which is often used as a tonic and purifying medicine for infants (Street et al. 2013).

2.2 Botanical description

Chicory is an erect perennial herb, growing mainly vegetatively during the first year, when it produces a rosette of broad leaves and a fleshy tap root that can grow up to 75 cm deep (Hare et al. 1990; Bais and Ravishankar 2001). During the second year, the plant flowers and seed production takes place (Street et al. 2013). The plant has broad oblong, oblanceolate or lanceolate leaves, which are crowded at the base, forming a rosette of six to eight leaves arranged spirally on a stem. The stems are angled or grooved consisting of spreading branches (Druat et al. 2000; Bais and Ravishankar 2001). During the first year of development, three growth phases occur in chicory. The first phase is the juvenile phase, consisting of structural growth forming a long primary root during the first two months, followed by the mature phase, characterized by radial growth, resulting in the formation of the tuberized root. This tuberized root develops due to extensive synthesis and accumulation of fructans at two to two and a half months old with maximum root thickening when the plant is four to five months old. This is

followed by the senescence phase, characterized by a decline in shoot growth and the cessation of fructan accumulation and N-compound accumulation in the root tuber (Stapelberg and Coertze 1995; Améziane et al. 1997; Druart et al. 2000). Chicory roots contain a thin outer covering that is brownish-yellow enclosing the white storage root. The central part of the roots contain a portion of the xylem together with various vessels (Ali 2013). The dry matter of the root consists up to 75-80% fructans (Améziane et al. 1997). Once overwintered, the plant develops an inflorescence that carries florets consisting of blue, lavender and occasionally white flowers containing 15 to 25 single flowers (Póvoa et al. 2011). Harvesting of roots is usually carried out 20 to 28 weeks after planting (Wilson 2013). Seeds are harvested 60 days after the appearance of first flowers (Bais and Ravishankar 2001). Seeds may be dark brown, straw-coloured, mottled or pale in colour and are 2-3 mm long; the seed cannot be separated from the pericarp and, therefore the entire seed is an achene (Minnar 1984).

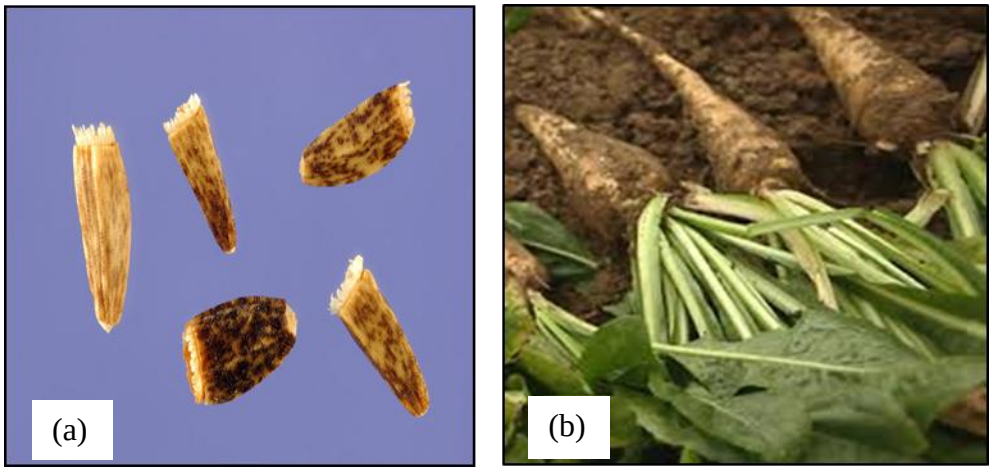

Figure 2.1: (a) Different seed colours in chicory (*http://www.plants.usda.gov*), (b) root chicory (*http://www.cargil.com*)

2.3 Agronomic requirements

Chicory requires a hot and humid climate and does well in calcareous, deep, fertile loam soils with a slight excess of clay (Stapelberg and Coertze 1995; Bais and Ravishankar 2001). Land preparation is crucial in chicory; seeds require a fine, level and firm seedbed (Schoeman 2010). The crop is usually planted using vegetable seed drills at shallow depth, not more than 1.5 cm due to its sensitivity to deeper planting. The crop can either be grown on ridges or flat rows (Stapelberg and Coertze 1995). A minimum temperature of 21℃ ensures successful germination while optimal temperatures between 18-24℃ are favourable for plant growth (Madani et al. 2004). Chicory requires a soil pH of 4.5-8.3 and its plant growth is greatly

influenced by soil pH, as this has an effect on nutrient availability (Bais and Ravishankar 2001; Street et al. 2013). The crop has a high potassium requirement, while root yield maybe improved by the application of manure (Bais and Ravishankar 2001). In order to transit from the vegetative to the reproductive stage, chicory requires chilling for about 90 days (Madani et al. 2004). In South Africa chicory can be planted during March to April, May to June, June to August and September to October, depending on the availability of moisture (NDA 2012). The recommended spacing is 60 cm to 80 cm between rows on no-ridged land; when planting on ridges the recommended spacing is not closer than 60 cm (Stapelberg and Coertze 1995). Thinning is carried out at four weeks after planting and a plant spacing of 20 to 25 cm is maintained between plants (Bais and Ravishankar 2001).

2.4 Worldwide production and cultivation of chicory in South Africa

Global production of chicory roots in 2013 was approximately 498 209 tons from a harvested area of 18 752 ha. The top five root chicory producing countries are Belgium (302 902 tons), France (51 100 tons), Netherlands (49 100 tons), Poland (24 120 tons) and South Africa (8 500 tons) (FAOSTAT 2017). In South Africa, the crop is grown on approximately 1 200 ha (Heraldlive 2016). Chicory is grown principally for its roots (Straatman et al. 1968). The crop was first cultivated commercially in 1985 in the Alexandria District of the Eastern Cape Province, South Africa (Orchard and Van Rooyen 1953; Young 1959; Straatman 1968). None of South Africa's chicory is exported; the locally produced chicory is only enough to supply the local coffee manufactures. This is despite the fact that South Africa is also one of the top five chicory consumers in the world (Kigozi 2003; Muvamengwana 2004). Nestlé, one of South Africa's leading manufacturer of coffee granules has since partnered with the South African government in an initiative to increase and extend chicory production in other provinces. The Province of KwaZulu-Natal has managed to establish a small scale chicory farming project, with the aim of empowering emerging local farmers in chicory production skills as means of income generation. According to the KwaZulu-Natal Department of Agriculture and Environmental Affairs, local chicory farmers were able to supply 449 tons of raw chicory to the Escourt factory which produced 90 tons of roasted chicory in the year 2010 (Baloyi 2011).

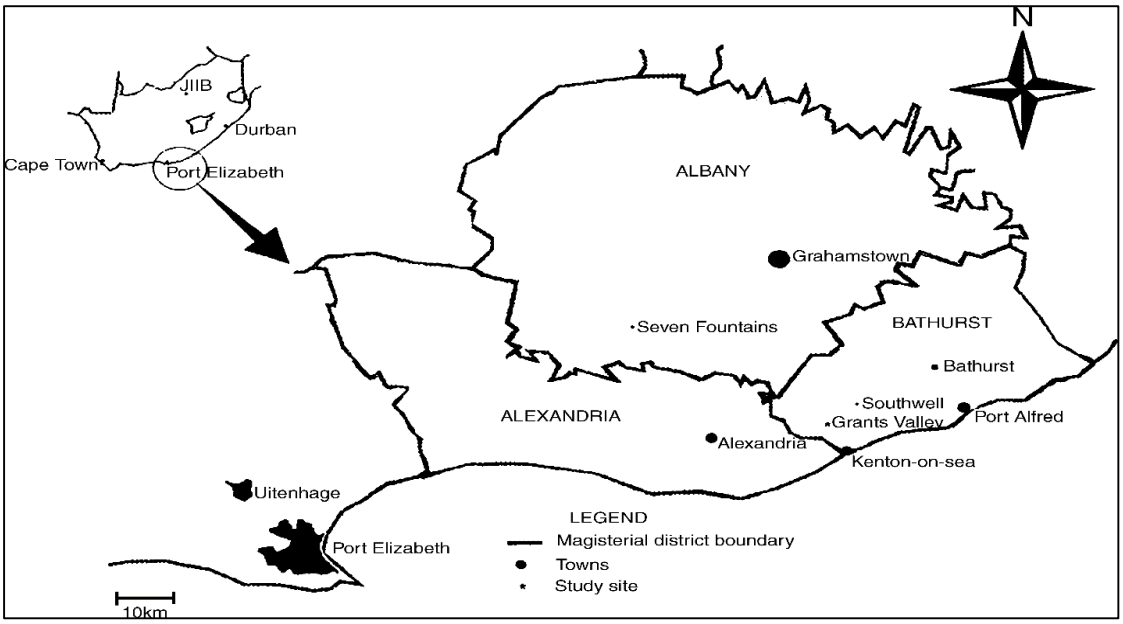

Figure 2.2: The three chicory growing districts in the Eastern Cape Province of South Africa are Alexandria, Albany and Bathurst (Kigozi 2003)

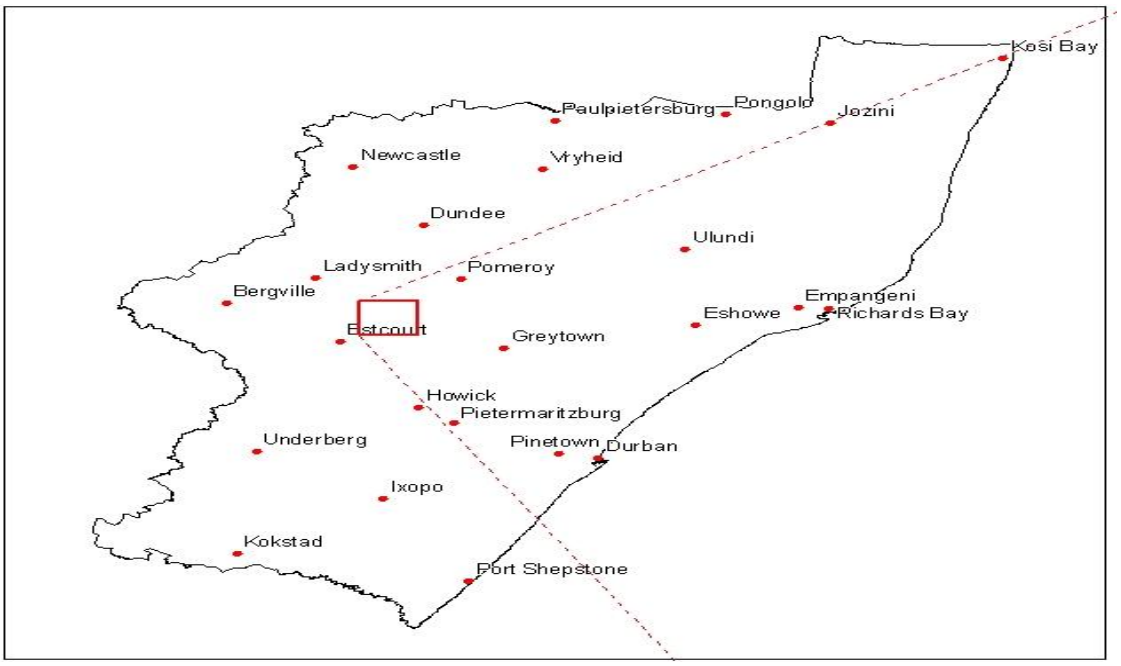

Figure 2.3: Chicory production area in the KwaZulu-Natal Province of South Africa: chicory production area (Mashele 2014, personal communication 2014)

2.5 Seed quality

2.5.1 Imbibition

Imbibition, the process of water uptake and swelling by the dry seed (Hershey 2010), is an essential step towards the initiation of biochemical changes leading to germination (Siddiqui and Khan 2010). During imbibition, the seed undergoes three main stages. Stage one is rapid water uptake, followed by the second stage, the plateau stage which, involves the initiation of nucleic acids and protein synthesis, thus preparing for the emergence of the radicle. The final stage of germination is the shortest stage in which the seed rapidly absorbs water resulting in cell enlargement as well as the elongation and emergence of the radicle (Sivritepe and Duorade 1995; Harb 2013). Among several important functions, the seed coat plays a role in the regulating of the imbibition process (Souza and Marcos Filho 2001). The seed coat provides protection to the seed by acting as a barrier to water penetration when excess water surrounds it or when the seed is exposed to imbibition stress. In soybean, McDonald et al. (1988) showed that, the seed coat assists in the tangential and radical movement in the embryo thus enabling the movement of water around the seed in such a way that both cotyledons are hydrated evenly. During imbibition, various substances such as amino acids, organic acids, sugars, phenolics, phosphate and potassium ions, gibberellic acid and proteins are leached out of the seeds (Ashraf and Nisar 1998). High water uptake causes imbibition damage, causing as high leakage of solutes from the embryo lead to the disruption of cell membranes (Powell and Matthew 1978). In addition to imbibition damage, high solute leakage provides an ideal medium for the development of pathogens on and around the seeds, leading to poor stand establishment (Nordin 1984; Perera and Cantliffe 1991; Tajbakhsh 2000). In species such as beans and peas (Rowland and Gusta 1977; Powell and Matthew 1978), *Glycine max* (soybeans) (Oliveira et al. 1984), *Brassica oleracea* (cauliflower) (McCormac and Keefe 1990) and *Solanum lycopersicum* (plum tomatoes) (Chachalis et al. 2008) imbibition damage was found to reduce germination and emergence, and increase the susceptibility of seeds to borne fungi. Therefore, such seeds have low viability and vigour (Woodstock 1988; Pekşen et al. 2004). Contrary to seeds with high water intake, seeds imbibing slowly are characterised by slight imbibition damage and have a high emergence (Matthew and Powell 2006).

Research suggests, that the rate of imbibition is closely associated with seed coat colour. In legumes (Powell 1989; Legese and Powell 1996), cowpea (Pekşen et al. 2004) and many other

crops (*Gleditsia triacanthos* (honey locust) (Ertekin and Kidar 2010), *Cyamapsis tetragonoloba* (guar) (Lui et al. 2007), *Brassica napus* (rapeseed) (Zhang et al. 2008), *Citrullus lanatus* (watermelon) (Mavi 2010) and *Trifolium pratense* (red clover) (Atis et al. 2011), seeds varying in seed coat colour were found to differ in their rate of water uptake. Pekşen et al. (2004) suggested, that seed coats of coloured, cowpea adhere close to the cotyledons, therefore, allowing slow water uptake while seed coats of white, cowpea allow rapid water uptake due to their loose seeds coats. In guar seeds, seed coat permeability was higher in black coloured seeds than in light coloured seeds (Lui et al. 2007). Permeable seed coats hold water between the seed coat and the cotyledons. Seeds with reduced permeability are found to have water only in the cotyledons (Powell 1989). Powell (1989) further established that pigmented pea seeds with genotype AA had slow water uptake compared with white seeds of the AA genotype which imbibed rapidly. He therefore, suggested the speed of water uptake to be heritable. Slow water uptake has also been correlated with seed pigmentation due to phenolic compounds in rape seed. Yellow-coloured rape seeds, with low melanin concentrations displayed higher water uptake than red or black coloured seeds which showed slow water uptake thus reducing solute leakage and imbibition damage (Zhang et al. 2008). Despite these findings, Singh et al. (1992) failed to establish a relationship between seed coat colour and imbibition in *Arachis hypogaea* (ground nut).

2.5.2 Electrical conductivity

Solutes leaked during imbibition are measured using the electrical conductivity test (Powell 1986; Khan et al. 2003) to identify seeds with high germination potential, which is aligned with the ability to leach a high number of solutes during imbibition therefore such seeds are not able to tolerate stressful conditions and are rated as low vigour seeds. On the other hand, vigorous seeds have a high germination percentage together with low solute leakage and are able to tolerate stressful conditions (Pekşen 2004). Rapid water uptake during early imbibition leads to the disruption of cellular membranes; however, cellular membranes of vigorous seeds are quickly re-established resulting in lower solute leakage than less vigorous seeds (Sørensen et al. 1996; Tajbakhsh 2000). Low quality seeds fail to re-establish membrane integrity consequently leaking more solutes. Solutes leaked from the seed act as electrolytes contributing to the electrical conductivity of water (Sørensen et al. 1996). According to Powell (1986), factors contributing to high solute leakage include the stage of maturity at harvest time, the degree of seed ageing as well as the incidence of imbibition damage. Data from previous

research on faba beans (Pekşen 2007), soybean (Khaliliaqdam et al. 2013), chickpea (Anuradha et al. 2009), horse gram (Singh et al. 2009), and pea seeds (Pekşen 2004) showed associations between seed coat colour and solute leakage during imbibition. Researchers have further reported the different relations between high solute leakage, reduced field emergence and seed coat colour in various plant species. For example, in an experiment conducted by Sinefu et al. (2011), white bambara seeds displayed higher solute leakage than red and brown seeds, which later corresponded with lower field emergence of white seeds. Pekşen et al. (2004) also reported high solute leakage and reduced field emergence in white-seeded cowpeas compared with coloured types. Similarly, yellow watermelon seeds were found to have higher solute leakage and a reduced emergence compared with brown and light-brown seeds (Mavi 2010). Uniform (dark grey) *Crotalaria juncea* L. (sunn hemp) seeds were reported by Pasculides and Ateca (2013) to have lower solute leakage and a higher emergence rate than variegated (brown spotted) seeds. However, no association between high solute leakage, reduced field emergence and seed coat colour was found in red and white common bean seeds (Borji et al. 2007).

2.5.3 Germination

Germination determines the potential of seedling emergence and establishment (Tylor et al. 1993), hence ensuring successful crop production (Atak et al. 2008). Seed coat colour has also been reported to play a role in germination. Dark chicory seeds were reported by Pimpini et al. (2002) to have faster and higher uniformity of germination than light-coloured seeds. Minnar (1989) found a lower germination percentage in white coloured chicory seeds compared with dark-coloured seeds. On the contrary, findings by Corbineau and Côme (1990) showed poor germination in black chicory seeds compared with light-coloured seeds. Several authors have also reported better germination in dark seeds in other species. For example, Lui et al. (2007) observed in guar, Agneiszka and Hołubowicz (2008) in *Viola tricolor* ssp. hortensis, Mavi (2010) in watermelon and Zhang et al. (2013) in canola seeds a higher germination percentage of dark seeds compared with any other seed colour. Furthermore, higher germination was observed in yellow honey locust seeds than dark seeds (Ertekin and Kidar 2010). In cowpea, Sinefu et al. (2011) found higher germination in brown and red cowpea seeds than in white seeds. In addition, light brown and reddish *Atriplex cordobensis* seeds were reported by Aiazzi et al. (2006) to have a higher germination percentage than dark brown-seeds. The variation in the germination capacity of seeds varying is seed colour has been associated with differences in seed maturity at harvest. Corbineau and Côme (1990) reported that black endive seeds,

contained a high percentage of incompletely developed embryos, while light-coloured contained well-developed embryos. Immature and non-viable embryos were found by Aiazzi et al. (2006) in dark brown *Atriplex cordobensis* seeds, while a high percentage of mature embryos were found in light-brown and reddish seeds. In pansy seeds, Agneiszka and Hołubowicz (2008) reported light-coloured seeds to have small embryos and some had none at all. According to Zhang et al. (2013) in canola, seeds from the main stem become mature earlier than those on branches and seeds from the bottom of basal stem position become mature earlier than those of the apical stem position consequently resulting in seed of different maturity stages and also seed coat colours.

2.5.4 Seed aging

The seed ages after reaching physiological maturity and continues aging during storage; this aging results in the loss of viability and vigour due to changes in biochemical and physiological procecess (Ghassemi-Golezani et al. 2010). Genetic constitution, storage conditions and seed physiological status determine the rate at which seeds age (Al-Maskri et al. 2003). The major cause of aging is lipid peroxidation (Murthy et al. 2003; Tian et al. 2008; Balešević-Tubić et al. 2011; Lubudda 2013), a process in which oxidants, such as free radicals attack lipids containing carbon-carbon double bonds (Ayala et al. 2014). Apart from lipid peroxidation, seed aging influences enzyme inactivation, protein degradation, cellular membrane disruption, as well as damages to nucleic acid integrity (Khan et al. 2003; Al-Maskri et al. 2003; Murthy et al. 2003).

Studies have evaluated the influence of seed coat colour on seed storability, for example Singh et al. (2009) and Durga et al. (2014) found improved storability in light-coloured horse gram seeds compared with dark-coloured seeds. Seed coat colour was reported to affect storability in soybean (Zhang et al. 2006) and *Vigna umbellata* (rice bean) (Srimathi and Malarkodi 2002). Zhang et al. (2006) suggested that the pigmentions of black soybean seeds might play a role in protecting seeds against aging. The accelerated aging test is used as one of the assessments to determine the potential seed storage (Balešević-Tubić et al. 2011) due to its ability to induce lipid peroxidation (Sung and Jeng, 2006) through seed exposure to high temperatures and high relative humidity (Santos et al., 2011). When submitted to the accelerated aging test, results showed that yellow and purple-coloured maize seeds had a higher germination percentage and higher vigour compared with orange, white and light orange seeds; this was associated with

higher amount of antioxidants found in yellow and purple seeds (Selvi et al. 2014). Accelerated aging of pea seeds leads to a decreased germination percentage in light and medium-coloured seeds compared with dark-coloured seeds (Atak et al. 2008). In addition, exposure of un-pigmented cowpea seeds to accelerated aging was reported by Asiedu et al. (2000) to result in higher deterioration through reduced germination and sensitivity to imbibition damage than in pigmented seeds. This was linked to chemical compounds present in pigmented seeds. Kharlukhi (2013) found colour not be aligned with storability following the the accelerated aging test in purple and yellow-coloured maize seeds.

2.5.5 Seedling emergence

According to Arnold et al. (1990) seedling emergence is the first appearance of a fully expanded leaf on the soil surface. Forcella et al. (2000) described seedling emergence as an important phenological event influencing the success of an annual plant. Successful seedling emergence is influenced by seed quality, which later affects plant stand establishment and yield potential (Rajal 2011). Seed of poor quality emerge erratically and fail to establish under field conditions, consequently resulting in low productivity (Ghassemi-Golezani et al. 2010). High quality seeds emerge rapidly and uniformly, resulting in enhanced seedling emergence and the production of vigorous plants, thus achieving optimal plant stands under various environmental conditions (Eskandari 2012). Several researchers have reported a strong influence of seed quality on seedling emergence in *Gossypium hirsutum* (cotton) (Wheeler et al. 1997; Ghassemi-Golezani et al. 2010), *Hordeum vulgare* (barley) (Rajal 2011) and *Glycine max* (soybean) (Diniz et al. 2013).

Pedersen and Toy (2001) also reported higher seedling emergence in red sorghum. Black canola seeds were found to have higher emergence percentage than dark-and light-brown seeds (Zhang et al. 2013). Charjan et al. (2012) made similar observations on the superiority of black seeds with regards to seedling emergence in black *Brassica nigra* L. (mustard) seeds and Omokanye (1996) described similar results in black horse gram seeds. Saeidi & Rowland (1999) reported that brown *Linum usitatissimum* (flax) seeds had higher field emergence than yellow seeds. Additionally, differences in seedling vigour has been reported for different coloured horse gram (Singh et al. 2009) as well for *Emblica officinalis* L. (Indian gooseberry) seeds (Karivarhadaraaju et al. 2001).

2.6 Seed priming

To enhance different aspects of germination and seed vigour, seed priming methods have been established for use in many horticultural crops (Farooq et al. 2007; Khan et al. 2011). Seed priming enhances germination and seedling growth through the process of hydrating and dehydrating seeds (Modi, 2005). Primed, seeds have completed the first two phases of imbibition and once rehydrated for sowing they immediately enter stage III, radicle emergence and seedling growth. The shortening of the time required for imbibition enhances seed performance by mobilizing storage proteins and activating or resynthesizing certain enzymes (Jisha et al. 2013; Sharma et al. 2014). Priming also induces other biochemical changes, like DNA replication, enhanced protein and RNA synthesis and reduced metabolite leakage (Hussain et al. 2013).

Several researchers have successfully primed chicory, for instance; Sambo et al. (2004) reported a higher germination percentage in response to hydro-priming. Osmo-priming improved chicory germination percentage, germination index and increased seedling length (Derkodhi et al. 2012). Osmo-priming experiments resulted in higher germination (Sambo et al., 2004) and in rapid germination and emergence (Tzortazakis 2009). In addition, Tzortazakis (2009) found hormonal priming to affect germination and emergence positively. Research by Mohammand et al. (2010) found improved germination and seedling properties in response to hormonal-priming.

Seed variation due to seed coat colour has been reported to affect germination (Minnar 1989; Corbineau and Côme 1990; Pimpini et al. 2002) and emergence (Kristum et al. 1994) in chicory. A study done by Sinefu et al. (2011) showed that white bambara seeds responded better to halo-priming and, therefore, displayed higher germination and emergence under water stress conditions compared with red and brown seeds. When comparing white and dark-red maize landraces following hydro-priming, Mabhaudhi (2009) found brown seeds to respond better than white seeds with regards to germination, whereas white seeds performed better than dark-red seeds with regard to seedling vigour traits; however, information describing whether variation in seed coat colour affects seed response to priming is still limited.

2.7 Crop growth

Hunt (2003) defined growth as an irreversible change in size, form and number with time. Suk et al. (2011) on the other hand described growth as a quantitative change of the plant involving an irreversible increase in size of tissues, organs and mass. Growth analysis allows to understand how processes such as photosynthesis contribute to growth and yield components (Lolliffe et al. 1982). In chicory, growth is generally measured by number of leaves, plant height, leaf area index, root weight, yield and biomass (Zafarbakhsh et al. 2011; Panahandeh et al. 2012; Madani 2012).

2.7.1 Leaf number

Leaves are important plant organs because they capture sunlight to produce carbohydrate during the process of photosynthesis. Carbohydrates produced during photosynthesis supply the plant with dry matter which is later allocated to vegetative and reproductive parts of the plants. Photosynthesis depends on the absorption of light by pigments in the leaves and on the acquisition of light by the plant is affected by leaf number. A low leaf number reduces light absorption and biomass production thus, have a negative influence on yield (Xiao et al. 2004).

The number of leaves per plant is affected by seed colour. According to Talukdar (2011) leaf numbers of whitish-yellow seed of *Lathyrus sativus* (grass pea) plants were higher than those of black seeds. Ikhajiagbe and Mensah (2012) on the other hand found no influence of seed colour on leaf number per plant grown from black, brown and light grey *Sphenostylis stenocarpa* (African yam bean) seeds. Under water stress conditions the lowest leaf number was reported on red bambara landraces compared with light-brown and brown landraces (Mabhaudhi et al. 2013).

Plant population also affect leaf numbers in chicory production. Lower plant populations tend to have a higher leaf number than higher plant populations. A report by Asghari et al. (2009) showed that plant population of 60 000 plants ha^{-1} produced the highest leaf number in chicory compared with plant stands of 90 000 and 120 000 plants ha^{-1}. This increase in leaf numbers was attributed to increased shoot production due to increased light absorption, compensating for low plant stands. Leaf numbers per plant were to reported to increase in low plant populations of root crops such as *Raphanus sativus* (radish) (Lavanya *et al*. 2004; Eldusuki et al. 2005), *Daucus carota* (carrot) (Dawuda et al. 2011; Kabir et al. 2013) and *Beta vulgaris* (sugar beet) (Khaiti, 2012). Contrary to that, higher populations of witloof chicory were found

by Zafarbakhsh et al. (2011) to produce the highest total number of leaves at an intra-row spacing of 20 cm compared with 25 and 30 cm spacing.

2.7.2 Plant height

Plant height is described by Pérez-Harguindeguy et al. (2013) as the distance between the upper boundary of the main photosynthetic tissue on a plant and the ground. Plant height plays an important role in determining light interception. Taller plants are reported to be more competitive for light interception than shorter plants (Yan et al. 2012).

Talukdar (2011) reported that plant height was also associated with seed colour in grass pea mutants. Plants of whitish-yellow seeds were taller than plants of black seeds. Plants produced from black African yam bean seeds were reported by Ikhajiagbe and Mensah (2012) to be taller than plants from brown and light grey seeds. A reduction in plant height of red bambara landraces compared with light-brown and brown seeds under water stress conditions was also reported by Mabhaudhi et al. (2013). A taller height of plants from cream bambara seeds compared with plants from red and black seed was observed by Onwubiko et al. (2011). Yao et al. (2010) found taller plants from brown *Chenopodium album* (lamb's quarters) seeds compared with those from black seeds under saline conditions. Taller plants in black *Phaselous vulgaris* (dry bean) seeds were also reported by Guimarães et al. (1989) compared with plants produced from beige seeds.

Plant population also has an effect on chicory plant height. Madani et al. (2012) observed differences of plant height in chicory stands of 160 000, 320 000 and 480 000 plants ha^{-1}. Seghatoleslami et al. (2014) found reduced plant height in high chicory stands (125 000 plants ha^{-1}) compared with low plant stands (50 000 plants ha^{-1}). Competition for nutrients and other resources was recognized as the main factor for reduced plant height. Different observations have been reported in carrot; Dawuda et al. (2011) observed no influence of plant population on plant height whereas, Bezerra Neto et al. (2005) and Kabir et al. (2013) found taller plants in high carrot stands. Taller plants in high carrot stands were associated with sufficient space for vegetative growth and less competition for nutrients (Kabir et al. 2013). An increase in plant height was reported by El-Dusuki et al. (2005), Bilekudari et al. (2005) and Lavanya et al. (2014) in higher compared with lower plant population of radish. The increased plant height in radish was attributed to limited space for lateral growth (El-Dusuki et al. 2005; Lavanya et al. 2014).

2.7.3 Leaf Area Index

Aboelghar (2011) defined leaf area index as the total one-sided leaf area per unit ground area. Processes within and below the canopy's microclimate, canopy water interception, loss of light energy, water and carbon exchange are determined by leaf area index (Bréda 2003). In crop growth, leaf area index is used as a reference tool for estimating photosynthesis capacity of a crops (Lan et al. 2009) and is critical for understanding roles of many crop management practices (Campillo et al. 2010). A high leaf area index is driven by the rate at which new leaves appear and enlarge, as well as the period over which they are retained by the plant (Lemaire et al. 2008). The capture of light energy within the canopy and dry matter production increases by increasing leaf area index, therefore, a low leaf area index reduces dry matter production, ultimately decreasing yield (Dalirie et al. 2010).

Mabhaudhi et al. (2013) failed to establish a relationship between seed colour and leaf area index in red, light-brown and brown bambara seeds. Ikhajiagbe and Mensah (2012) reported differences in leaflet area index in black, brown and light grey African yam seeds. Plants produced from brown seeds had the highest leaflet area and light-grey seeds produced the lowest leaflet area.

Plant population has an important effect on vegetative growth of plants hence, the leaf area index. A high leaf area index with increasing plant stands has been reported in several crop species. For instance, this was shown in sugar beet (Hussain and Field 1991; Pospišil et al. 1999; Çakmaski and Oral 2002), *Colocasia esculenta* (taro) (Tumuhimbise et al. 2009), *Manihot esculenta* (cassava) (Streck et al. 2014), *Vigna unguiculata* (cowpea) (da Lacerda et al. 2011; Soratto et al. 2012) and *Zea mays* (maize) (Remison and Lucas 1982; Hassan et al. 2007; Lui et al. 2010). However, increasing the plant population from 125 000 to 500 000 plants ha^{-1} in chicory did not affect leaf area index (Panahandeh et al. 2012).

2.7.4 Yield parameters and yield

Studies have shown that there is a relationship between seed colour and mass of harvested plant organs in several crops. For example, Onwubiko et al. (2011) reported higher mass of fresh pods in cream than in red and black bambara seeds. Research by Sabatino et al. (2014) reported the highest fruit mass in fruit produced from light brown *Lagenaria siceraria* (sicilian bottle

gourd) than from grey white and brown seeds. In black seeded cowpeas, higher pod mass was found compared with white seeded plants (Peksen 2004).

Varying plant stands affect storage root mass in chicory. Several authors have reported a decreased root mass and size, with increasing plant stand density. For instance, the highest root mass was recorded in the lowest stand of 125 000 plants ha^{-1} compared with 250 000 and 500 000 plants ha^{-1} (Panahandeh et al. 2013). Madani et al. (2012) found heavier roots at 160 000 at 320 000 and 480 000 plants ha^{-1}. Findings by Zafarbakhsh et al. (2011) showed the highest root mass at an intra-row spacing of 30 cm compared to 25 cm and 20 cm. Similar findings have been reported in sugar beet (Ali 2004; Hozayn et al. 2013), and carrot (Kabir et al. 2013). In sugar beet, Hozayn et al. (2013) explained that low plant stand density allow optimum use of soil and other resources due to less interplant competition. Ideal chicory roots should weigh 250 to 400 g, as smaller roots may not be accepted by the processor.

Good quality seeds are known to improve crop yields by producing vigorous plants and optimal plant stands under various environmental conditions. Several studies have established a relationship between seed colour and crop yield. For instance, black African yam bean seeds were found to produce higher grain yield than light grey and brown seeds (Ikhajiagbe and Mensah 2012). Guimarães et al. (1989) reported dry bean yields to be affected by seed coat colour, with light beige seed producing higher yields than beige seeds. Seed coat colour was also associated with yield per plant in *Sesamum indicum* (sesame) seeds (Khidir and El Gizouli Osman 1970). Findings by Yao et al. (2010) showed that brown *Chenopodium album* (lamb's quarters) seeds produced higher total seed output than black seeds grown under various saline concentrations. Very small effects of seed coat colour in chickpea yields were observed by Knights and Mailer (1989). Bambara landraces differing in seed colour produced under different water regimes were found not to differ in yield (Mabhaudhi et al. 2013).

Space occupied by the roots in the soil affects root growth, which, in turn, influences yield in crops. Therefore, plant population critically influences root size and yield of root crops. According to Meijer and Mathijssen (2014) plant stands are among important factors affecting variation of root yield in chicory. Research by Madani et al. (2012) on chicory reported that the lower plant stand (160 000 plants ha^{-1}) produced higher yields than closer plant stands (320 000 and 480 000 plants ha^{-1}). Meanwhile, Panahande et al. (2012) found no differences in yield between plant stands of 500 000, 250 000, 166 700 and 125 000 plants ha^{-1}. In other

root crops, such as radish (El-Dusuki et al. 2005; Lavanya et al. 2014), carrot (Evers et al. 1997; Dawuda et al. 2011) and sugar beet (Hozayn et al. 2013), higher plant stand have been shown to increase yield. Higher yields due to increased plant stands were associated with production of more plants per unit area in radish (Lavanya et al. 2014) and carrot (Dawuda et al. 2011). In radish, El-Dusuki et al. (2005) linked higher yields to higher ground cover by leaves which resulted in higher light interception and consequently, in higher assimilate production. Low plant stands were reported by Anjum et al. (2004) to produce higher yields compared with higher plant stands in sugar beet stands, and this was associated with greater tuber size in lower plant stands due to the availability of space for root growth.

2.8 Effect of weed infestation on chicory

Weed infestation is a major problem in chicory, particularly in high rainfall years resulting in reduced chicory yields (Stapelberg and Coertze 1995). Schnieders (1999) found reduced chicory root size, increased percentage of roots with small diameters and reduced economic value due to weed competition. Furthermore, the efficacy of root harvesters in chicory fields is negatively affected by poor weed control (Mersie and Elliott 1993) as weeds present generally block the root lifters.

2.8.1 Weed control in chicory

2.8.1.1 Manual and mechanical weed control

In chicory, weed control is either carried out through hand-weeding, by physical pulling of weeds, or by hand-hoeing. This first control is applied immediately after crop emergence. Inter-row cultivation (normally achieved after hand hoeing) offers effective weed control during vigorous crop growth; hence, selecting wide inter-row spacing is of great importance to ensure satisfactory weed control. Shallow cultivation of newly germinated weeds is important to prevent injury to chicory roots and commences when leaves are less susceptible to damage from tractor wheels. Effective weed control involves cultivating shortly after weed emergence with the frequency of cultivation determined by the level of weed infestation (Stapelberg and Coertze 1995). Hand-weeding is essential however; it reduces the profitability of the crop due the rise of crop production costs associated with manual labour (Ghanizadeh et al. 2011; Rahman et al. 2011).

2.8.1.2 Chemical weed control

Pre-plant soil incorporation of benfluralin and pronamide plus asulam and pre-emergence carbetamide have been successfully used for early season weed control in Belgium and France (Baert and Van Bockstaele 1993; Wilson et al. 2004). These two countries are the world's leading producers of chicory. Chlorpropham is also recommended for weed control in the leafy crop *Cichorium endivia* in Europe (Mersie and Elliot 1993).

In 'Grasslands Puna' chicory, thistles and broad-leaf weeds are controlled with the application of pre-plant soil incorporation of trifluralin and eradicane super (EPTC). Chlorpropham and propyzamide are applied as pre-emergence herbicides. Post-emergence herbicides bentazon and metribuzin are usually applied for broadleaf weed control two to three months after planting while carbetamide, haloxyfop, propyzamide and fluazifop-P-butyl control grass weeds. In mature 'Grasslands Puna' chicory crops (three to four months after crop establishment) atrazine, paraquat and asulam are applied for controlling a wide range of both grass and broadleaf weeds (Hare et al. 1990).

Herbicides registered for weed control for chicory in Europe have been evaluated in the United States of America for potential registration under local US conditions. Mersie and Elliot (1993) reported that combining pronamide in with sethoxydim or applying it alone improved weed control compared with trifluralin. The authors further reported that grasses and broadleaf weeds can be controlled for the entire season by applying of pronamide alone. Out of the three pre-plant incorporated and two post-emergence herbicides, pronamide pre-plant incorporated plus benfluralin or trifluralin were most effective in controlling weed populations, while in the second experiment the other herbicides, pre-plant incorporated trifluralin followed by post-emergence application of imazamox, provided better weed control (Wilson et al. 2004).

2.8.1.3 Benefin use in chicory

Benefin is currently the only one registered herbicide (soil applied pre-emergent) for chicory weed control in South Africa and is applied at 6-8 L ha^{-1} (van Zyl 2012). Benefin is used in several agronomic and horticultural crops for controlling annual grass and broadleaf weed species (Golab et al. 1970). It is, however, the only registered herbicide for chicory use in South Africa (van Zyl 2012). Benefin prevents root and shoot development in germinating weed seeds (Reicher 1988; Tickes and Kerns 1996). The herbicide inhibits cell division through binding to

tubulin thus preventing polymerization of microtubules at the growing end of the tubule (Murphy 1996). Growers in the KwaZulu-Natal chicory production area commonly apply benefin as a pre-emergence herbicide and incorporate paraquat, a non-selective herbicide for post-emergence weed control. Shielded sprayers are used to apply paraquat to avoid crop damage, while delivering effective weed control (Gordon 2011).

Table 2.1: Benefin nomenclature and classification (Murphy 1996; Weber 1900)

Common name	Trade name	Chemical name	Herbicide family
Benefin/benfluralin	Balan®	*N*-butyl-*N*-ethyl-2,6-dinitro-4(trifluoromethly)benzenamine	Dinitroaniline

2.9 Conclusion

Chicory is an important root crop cultivated for the preparation of a coffee powder substitute; however, chicory seeds do not germinate and emerge as expected, often resulting in poor plant stands and low yields. The crop is cultivated from seeds varying in seed coat colour. Research has indicated that seed colour is associated with seed quality. A review of literature showed that seed quality studies in chicory require broader investigation. The response of seeds varying in seed coat colour to priming is not well understood and requires investigation to mitigate the effects of low seed quality. Presently, no attempts have been made to examine the impact of seed coat colour on chicory production under field conditions, especially under local environments. Chemical weed control of chicory is limited and investigations into how different weed management methods affect the crop productivity has not yet carried out.

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CHAPTER 3

EFFECT OF SEED COLOUR VARIATION ON CHICORY (*CICHORIUM INTYBUS* L.) SEED QUALITY WITH RESPECT TO GERMINATION AND VIGOUR

3.1 Introduction

Chicory (*Cichorium intybus* L.) is a perennial tap-rooted herb. In South Africa roasted chicory roots are ground and then blended with coffee seeds for the preparation of coffee powder (Bais and Ravishankar 2001, Mavumengwana 2004). Chicory has been commercially cultivated in South Africa since 1985 at Alexandria, Albany and Bathurst in the Eastern Cape Province (Orchard and Van Rooyen 1953; Young 1959; Straatman 1968). South Africa's chicory producers need to increase production to meet the demand as possible (Schoeman 2010). The leading producers of chicory are Belgium (300 902 tons), followed by France (51 100 tons), the Netherlands (49 100), Poland (24 120) while South Africa is ranked 5th, producing 8 500 tons (FAO 2017). Due to low yields, there is still no exportation of chicory from the country (Kigozi 2003). As in most field crops, successful production of chicory relies on the availability of high quality seed. Chicory farmers in South Africa rely on imported seed, which is often of poor quality (Schoeman 2010). No local seed is available because, although chicory growing environments are suitable for the growth of chicory; they are not suitable for seed production due winter temperatures which are too mild to stimulate seed production (Minnar 1984). Therefore, the availability of low quality seed is one of the obstacles to chicory production, often resulting in poor crop establishment and low yields (Corbinaeu and Côme 1990; Pimpini et al. 2002).

Seed quality is a multifaceted concept comprising different aspects which include germination, vigour, genetic and physical purity as well as seed health (Brasa 2005). To a seed physiologist, germination refers to "emergence of the radicle through the seed coat" and to a seed analyst, it refers to "emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions" (Copeland and McDonald 2001). Seed vigour is defined as "the total sum of those properties of seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence" (Hampton and TeKrony

1995). The electrical conductivity and accelerated aging tests are among several seed vigour tests used to obtain further information about a certain seed-lot.

Chicory seed colour is dark-brown upon seed maturity (Reaume 2010); however, different types of seed colour exist within a single batch of seeds. Different seed colours within a cultivar are associated with the harvesting of seeds at different developmental stages and some genetic differences (Atis et al. 2011; Zhang et al. 2013). Investigations by several researchers have suggested that seed colour can be associated with seed quality. As such, the rate of water uptake by *Pisum sativum* (Powel 1989; Chachalis and Smith 2000), *Gleditsia triacanthos* (Ertenkin and Kirdar 2010) and *Cyamopsis tetragonoloba* L. (Taub) (Lui *et al*. 2007) seeds vary with seed coat colour. Dark chicory seed were reported to perform better than light-coloured seeds with regards to seed germination (Adlakha and Chhibber 1963; Minnaarr 1984; Pimpini et al. 2002). On the other hand, findings by Corbineau and Côme (1990) demonstrated poor germination of black chicory seeds compared with light-coloured seeds. The capability of seeds to reorganise and repair cellular membranes from damage that may have occurred during early imbibition, influences electrolyte leakage from the seed. The quicker seeds able to re-establish membrane integrity, the lower is their electrolyte leakage; therefore, seeds with high vigour are able to able to reorganize t cellular membranes more rapidly than low vigour seeds (Hampton and TeKrony 1995). Differences in the electrolyte leakage of seeds varying in seed coat colour have been reported by Pekşen et al. (2004) for *Vigna unguiculata* (cowpea), Mavi (2010) for *Citrullus lanatus* (watermelon), and Atis et al. (2011) for *Trifolium pratense* (red clover). Research by Durga et al. (2014), Jagadish et al. (2013), Srimathi and Malarkodi (2002) and Zhang et al. (2006) suggested that seed coat colour could be associated with the aging of seeds. Presently, there is limited information describing seed quality of chicory and its possible association with seed coat colour.

Seed colour assessment is gradually gaining importance as a measure of ensuring seed quality (Agnieszka and Hołubowicz 2008; Atis et al. 2011; Whan et al. 2014). Generally, seed colour differences are assessed by visual inspection; however, visual assessment is highly subjective and has a high likelihood of inconsistency due several factors such as fatigue, eyesight, mental state, improper lighting and other environmental factors (Sidnal et al. 2013). To reduce the subjective nature of visual assessment and to be able to differentiate accurately between seed colours, image analysis has been used to assess seed colour. Image analysis is a tool that partitions a digital image into multiple parts (set of pixels on which hue, saturation and intensity

are recorded) in order to change the presentation of an image into data that can be analysed (Rodriguez-Pulido et al. 2012). This tool can be used in research studies to objectively assess seed colour. Image analysis was reported by Odindo (2007) to be an objective tool for discriminating seed colour in cow pea. Further research is needed to identify objective methods that can be used to assess seed colour in other crop species.

The aim of this study was to evaluate the use of image analysis as a method to determine seed coat colour differences in chicory, as well as to determine the association between seed quality and seed coat colour with respect to germination and vigour.

3.2 Materials and methods

3.2.1 Plant material

Chicory (*Cichorium intybus* L. cv. Orchies) seeds, were obtained from Nestle® Estcourt, KwaZulu-Natal.

3.2.2 Seed image analysis

For colour determination seeds from one batch obtained from Nestle® were separated visually into eight seed colour categories: cream, cream with light brown speckles, cream with dark brown speckles, roasted, brown with yellow speckles, cream and brown, light brown and dark brown (Figure 3.1). Seed colour was captured using images produced by a Leica MZ16 stereomicroscope (Meyer instruments, Inc., USA). Eight seeds from each of the categories were used to measure the colour parameters hue angle, colour intensity and saturation using the image analysis system AnalySIS®. The hue, intensity and saturation were based on pixel values recorded by randomly clicking at five different points (per seed) on eight individual seeds.

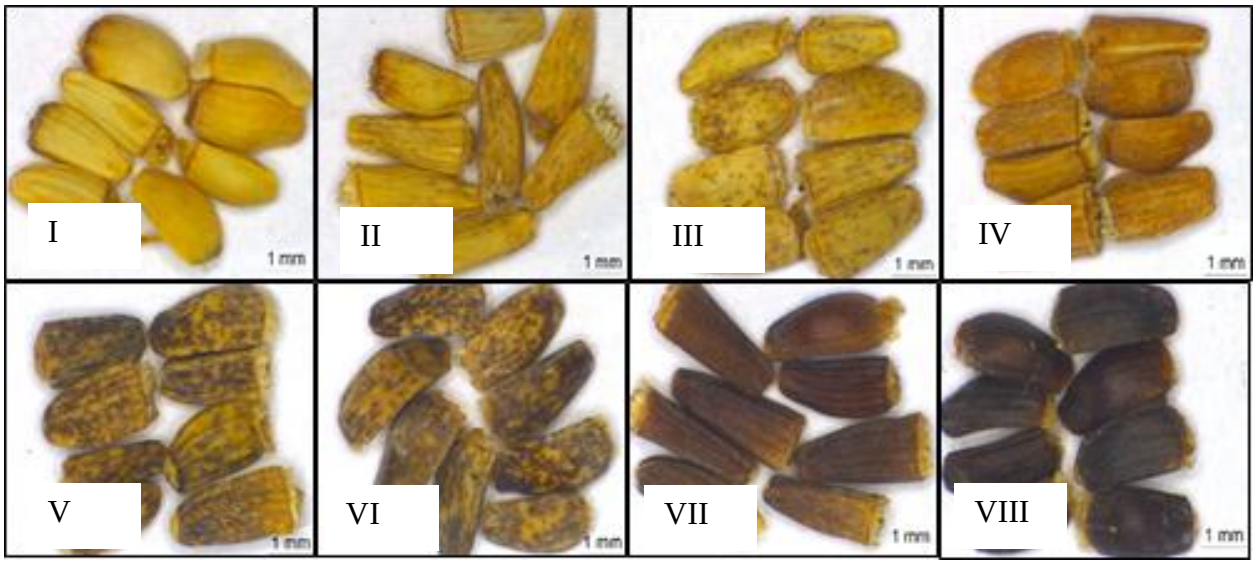

Figure 3.1: Colour categories of 'Orchies' chicory seeds. (I) cream, (II) cream with light brown speckles, (III) cream with dark brown speckles, (IV) roasted, (V) brown with yellow speckles, (VI) cream and brown, (VII) light brown and (VIII) dark brown

3.2.3 Standard germination

Seed germination of different colour seeds was assessed using the standard germination test (ISTA 2011). Four replicates of 50 seeds were germinated in 9 cm (diameter) petri dishes on Whatman No. 1 filter paper moistened with 10 ml distilled water. Seeds were incubated for 14 days in growth chambers at 20/30°C day/night (16/8 h). The GVI (germination velocity index), indicating the relative speed of germinant was determined daily by counting the number of seeds that had geminated from day one until day 14. The value of GVI is higher, the more seeds germinate in the least number of days (Raizada and Raghubanshi 2010). Seeds were considered germinated upon radicle emergence from the seed. The germination velocity index (GVI) was calculated according to the formula established by Maguire (1969):

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \qquad \text{Eq. 1}$$

Where:

GVI = germination velocity index,

G1, G2…Gn = number of germinated seeds in first, second… last count

N1, N2…Nn = number of sowing days at the first, second… last count.

The mean germination time was (MGT) calculated according to Ellis and Roberts (1981):

$$\text{MGT} = \frac{\Sigma Dn}{\Sigma n} \qquad \text{Eq. 2}$$

Where:

n= the number of seed which were germinated on day D

D= number of days counted from the beginning of germination.

3.2.4 Germination vigour characteristics

3.2.4.1 Accelerated aging

Seeds were aged using the accelerated aging (AA) test as described by ISTA (2011). Samples were weighed and spread on a wire mesh tray (10.0 x 10.0 x 3 cm) inside plastic boxes (11.0 x 11.0 x 3.5 cm). There so-prepared seeds were then placed in a MD1400 modular climate chamber (Snijders Scientific, Jumo Imago 500, Netherlands) maintained at 41℃ and ≈95% relative humidity for 72 h. After removal from the chamber, seeds were evaluated for germination as described in 2.3.

3.2.4.2 Imbibition

Water uptake for each colour was determined using four replicates of 100 individual seeds. Seeds were weighed on an analytical balance and placed on Whatman No. 1 filter paper in 9 cm diameter petri dishes and moistened with 10 ml distilled water. Change in mass (%) was measured at 0, 15, 30, 60 min, 2, 3, 4, 5, 6, 7 and 8 h. At each sampling interval, seeds were removed patted dry, weighed and immediately returned to the petri dishes. The percentage change in seed mass at each time interval was recorded accordingly using the following formula:

Change in mass (%) = [(Final mass – Initial mass)/ Initial mass] x 100

3.2.4.3 Electrical conductivity

Electrical conductivity (EC) was tested according to ISTA (2011) using four replicates of 100 seeds (weighed to two decimal places, 0.01 g) for each colour. Seeds were soaked in glass beakers (500 mL) containing 250 mL distilled water for 24 h at room temperature (25°C). Thereafter, the EC of the soaking solution was determined using a Hanna HI portable

pH/EC/TDS/Temperature meter (Hanna Instruments, UK). Electrical conductivity was expressed as µS cm^{-1} g^{-1} seed.

3.2.5 Data analysis

In order to bring data closer to normal distribution and to reduced skewness, germination percentage, GVI, MGT and imbibition data were transformed according to the angular transformation method and subjected to ANOVA using GenStat® (Version 18, VSN International, UK). Means were separated using Least Significant Differences (LSD, P = 0.05).

3.3 Results

3.3.1 Seed image analysis

Highly significant differences (P < 0.001) were observed between images taken of the different seed colour categories with respect to hue angle, intensity and saturation (Table 3.1). The dark brown colour component exhibited the highest pixel values for hue (233.05) with cream and brown seeds displaying the lowest values (34.98). Mean separation of hue showed dark brown seeds to be significantly different from all other seed colours. No significant differences were observed amongst the other categories. Image analysis indicated that two colour categories existed with respect to hue. These were categorized as light- and dark-coloured seeds and seed quality tests carried out were based on the hue values of the image analysis.

The highest pixel value for saturation was recorded for roasted seeds (107.83) and the lowest for dark brown seeds (15.05). Mean separations also showed the saturation of cream, cream with dark brown speckles, cream with light brown speckles and roasted seeds to be not statistically different (P > 0.05).

Colour intensity was highest in cream seeds (128.15), while light brown seeds displayed the lowest colour intensity (62.45). In addition, mean separation showed the colour intensity of brown seeds with yellow speckles to be not statistically different to cream and brown seeds (P > 0.05), while cream seeds were found to be statistically similar to cream seeds with dark brown speckles; cream seeds with dark brown speckles were not statistically different to cream seeds with light brown speckled.

Table 3.1: Discrimination of seed colour in chicory using image analysis

Categories	Hue	Saturation	Intensity
I Cream	46.97 a	105.23 ef	128.15 f
II Cream with light brown speckles	44.64 a	104.43 ef	113.52 e
III Cream with dark brown speckles	45.70 a	93.58 e	121.65 ef
IV Roasted	37.79 a	107.83 f	99.82 d
V Brown with yellow speckles	57.95 a	52.73 c	94.77 cd
VI Cream and brown	34.82 a	66.93 d	88.72 c
VII Light brown	69.15 a	38.15 b	76.97 b
VIII Dark brown	233.05 b	15.05 a	62.45 a
LSD	36.73	13.04	8.25
F.Pr	<0.001	<0.001	<0.001
CV%	23	4.7	3.8

Values followed by the same letter in a column are not significantly different from each other ($P < 0.05$)

3.3.2 Germination

In terms of germination percentage, the interaction between seed colour and seed quality test was significant ($P < 0.05$) (Figure 3.2). Dark-coloured seeds showed the highest germination percentage of both treatments. Light-coloured seeds had 59% germination; dark-coloured seeds on the other hand, had 76% germination in the SG test. In the AA test dark-coloured seeds had 60% germination, while 46% of the light coloured-seeds germinated.

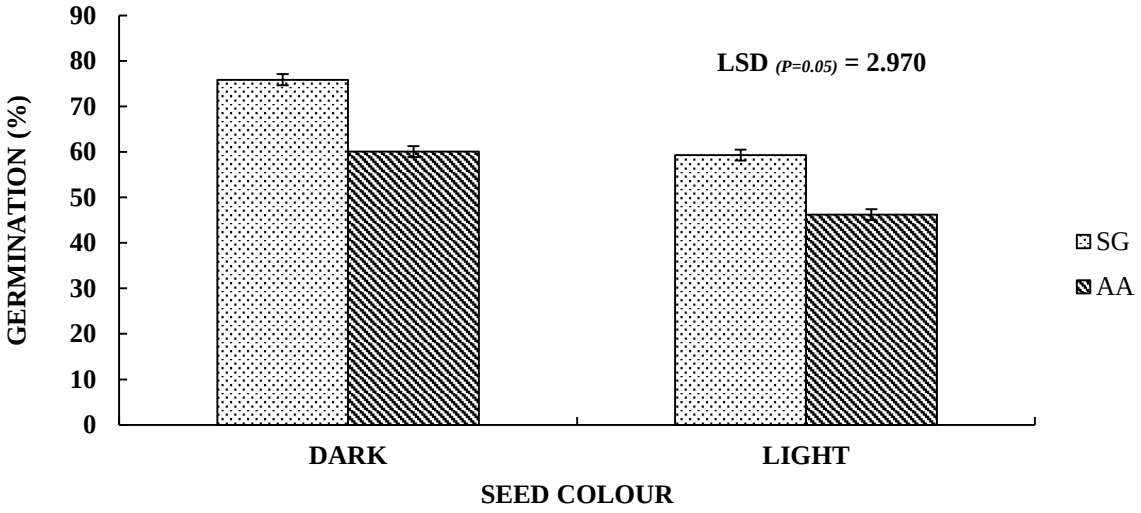

Figure 3.2: Germination percentage of chicory seeds varying in seed colour (dark- and light-coloured) as observed in the standard germination (SG) and accelerated aging test (AA)

With regard to GVI, the interaction between seed colour and seed quality was highly significant ($P < 0.001$) (Figure 3.3). A higher GVI was observed for dark-coloured seeds (26) than for light-coloured seeds (16) in the SG test. Aging severely reduced germination velocity, light-coloured seeds had higher GVI (5) than dark coloured seeds (3), although it was not significantly different.

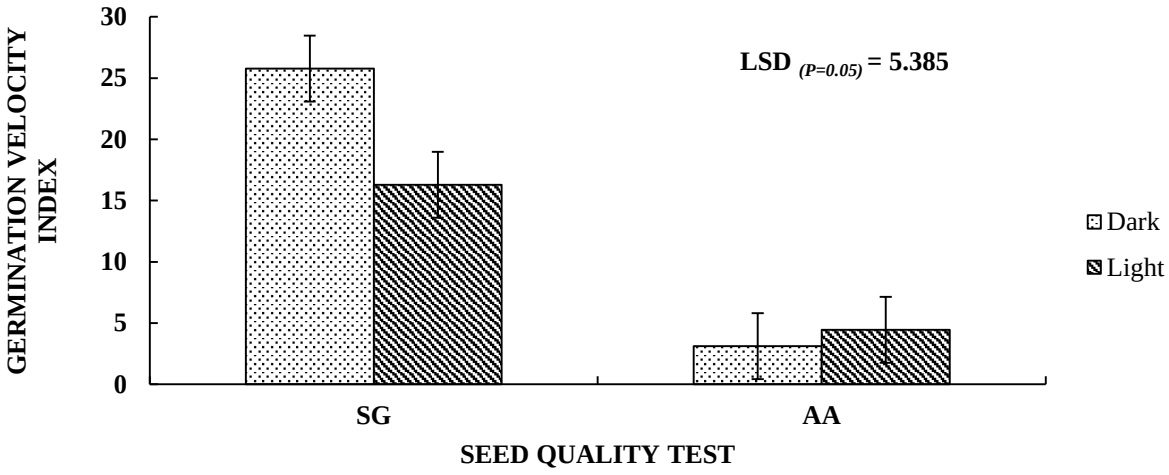

Figure 3.2: Germination velocity index of chicory seeds varying in seed colour (dark- and light-coloured) as observed in the standard germination (SG) and accelerated aging test (AA)

The interaction between seed colour and seed quality test for MGT was significant ($P < 0.05$) (Figure 3.4). In the SG test MGT of dark-coloured seeds was three days, while light-coloured

seeds took four days. In the AA test, light-coloured seeds had a MGT of 12, days while dark coloured seeds had a MGT of 18 days.

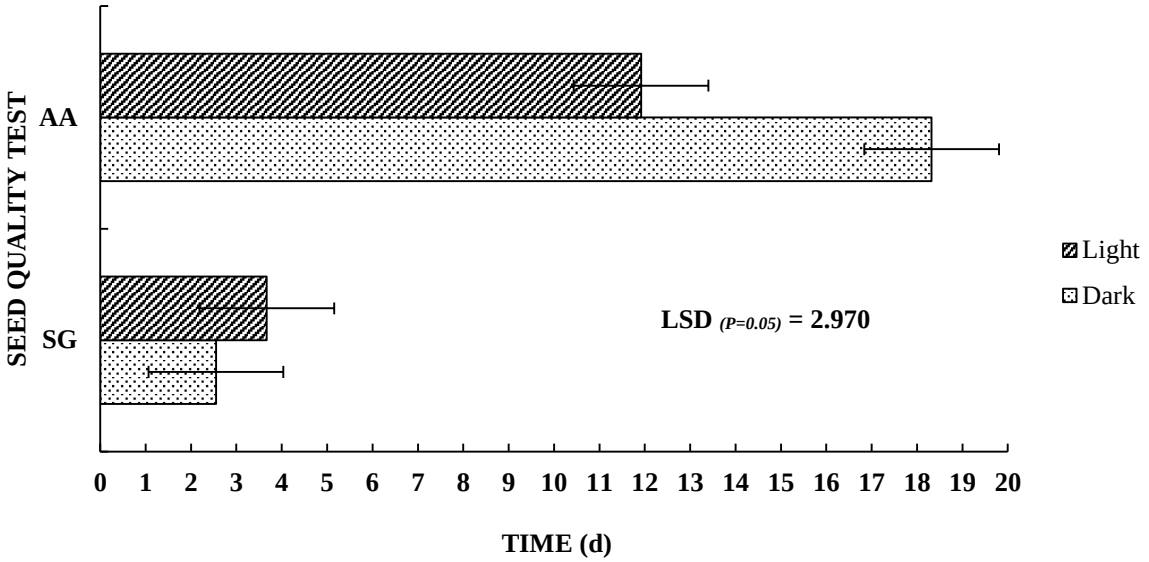

Figure 3.3: Time (days) taken by chicory seeds varying in seed colour (dark- and light-coloured) to 50% germination in the standard germination (SG) and accelerated aging test (AA)

3.3.3 Seed Imbibition

The percentage change in seed mass was used to measure the rate of water uptake by the seed. Results showed highly significant differences ($P < 0.001$) in the interaction between seed colour and imbibition time (Figure 3.5). Fastest water uptake occurred within the first 15 minutes in both seed colours. During this initial period, light-coloured seeds had a tendency to imbibe more water (20%) compared with dark-coloured seeds (17%), although there were no significant differences. Light-coloured seeds displayed a decrease in mass after 240 and 480 minutes of water uptake, while a decline in water uptake by dark-coloured seeds was observed after 360 minutes of soaking seeds. Overall, the change in mass was significantly different, with dark-coloured seeds imbibing more water (mass increase of 44%) than light-coloured seeds (mass increase of 41%).

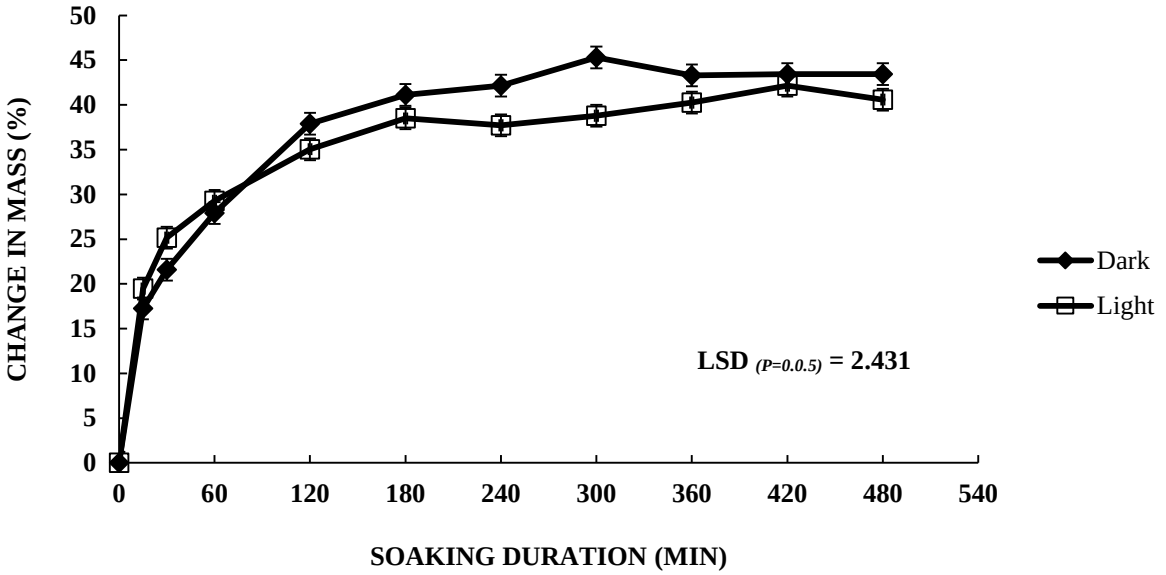

Figure 3.4: Percentage change in mass of chicory seeds (dark- and light-coloured) during imbibition

3.3.4 Electrical conductivity

There were no significant differences (P > 0.05) between the two seed colours with respect to leakage of electrolytes from the seed (Figure 3.6); however, dark-coloured seeds had the tendency of a higher electrolyte leakage (8.16 µS cm^{-1} g^{-1}) compared with light-coloured seeds (5.25 µS cm^{-1} g^{-1}).

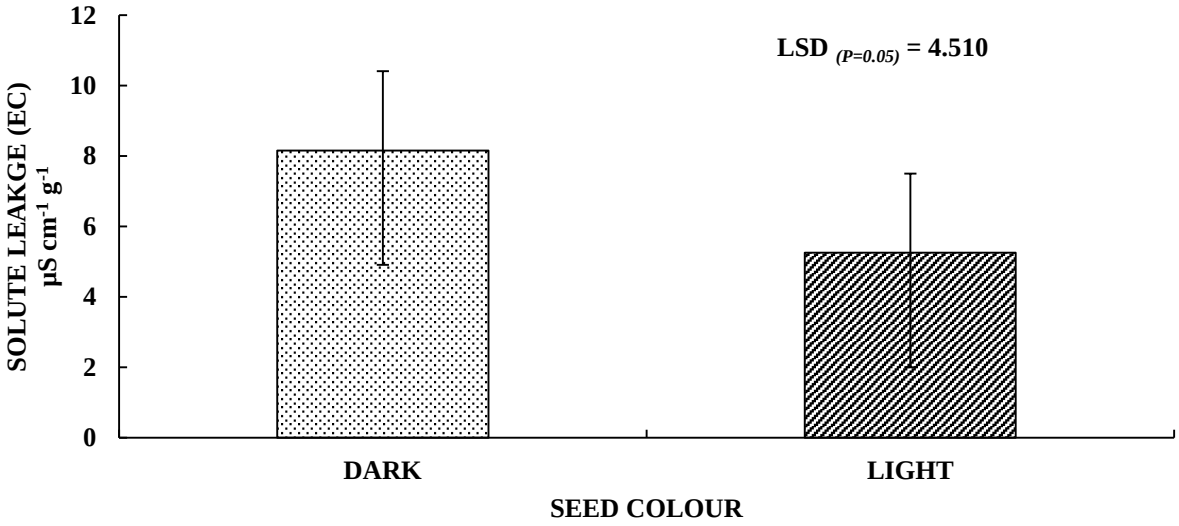

Figure 3.5: Electrical conductivity of chicory varying in seed colour (dark- and light- coloured) measured over a 24 h period

3.4 Discussion

Visual assessment of seed colour is highly subjective (Farahani 2012) and likely to produce inconsistent results (Whan et al. 2014). In this study, the subjective nature of visual assessment made it difficult to accurately distinguish between seed colours. An objective method, such as image analysis can help reduce the subjective nature of visual assessment. Image analysis provides numerical data of an acquired image (Verma et al. 2013). Soft image analysis measures pixel values by utilizing hue angel, colour saturation and colour intensity. Hue is the measure of the dominant colour (by wavelength) as perceived by the human eye, while saturation refers to relative colour purity and intensity measures the brightness of colour (Lui and Chung 2011).

Hue, saturation and intensity were highly significant between the visually identified 'Orchies' seed colour categories. Furthermore, mean separation showed dark-coloured seeds to be clearly different from all other seed colours with respect to hue. According to Ikonomakis et al. (2000) hue is the most useful characteristic for colour description and has the greatest discriminating power between the three values (L, a, b) because of its lower dependency on intensity.

Seed colour is one of the factors influencing seed quality. Different seed colours within a cultivar are often associated with the harvesting of seeds at different developmental stages or due to genetic differences (Atis 2011; Zhang et al. 2013). Different developmental stages contribute to seed maturity; hence, seed colour affects seed quality. Ochuodho (2005) associated hue with seed maturity in *Cleome gynandra.* Seed colour may also be influenced by the concentration of phenolic compounds in the seed coat (Slattery et al., 1982; Asiedu et al., 2000; Troszyńska and Ciska, 2002; Tango et al., 2014). The phenolic concentration of seeds has been linked to seed performance (Debeaujon et al., 2000; Simic' et al., 2004; Simic' et al., 2005).

Seed colour variation exists in several crop species. In this study, the aim was to evaluate the association between seed colour and seed quality with respect to seed performance during germination. The observed interaction between seed colour and seed quality test coupled with slow and delayed germination of dark-coloured seeds in the AA test showed, that seed quality is influenced by the physiological processes determining germination percentage, GVI and MGT. Therefore, even though germination may remain high in the standard germination test,

some deterioration may be present; as a result, vigour tests provide more information about the physiological quality of seed than the standard germination test (Elias and Copeland 1994).

With regard to germination percentage, dark-coloured seeds performed better than light-coloured seeds in the SG test. Previous researchers have also reported differences in seed performance of chicory seeds varying in seed colour. Dark chicory seeds were found to outperform light-coloured seeds by having higher germination percentage (Adlakha and Chibber 1963; Minnaar 1989, Pimpini et al. 2002). Contrary, Corbineau and Côme (1990) reported a higher germination percentage in light-coloured seeds than in dark-coloured chicory seeds. The chicory inflorescence is a raceme with five flower heads opening per day per plant and only one flower head per cluster is open at once; moreover, it takes 12 days until another flower head opens within the same flower cluster (Reaume 2010). This large time difference between anthesis of the first and last flower in an inflorescence could explain why seeds from one plant are at different stages of development. Germination differences of chicory seeds varying in colour have been associated with seed maturity, resulting from incomplete embryo development (Pimpini et al. 2002; Aiazzi 2006; Charjan et al. 2012). The flowering pattern of chicory could possibly contribute to differences in seed maturity and seed colour, as some seeds may mature on one part of the inflorescence, while other seeds continue to develop within the same inflorescence (McDonald and Copeland 1997). Aged seeds have a low germination percentage; this was observed in both seed colours following the AA test; however, dark-coloured seeds still performed better compared with light-coloured seeds. These results are consistent with those for *Brassica napus* L. (Zhang et al. 2006), *Vigna unguiculata* L. (Marwanto (2004) and *Glycine max* L. Merill (Jagadish et al. 2013). The better performance of dark-coloured seed could be related to the high concentrations of phenolics in dark-coloured seeds. Phenolics play a protective role against plant stress and therefore, could provide tolerance to seed deterioration during artificial aging (Zhang et al. 2006).

A high GVI represents a relatively high speed of seed germination, meaning a high number of seeds germinated per day. Aging of seeds slows germination. Differences in germination speed has been observed between seeds varying in seed colour in response to seed storability (Pimpini et al. 2002). Dark-coloured seeds germinated faster in the SG test than light coloured seeds (Figure 3.3). In certain species, slower germination of light-coloured seeds is attributed to seed hardness; even if seeds germinate under desirable conditions, germination speed of hard seed is still low (Lui et al. 2007). Following the AA test, light-coloured seeds had a tendancy to

germinate faster than dark-coloured seeds. This is contrary to findings by Durga et al. (2014) who assessed storability of *Macrotyloma uniflorum* seeds and observed faster germination in light straw-coloured seeds than in straw- and black-coloured seeds. Time taken by dark-coloured seeds to reach 50% germination (MGT) was longer in the AA test. Findings from this study are contrary to that by Atak et al. (2008) in *Pisum sativum* L. who observed shorter MGT of dark seeds following accelerated aging treatment compared with light and medium coloured seeds. Better performance of light- than dark-coloured seeds following accelerated aging could be attributed to high levels of free radical scavenging activity in light-coloured seeds (Selvi et al. 2014).

Seed coat characteristics play a crucial role in the rate of imbibition; hence, speed of imbibition has been associated with seed colour in several crop species (Liu et al. 2007; Borji et al. 2007; Zhang et al. 2008). Seeds that imbibe more rapidly lose high amounts of electrolytes, consequently leading to cell death (Powell 1989); such seeds are known to be susceptible to imbibition damage (Peksen et al. 2004; Siddiqui and Khan 2010). The higher water uptake of dark-coloured seeds (Figure 3.5) could be attributed to the higher permeability of the seed coat; such a fast water-uptake by the seed has been associated with a thinner seed coat, cracking of the seed coat, as well as scarifying which in turn influenced water uptake (Lui et al., 2007). In this study, the rate of imbibition was influenced by the interaction between seed coat colour and soaking duration. This is consistent with finding by Ertekin and Kirdar (2010) on *Gleditsia triacanthos*, Lui et al. (2007*)* on *Cyamopsis tetragonoloba L.* Taub and Pekşen et al. (2004) on *Vigna unguiculata L. walp* seeds differing in colour. In honey locust, final water uptake of dark-coloured seeds explained higher germination obtained in dark coloured seeds compared to light coloured seeds (Ertekin and Kidar 2010). According to Siddiqui and Khan (2010) when seeds are imbibed in water, they not only absorbed water but some metabolites from the seeds are also leached into the surrounding medium; hence, the leaching of metabolites from the seeds could give an explanation for the change in mass at certain time intervals.

The standard germination test has been found to be a poor indicator of field emergence, as it is conducted under controlled laboratory conditions (Salinas et al. 2010). Seed vigour tests, which measure the ability of seeds to establish under a wide range of environmental conditions, are more accurate in predicting field emergence (Khaliliaqdam et al. 2013). The EC test is used to determine the amount of solutes leached from seeds during imbibition (Tajbakhsh 2000) and high electrical conductivity of the leachate is an indicator of a greater loss in solutes from the

seed (Powell 1986) and, hence, poor seed quality (Ramos et al. 2012). This EC is influenced by seed maturity, ageing and imbibition damage (Powell 1986). Several researchers have associated seed colour and EC, as described in *Pisum sativum* L. (Nascimento 1994), *Crotalaria juncea* L. (Fabaceae) (Pascualides and Ateca 2013), *Phaseouls vulgaris* L. (Borji et al. 2007) and *Trifolium pratense* (Atis 2011); however, results of this study found no association between seed colour and EC (Figure 3.6).

3.5 Conclusion

The separation of 'Orchies' chicory seeds according to seed colour was possible using an Image Analysis System; further seed colour is associated with seed quality. Dark-coloured seeds had a higher germination percentage than light-coloured seeds; however, this did not indicate higher vigour. Based on the GVI results and mean germination time, the accelerated aging test is useful in determining storability of chicory seeds differing in seed colour. These results suggest the need to evaluate seed performance of chicory seeds varying in seed colour using different seed vigour tests. In the present study, no relationship between seed colour and electrolyte leakage could be established. Therefore, electrolyte leakage does not seem to be reliable indicator of 'Orchies' chicory seed vigour. Sorting chicory seeds according to seed colour is likely to result in separation of seed according to performance. Due to seed size this exercise is, however, impractical on a large scale. Therefore, in order to avoid seed quality differences, seed harvesting should be carried out once seeds have attained uniform seed colour. Future research should determine whether chicory seeds varying in seed colour differ in phenolic composition as well as determine whether variations in seed performance may be linked to differences in the phenolic concentrations. In addition, differences in seed colour could suggest differences in stored proteins and soluble sugar composition. Future research should also determine whether chicory seeds varying in seed colour differ in genetic composition and whether such a potential genetic variation could be aligned with seed quality.

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CHAPTER 4
CHICORY (*CICHORIUM INTYBUS* L.) SEED QUALITY RESPONSE TO SEED COLOUR SELECTION AND VARIOUS PRIMING METHODS

4.1 Introduction

The availability of low quality seed is one of the major obstacles to growing chicory in South Africa, often resulting in poor crop establishment and low yields. Poor seed quality affects germination and vigour, produces non–uniform plants with low plant stands (Soares 2013) particularly under adverse environmental conditions (Elias and Copeland 1997). Seed germination and seedling growth are critical stages required for successful crop production (Sharma et al. 2014; Aghbloghi and Sedghi 2014). A high germination rate and a high emergence ensure good crop establishment (Mabhaudhi and Modi 2011) and high crop establishment enhances crop yield (Ghobadi et al. 2012; Afzal et al. 2012). A possible solution to low seed quality in chicory is improving seed quality characteristics that occur prior to seed germination. Strategies used to improve seed germinability include seed sorting and seed priming (Agneiszaka 2008; Jisha 2013).

Seed priming is a pre-sowing strategy used to achieve rapid germination and emergence, uniform emergence, improved emergence rate and seedling stand, consequently leading to better crop establishment (El-Araby and Hegazi 2004; Farooq 2007). Hydro-priming (soaking seeds in water), halo-priming (soaking seeds in organic salt solutions), osmo-priming (soaking seeds in osmotic solutions) and hormonal-priming (use of plant growth regulators in soaking solution) are some of the priming methods available. During priming, seeds are hydrated to allow metabolic activities necessary for imbitition to occur; however, the germination process is halted as radicle protrusion through the seed coat is prevented (Jahangir et al. 2009). Among several factors, successful priming depends on exposure duration of seeds to the priming solution (Nascimento et al. 2013). Hydro-priming was reported to improve germination percentange of chicory (Sambo et al. 2004). Dehkordie et al. (2012) found osmo-priming to improve germination percentage, germination index, reduce mean germination time and produce longer seedlings. Mohammand et al. (2010) found increased germination rate and

caulicle length in osmo-primed chicory seeds. Tzortzakis (2009) reported reduced mean germination time in hormonal-and halo-primed chicory seeds.

In this study, osmo-priming using polyethyleneglycol (PEG) and hydro-priming using distilled water were selected to improve the low germination of chicory seeds. Hydro-priming is a simple, low cost, safe method that raises the capacity of seeds towards osmotic adjustment for improved seedling establishment and crop production under abiotic stresses by simply soaking seeds in water (Jisha et al. 2013). This method is easy to use and can even be applied by resource-constrained farmers, wishing to improve seed quality attributes of chicory. Other advantages of hydro-priming include the accomplishment of pre-germination processs for instance, the repair and synthesis of nucleic acids (DNA and mRNA), protein, restoration of membranes and the initation of a variety of biochemical changes and enzyme activation (Dastanpoor et al. 2013). Positive seed quality effects of hydro-priming on chicory have already been reported by Dehkordi (2012).

Osmo-priming prolongs the hydration period of seeds during early imbibition, triggering gradual progression of various pre-germinate metabolic activities establishment (Jisha et al. 2012). Improvement in seed performance of primed seeds has been linked to altered physiological condition of the embryo and release of enzymes which are involved in the increase availability of soluble food nutrients (Abbasi et al. 2012).

Seed quality can be improved by seed priming, a pre-sowing strategy that improves germination by inducing a range of biochemical changes in the seed required to initiate the germination process, resulting in rapid and uniform seedling emergence (Arif et al. 2008). Seed quality of seed lots of the same species vary in their response to priming. Research suggests that seed coat colour maybe associated with seed quality in chicory (Minnar 1984; Corbineau and Côme 1999; Pimpini et al. 2002). Seed priming of different coloured seeds has been reported to result in differential seed quality responses (Adebisi et al. 2011; Sinefu et al. 2011). It could be that, an optimal priming method varies for chicory differing in seed coat colour. Therefore, the aim of this study was to assess the effect of seed coat colour on germination, seedling growth and development of chicory in response to different priming solutions and durations.

4.2 Materials and Methods

4.2.1. Plant material

Chicory seeds (*Cichorium intybus* L. cv. Orchies) were obtained from Nestle® Estcourt, in the KwaZulu-Natal province of South Africa. Seeds were visually separated in to light- and dark-coloured seeds.

4.2.2 Experimental design and seed priming procedure

The experiment used two seed colours (light- and dark-coloured seeds), three seed priming methods (hydro-priming, osmo-priming and untreated control) and three exposure durations (six, nine and twelve hours). For hydro–priming, seeds were soaked in distilled water at room temperature. Osmo-priming was achieved through use of polyethylene glycol (PEG). For osmo–priming, seeds were soaked at two PEG 6000 osmotic potential levels (-0.5 and -0.9 MPa). The osmotic potential of PEG 6000 was determined according to Michel and Kaufmann (1973). After osmo-priming, seeds were washed thoroughly under running tap water for five minutes, and, thereafter, left to dry at room temperature for 48 hours (Lee and Kim 1999).

4.2.3 Germination test

Seeds were germinated using the standard germination test (ISTA 2011). Three replicates of 10 seeds were germinated in 9 cm (diameter) petri dishes on Whatman No. 1 filter paper moistened with 10 ml distilled water. Seeds were incubated for 14 days in a germination chamber at 20/30°C day/night (16/8 h). The GVI (germination vigour index) indicating the relative speed of germination and was determined by daily records (counting the number of seeds that had germinated) from day one until day 14. The GVI value is higher, when more seeds germinate fast (fewer number of days) (Raizada and Raghubanshi 2010). Seeds were considered germinated upon radicle protrusion. Germination velocity index (GVI) was calculated according to the formula established by Maguire (1969):

$$GVI = G1/N1 + G2/N2 + \ldots + Gn/Nn \quad \text{Eq. 1}$$

Where:

G1, G2…Gn = number of germinated seeds in first, second… last count

N1, N2…Nn = number of sowing days at the first, second… last count

The mean germination time (MGT) was calculated according to Ellis and Roberts (1981):

$$\text{MGT} = \frac{\Sigma Dn}{\Sigma n} \quad \text{Eq. 2}$$

Where:

n= the number of seed which were germinated on day D

D= number of days counted from the beginning of germination.

4.2.4 Seedling growth and development

Seedlings were established in a plant growth chamber (20/30°C day/night 16/8 h) in seedling trays. Pine bark was used as growth medium in a factorial experiment replicated three times. Seeds were watered on alternate days and seedling emergence measured daily for 14 days. The experiment was terminated after 30 days. Seedling emergence was defined as when at least 2 mm hypocotyls appeared from the above the soil surface. Seedling growth was assessed using the following parameters: Seedling shoot and root lengths (mm), fresh and dry root and shoot mass (g), and root to shoot ratio. A ruler was used to measure root and shoot length. To measure dry mass, first the root and shoot were separated, and individual parts dried at 70°C in an oven for 72 hours. Thereafter, root and shoot mass was determined. Mean time to emergence (MET) was calculated using the following formula by Bewley and Black (1994):

$$\text{MET} = \frac{\Sigma\ (fx)}{\Sigma f} \quad \text{Eq. 3}$$

Where:

f= the number of newly germinating seeds at a given time (day), and

x= number of days from date of sowing.

4.2.5 Data analysis

Data was subjected to ANOVA using Genstat® (Version 18, VSN International, UK). Means were separated using Least Significant Differences (LSD, P = 0.05).

4.3 Results

4.3.1 Germination percentage

The interaction of seed coat colour, priming solution, and priming duration significantly ($P < 0.001$) affected final germination percentage (Figure 4.1). Differences in final germination of

seeds primed for 6 and 12 hours were not significant (P > 0.05); however, the were significant differences in seeds primed for 9 hours with light-coloured seeds having a lower final germination percentage than dark-coloured seeds. Overall, maximum germination (76%) for both seed colours was achieved in unprimed seeds. Priming reduced final germination of seeds of both colours.

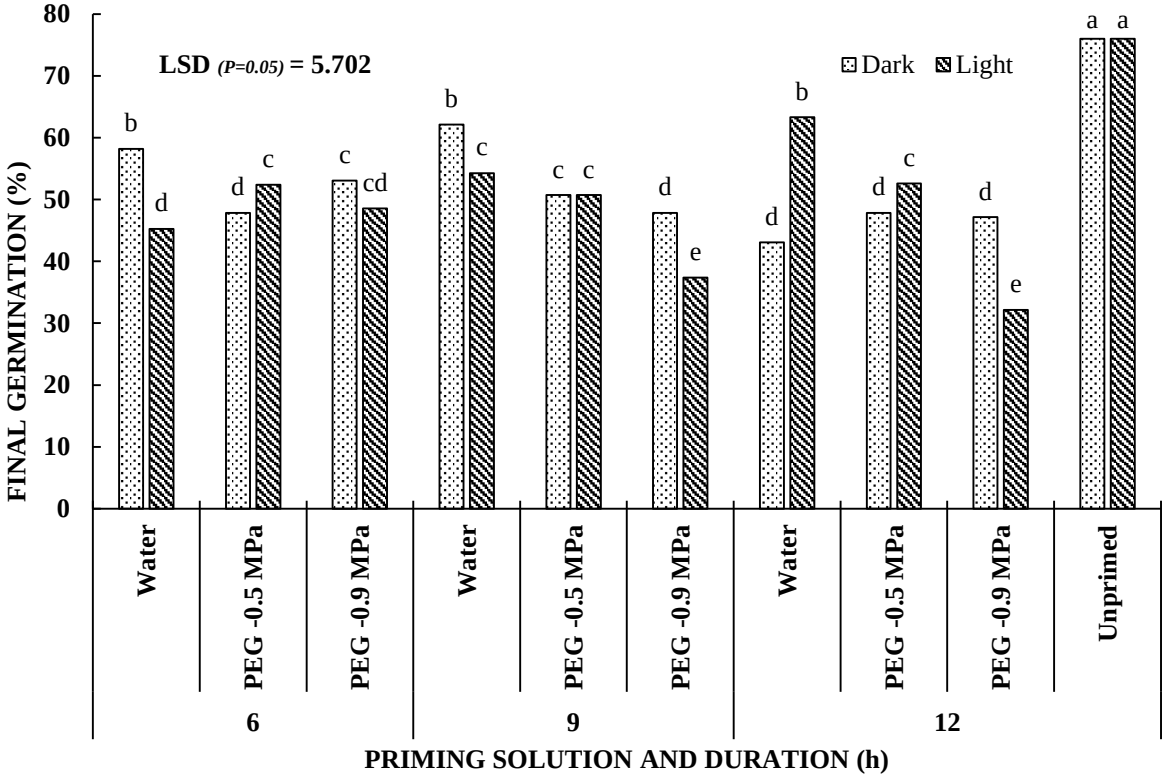

Figure 4.1: Final germination percentage of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.3.2 Mean germination time (MGT)

The interaction of seed coat colour, priming solution, and priming duration significantly (P ≤ 0.001) affected mean germination time (Figure 4.2). Unprimed controls took longer to germinate, whilst priming seeds with PEG -0.9 MPa solution for 12 hours resulted in the lowest observed germination time. Hydro-priming and osmo–priming significantly reduced MGT relative to unprimed controls. Priming using either hydro- or osmo-priming resulted in relatively similar reductions in MGT.

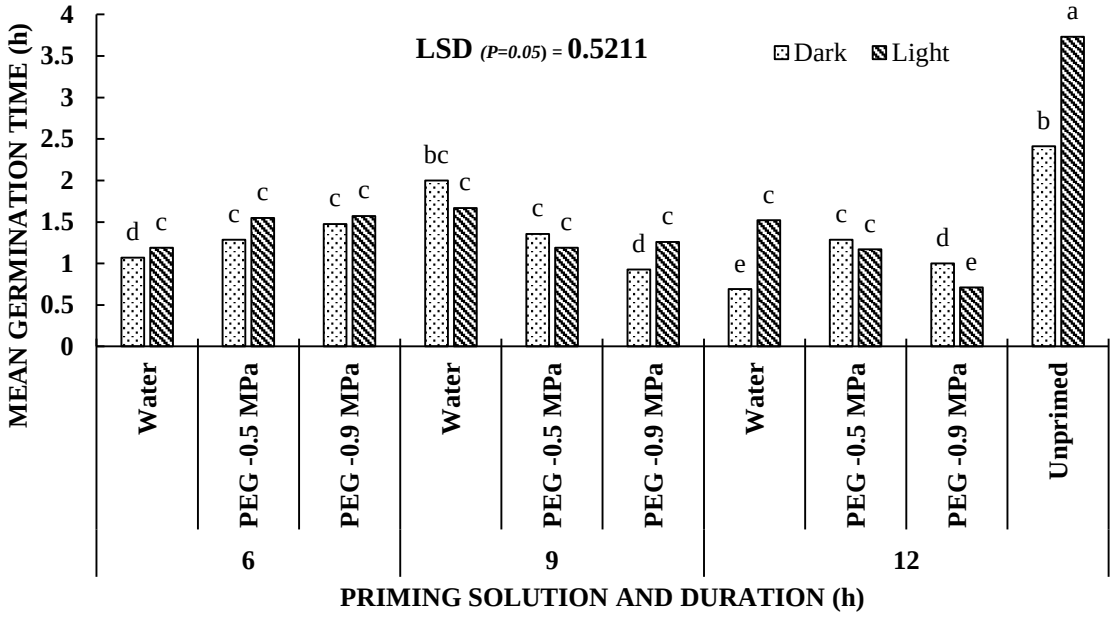

Figure 4.2: Mean germination time of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.3.3 Germination vigour index (GVI)

The interaction of seed coat colour, priming solution, and priming duration significantly ($P < 0.001$) affected germination vigour index (Figure 4.3). Unprimed control seeds had a significantly higher GVI relative to primed seeds. As individual factors, seed colour, priming solution or priming duration did not significantly ($P > 0.05$) affect GVI.

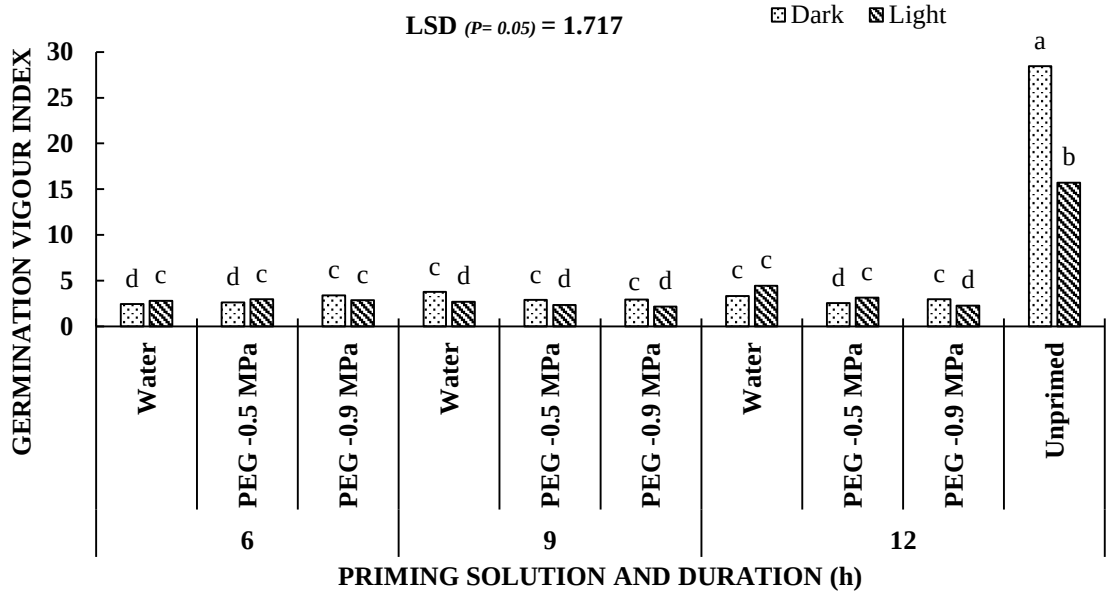

Figure 4.3: Germination vigour index of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.3.4 Mean emergence time (MET)

The interaction of seed coat colour, priming solution, and priming duration did not significantly ($P > 0.05$) affect mean emergence time (Figure 4.4). Significant differences ($P < 0.05$) were only observed between differences in priming solution. Seeds primed using PEG -0.5 MPa had the highest MET (12.15 days), followed by PEG –0.9 MPa (10.53 day) and, lastly, hydro primed seeds (8.07 days). Unprimed controls had the lowest MET (5.48 days).

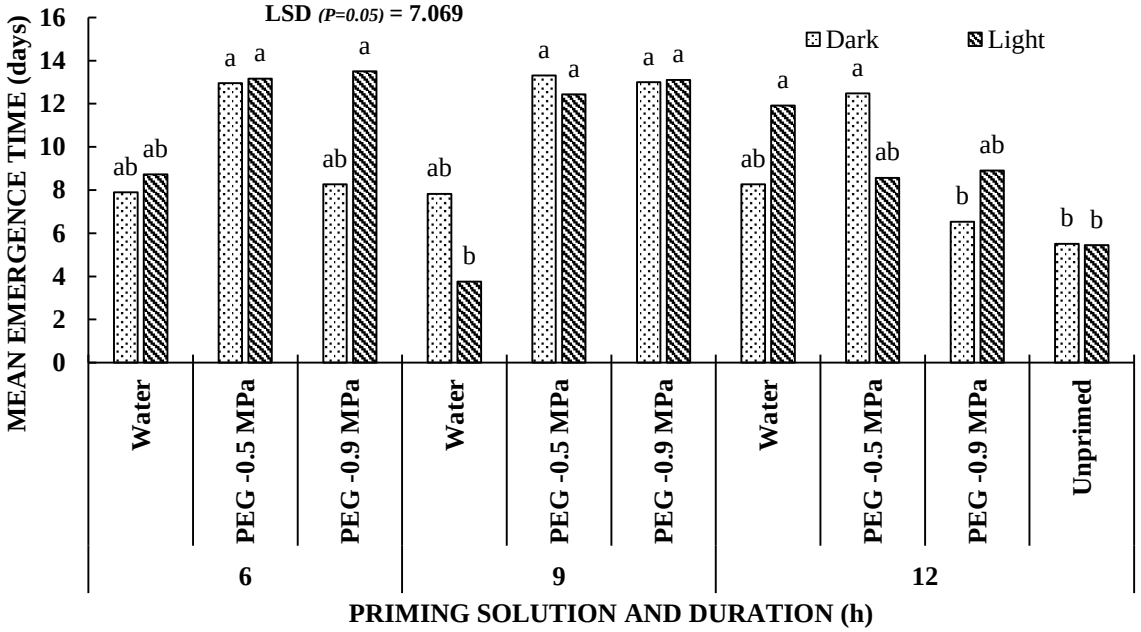

Figure 4.4: Mean emergence time of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.3.5 Seedling length

The interaction of seed coat colour, priming solution, and priming duration did not significantly ($P > 0.05$) affect seedling length (Figure 4.5); however, the interaction of priming solution and priming duration significantly ($P < 0.05$) affected seedling length. The interaction of priming solution and priming duration that exhibited the tallest (106.9 mm) seedlings was PEG -0.9

MPa following nine hours of priming. Unprimed controls had the shortest seedlings (38.6 mm). Priming solution significantly ($P < 0.001$) affected seedling length. The most effective priming solution, in terms of improving seedling growth, was PEG -0.9 (95.0 cm), followed by PEG -0.5 MPa (88.8 cm), and lastly water (81.6 cm). Unprimed seeds were 38.7 cm tall.

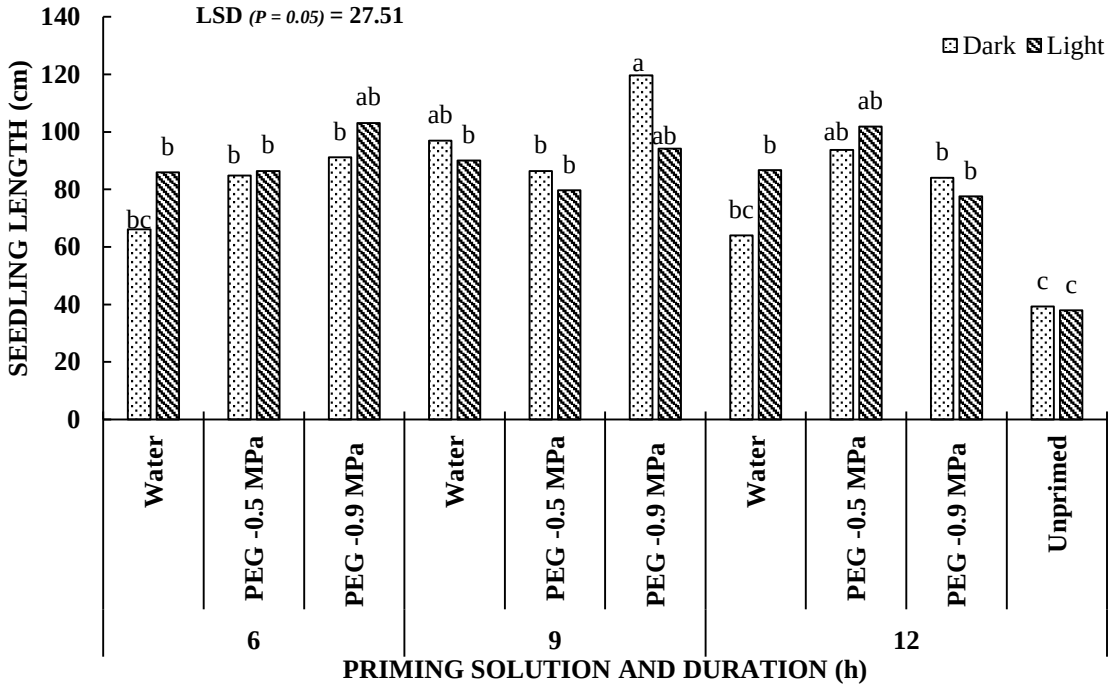

Figure 4.5: Seedling length of differently coloured chicory seeds when primed using different priming solutions at various priming duration

4.3.6 Shoot length

The interaction of seed coat colour, priming solution, and priming duration did not significantly ($P > 0.05$) affect shoot length (Figure 4.6). The only factor that significantly ($P < 0.05$) affected shoot length was priming solution. The PEG -0.5 MPa solution had the tallest shoots (44.8 cm), followed by PEG -0.9 MPa (43.2 cm), and lastly water (36.5 cm). Unprimed controls had the shortest shoots (23.2 mm).

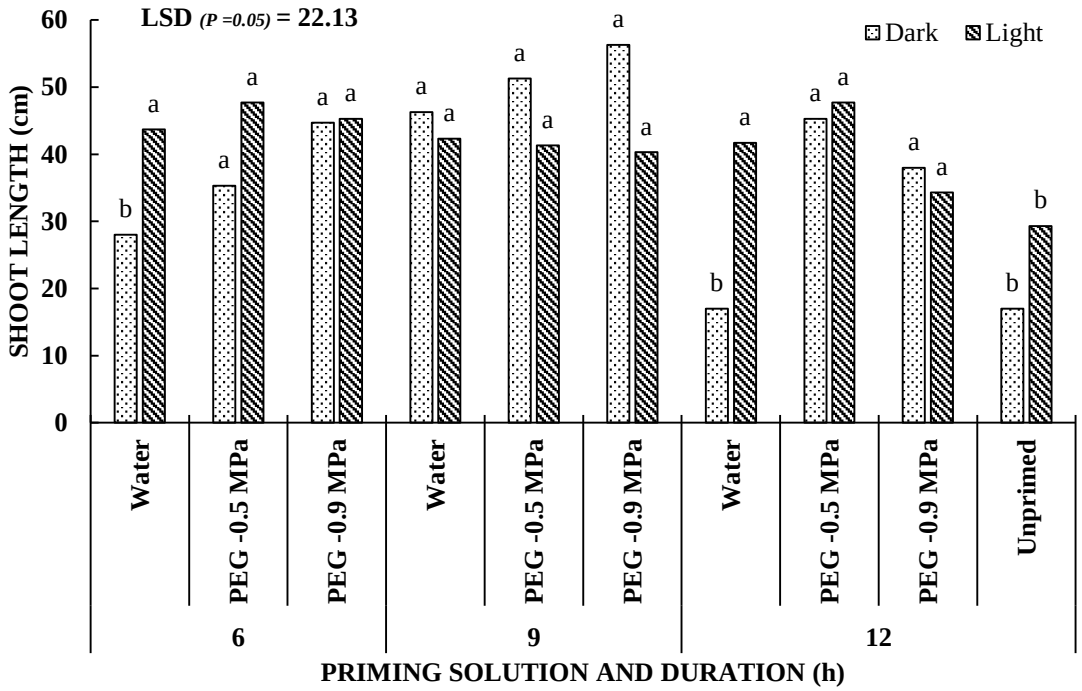

Figure 4.6: Shoot length of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.3.7 Root length

The interaction of seed coat colour, priming solution, and priming duration did not significantly ($P > 0.05$) affect root length (Figure 4.7); however, the interaction of priming solution and priming duration highly and significantly ($P < 0.001$) affected root length. This was largely due to seed coat colour differences not having a significant ($P > 0.05$) influence on root length. The interaction of priming solution and priming duration that exhibited the highest (58.6 mm) was PEG -0.9 MPa solution at nine hours priming duration. Unprimed controls had the lowest seedling length (15.5 mm). This trend was similar to seedling length of unprimed controls.

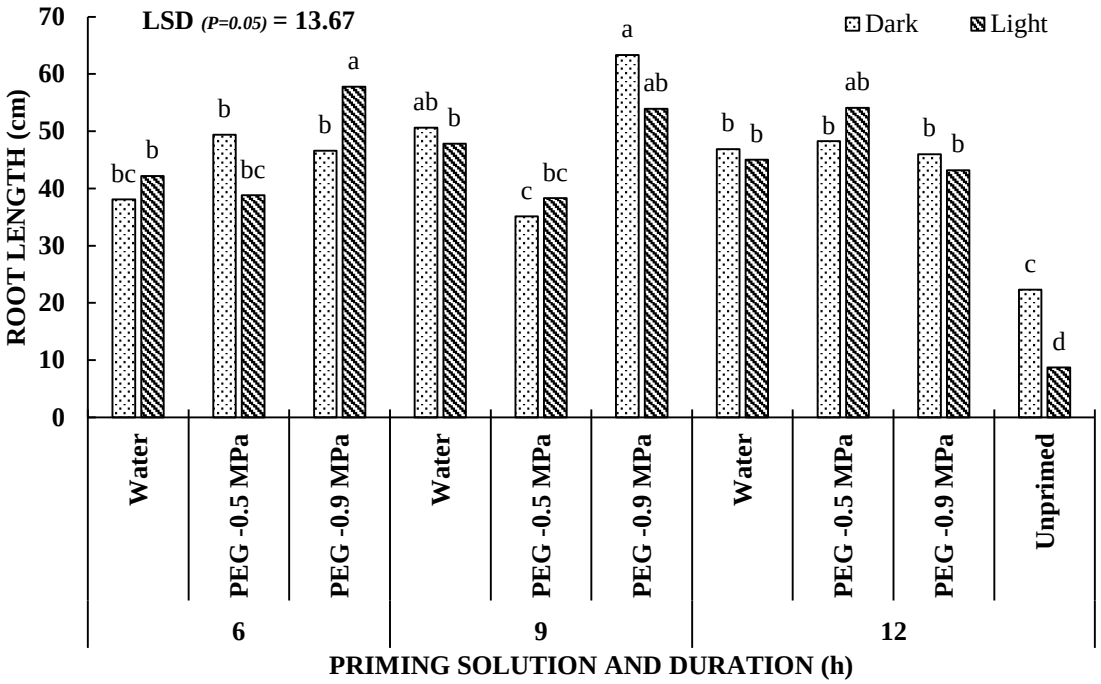

Figure 4.7: Root length of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.3.8 Fresh mass, dry mass and root to shoot ratio

The interaction of seed coat colour, priming solution, and priming duration did not significantly ($P > 0.05$) affect fresh mass (Figure 4.8); however, the interaction of priming solution and priming duration significantly ($P < 0.05$) affected fresh mass. Following priming for nine hours and, priming in PEG -0.9 MPa resulted in the highest fresh mass (0.3700 g). Unprimed controls had the lowest mass (0.1583 g). The interaction of priming solution and seed coat colour also significantly ($P < 0.05$) affected fresh mass. Dark-coloured seeds, primed using PEG -0.5 or PEG -0.9 MPa had the highest fresh mass (0.3233 g). Both unprimed controls had the lowest fresh mass (dark-coloured seeds, 0.1067 g; light-coloured seeds 0.21).

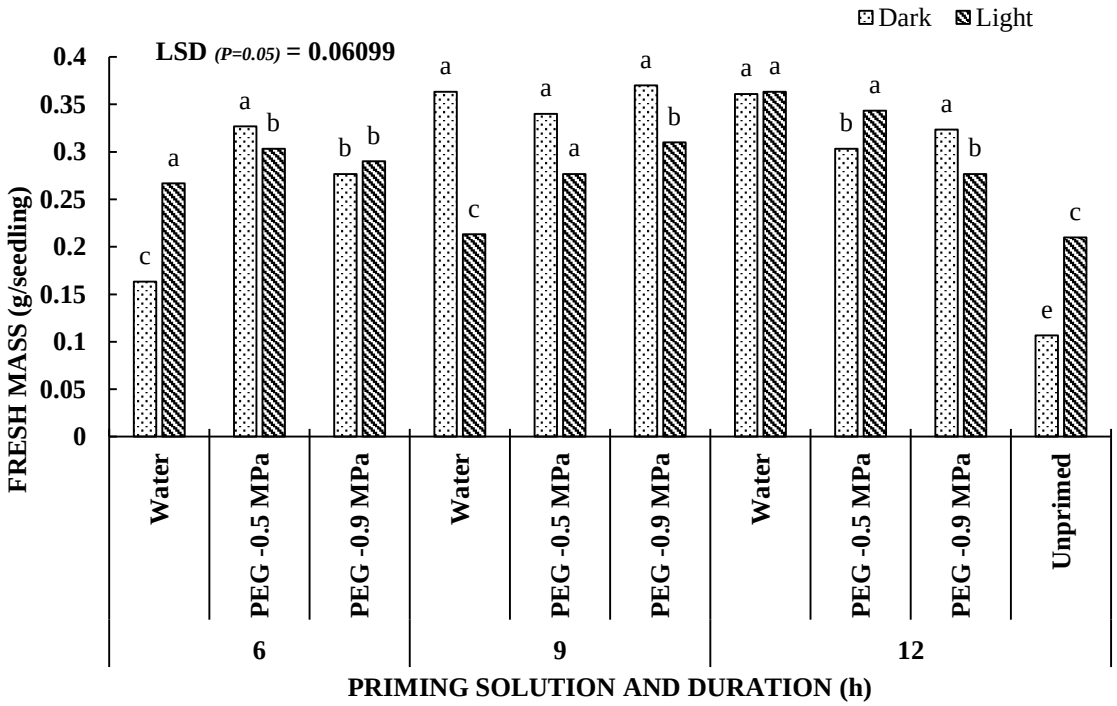

Figure 4.8: Fresh mass of differently coloured chicory seeds when primed using different priming solutions at various priming durations

There was no single factor nor interaction of factors, which significantly affected differences in dry mass (Figure 4.9). Light-coloured seeds primed using water for six hours had the highest fresh mass (0.053 g). This finding was probably an outlier, given that all other treatments were within similar ranges; and given that dark-coloured, water-primed seeds were also within that range. Unprimed controls had the lowest dry mass (dark-coloured seeds, 0.0175 g; light-coloured seeds, 0.0173 g).

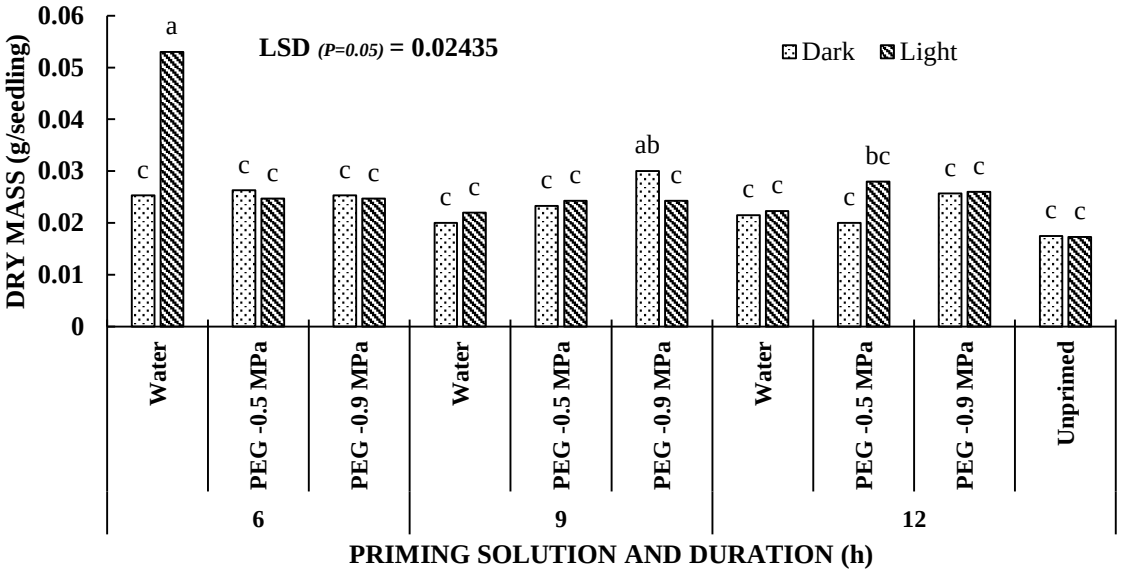

Figure 4.9: Dry mass of differently coloured chicory seeds when primed using different priming solutions at various priming durations

The interaction of seed coat colour, priming solution, and priming duration did not significantly ($P > 0.05$) affect root to shoot ratio (Figure 4.10). The only factor that significantly ($P < 0.05$) affected root to shoot ratio was priming solution. Priming seeds using PEG -0.9 MPa had the highest root to shoot ratio (1.391), followed by water (1.367), and lastly PEG –0.5 MPa (1.067). Unprimed seeds had the lowest root to shoot ratio (0.748).

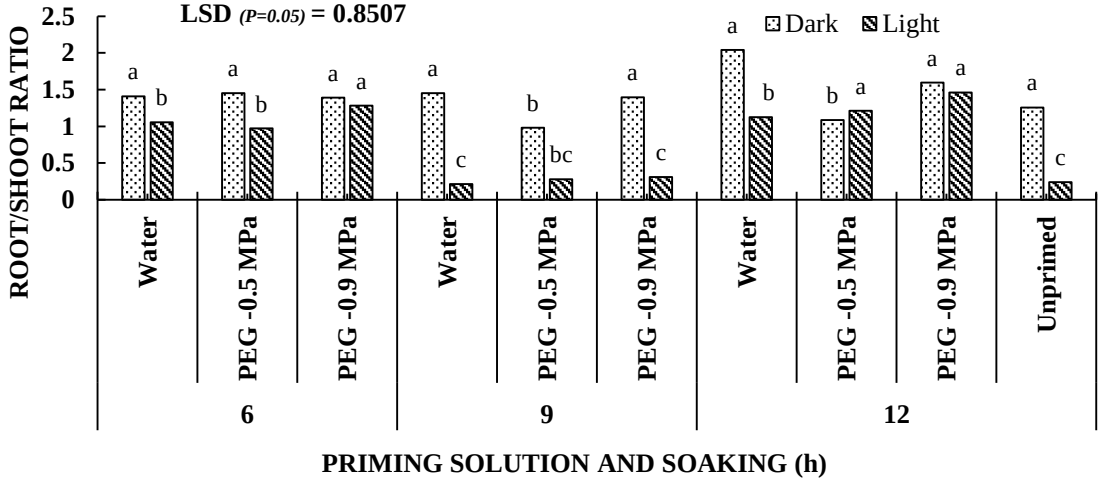

Figure 4.10: Root to shoot ratio of differently coloured chicory seeds when primed using different priming solutions at various priming durations

4.4 Discussion

The aim of this study was to determine the effect of seed coat colour on seed quality of chicory in response to different priming solutions and priming durations to establish, if dark- and light-coloured seed responded differently to priming, so as to improve germination and crop establishment.

4.4.1 Seed germination and vigour

Priming significantly and negatively impacted germination of chicory seeds. The findings are contrary to those by other authors (Tzortzakis 2009; Dehkordi et al. 2012), who found osmo-priming and hydro-priming to improve the seed germination percentage of chicory. The reason for decreased final germination percentage observed in the study could not be explained.

Regarding MGT, hydro-priming and osmo-priming significantly reduced MGT relative to unprimed controls with comparable reductions between the two priming methods. Selecting for differences in seed coat colour or priming time did not significantly affect improvements in MGT that are due to seed priming. These results suggest that seed priming pre-sowing treatment could be a useful tool to reduce the time to seed germination of chicory. Similar findings have been reported by Soughir et al. (2012) and Aymen and Cherif (2012) who reported a significantly improved MGT following priming of fenugreek (*Trigorella foenum-graecum*) and safflower (*Carthamus tinctorius*) with various solutions and priming durations.

A seed lot showing higher seed vigour index is considered to be more vigorous. In this study, osmo-priming and hydro-priming improved GVI similarly, irrespective of seed coat colour and priming duration. This suggests that using either hydro-priming or osmo-priming as a pre-sowing seed treatment can significantly and exponentially improve seed vigour. To save input costs and improve GVI, two recommendations can be derived from this study. Firstly, sorting seeds according to seed colour is not recommended before the application of priming treatments. Secondly, priming duration of six hours suffices to save time and other inputs.

4.4.2 Seedling establishment: Seedling growth

In crop production, appropriate seedling emergence is important for obtaining good crop establishment and optimum plant population. Faster emergence is crucial for successful crop establishment (Nawaz et al. 2013). The use of different priming solutions significantly

increased MET. Osmo-primed seeds took longer to emerge than hydro-primed seeds. Similar to this study, Ahmadi et al. (2007) in lentil (*Lens culinaris*) and Ghassemi-Golezanik et al. (2008) in wheat (*Triticum aestivum*) found hydro-priming to be more effective in accelerating time to emergence than osmo-priming. Rapid emergence of hydro-primed seeds could be related to faster imbibiton and earlier initation of metabolic processes than osmo-primed seeds (Ghassemi-Golezanik et al. 2008). In addition, osmo-priming with PEG may negatively affect the synthesis and degradation of proteins due to its viscous nature, resulting in delayed respiration during germination (Ahmadi et al. 2007).

For successful crop establishment, rapid development of the root and shoot system is important as it enables seedlings to capture, preserve, and use growth inputs from the soil and atmosphere (Aguirre and Johnson 1991). Overall, seedling length was significantly improved by the interaction of priming solution and priming duration. The most effective priming solution was PEG -0.9 MPa, with water showing the least, yet significant, improvement in seedling length. The trend in effectiveness of priming on seedling growth was similar to that of root growth. Therefore, to improve seedling water capture in the soil, osmo-priming is recommended, where affordable.

The shoot portions of the crops is where plants derive the canopy, which is the photosynthetic portion of the crop. When separating seedling length to its root and shoot component, results showed that shoot length was significantly affected by priming solution. The trend was similar to results of seedling length in that osmo-priming was more effective in improving shoot length compared to hydro-priming. This suggests that osmo-priming is the best treatment for improving the ability of a seedling to exploit the available water, thereby improving seedling establishment. The difference in the trend was that PEG -0.5 was more effective than PEG -0.9 MPa in improving shoot length.

4.4.3 Seedling establishment: Seedling mass

Seedling fresh mass has been a profound impact on a crop's ability to survive and grow after planting (Garcia et al. 1994). The interaction of priming solution and priming duration significantly ($P< 0.05$) improved seedling fresh mass. Unprimed controls had the lowest seedling fresh mass. Priming seed for 9 h in PEG -0.9 MPa resulted in the highest fresh mass. The interaction of priming solution and seed coat colour also significantly improved fresh

mass. Dark-coloured seeds, primed using PEG -0.5 or PEG -0.9 MPa had the highest fresh mass. This suggests that osmo-priming was more effective than hydro-priming in improving biomass accumulation. Seedling dry mass translates directly to how much of the seedling is actual solid biomass. This indicates conversion of inputs into actual crop biomass. Unfortunately, improvements in seedling establishment due to pre-sowing seed priming did not translate to significant improvements in dry mass. This suggests that improvements in seed germination and vigour, seedling establishment, and seedling fresh weight may not necessarily translate to improvements in biomass accumulation in chicory. Similar to this study, Ghassemi-Golezanik et al. (2011) found priming not to be effective in improving seedling dry mass in soybean.

The root to shoot ratio indicates the partitioning of photosythates between roots and shoots (Rogers et al. 1996). The only factor that significantly ($P< 0.05$) affected root to shoot ratio was priming solution. The most significant improvements in root/shoot ratio were observed, when osmo-priming with PEG -0.9 MPa solution, and the least significant improvement when osmo-priming with PEG -0.5 MPa solution. Benefits of hydro-priming on increasing root/shoot ratio were moderate. This means that both, hydro- and osmo-priming employed in this study are likely to favour increasing canopy development and light capture efficiency, compared with the significantly higher water and nutrient uptake in unprimed controls. This is probably due to seed priming methods employed in this study which had already exhibited significant increases in root length, therefore minimising trade-offs and negative impacts that a high root/ shoot ratio might have on water and nutrient capture (Silva et al. 2012).

4.5 Conclusion

Osmo- and hydro-priming chicory seeds improved seed quality through improvements in seed vigour (GVI) and time to germination (MGT); however, deleterious effects were observed on final germination percentage. Osmo-priming resulted in relatively high improvements in seedling length, shoot length, fresh mass and root to shoot ratio compared to hydro-priming. Despite osmo-priming proving a more effective method of combating seed quality issues in chicory, hydro-priming remains an affordable, effective pre-sowing treatment for chicory seeds. Priming improved seedling establishment (seedling length, shoot length, root length, fresh mass, root to shoot ratio), without significant improvements in biomass accumulation. The benefits of improved seedling establishment may potentially be significant and noticeable

on biomass accumulation under stress conditions or during later growth stages. It is, therefore important to conduct field trial experiments to conclusively determine effects of seed priming on chicory growth, development and yield.

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CHAPTER 5

DETERMING THE OPTIMAL PLANT STAND FOR ROOT CHICORY (*CICHORIUM INTYBUS* L.) WITH RESPECT TO SEED COAT COLOUR AND WEED MANAGEMENT

5.1 Introduction

Chicory is a root crop from which a caffeine-free coffee substitute can be produced; the crop is, hence, important to the human diet in the 21st century. Other uses of *Cichorium intybus* include animal feed stocks and leaf consumption through of various botanical varieties of the species. Currently, 80% of root chicory consumed in South Africa is imported from India (Nestlé 2012); however, there is interest in increasing local production. A strategy to achieve this is the inclusion of smallholder farmers in chicory production. Smallholder farmers are largely characterised by traditional, low input farming systems (FAOSTAT 2013). These characteristics impact majorly on crop management decisions. This necessitates development of best management practices for chicory production that accommodate smallholder farmers. Various agronomic determinants, such as planting density, weeding frequency and method and seed selection form part of crop management strategies that can be considered by farmers to improve yield and quality of the end product.

Weeds compete with crops for resources, and can suppress crop growth and, subsequently, yield (Alfakpui and Bolfrey-Arku 2007) when resources are in short supply (Park et al. 2003). Smallholder farmers prefer hand-weeding due to financial and resource constraints. Lack of weed control can result in a substantial reduction in quantity (root yield) and quality (harvestable roots) in chicory (Schnieders 1999). Chicory is highly susceptible to weed competition during early growth stages, when plants are relatively small and are, therefore, poor weed competitors (Baert and Van Bockstaele 1993). Weed suppression generally increases with plant canopy and crop growth. Early crop establishing cultivars, high plant populations, narrow crop rows and mechanical cultivations are tools that can be incorporated into successful weed management strategies for root chicory production (Wilson et al. 2004).

Seed coat colour forms part of the seed selection criteria by farmers in many crops as seed coat colour can be associated with differences in plant metabolites and growth characteristics under

variable environments (Chibarabada et al. 2015). Research on whether chicory seed coat colour is associated with differences in growth and development characteristics seems to be lacking; however, research on other crops suggests that there might be a link. For instance, Chibarabada et al. (2015) observed differences in seed quality and crop growth, development and yield of different seed coat colours for Bambara groundnut. It could also be, that the different seed coat colour in chicory relates to differences in seed maturity; hence, differences in colour reflect in seed quality and vigour variation. Therefore, it is important to establish potential effects of seed coat colour on growth and development of chicory. More so, when the seed available is of different seed quality, the choice of certain seed is necessary to increase the productivity of chicory.

Manipulation of planting density in chicory can influence yield, canopy development and weed growth by manipulating crop competition for resources (Asghari et al. 2009a; Asghari et al. 2009b; Zafarbakhsh et al 2011; Panandeha et al. 2012; Asghari and Farahani 2014). Previous studies have reported environmentally specific variations in optimal planting density, with a consensus on 150 000 chicory plants per hectare as an optimal planting density (Baert and Van Bockstaele 1993; Asghari et al. 2009b). The genotype and the environment in which a plant is grown determine the production efficiency of a plant. Thus, specific planting populations have to be determined for new production sites and these should be included in best management recommendation to farmers. This study aimed at determining optimal planting population with respect to seed coat colour and weed management strategies.

5.2 Materials and methods

Chicory (*Chicorium intybus* L.) seeds from obtained from Nestle® Estcourt, KwaZulu-Natal and sorted according to seed colour into dark-, light- and mixed-coloured seeds. The chicory cultivar used in this study was ‘Orchies’.

5.2.1 Site description

A field experiment was planted at Ukulinga Research Farm [30°24'S, 29°24'E, 805 m above sea level (a.s.l)] from March to September under rain-fed conditions during the 2014/15 season. The farm is situated in Mkondeni, Pietermaritzburg, in the subtropical hinterland of KwaZulu-Natal province. Ukulinga represents a semi–arid environment and the soil is classified as Arcadia form, Lonehill family (Soil Classification Working Group 1991). Rain falls mostly in

summer, between September and April with rainfall distribution variying during the growing season (Swemmer et al., 2007), but the bulk of rain falls in November, December and early January. Occasionally, light to moderate frost occurs in winter (May - July).

5.2.2 Experimental design

The experiment was laid out in a split–split plot design, with three factors replicated three times in a completely randomized block design. The main factor was weeding strategy, with two levels: hand-weeding plus herbicide and hand-weeding only. Planting density was a sub-factor with three levels: 100000 (low), 150000 (mid), and 200000 (high) plants per hectare. The third factor considered was seed colour, separated into dark-, light- and mixed-coloured seeds. Seed colours were separated visually into different catergories, as differences were very distinct. The pre-emergence herbicide benfluralin (Balan®, Dow Agro-Sciences) was applied at 3 L ha^{-1} three weeks prior to planting. Application was carried out with a knapsack sprayer fitted with a flat fan nozzle. Plots were hand-weeded using hand-hoes at two and 10 weeks after planting. Low, mid and high planting densities were achieved by hand-sowing seeds at 0.2 m x 0.5 m, 0.15 m x 0.5 m, and 0.1 x 0.5 m intra-row and inter-row spacing, respectively. Seeds were sown at a planting depth of 5 cm into the soil. Individual plots were 1.5 m length by 2 m breadth. Main plots were 5.5 m length by 8 m breadth with 1 m pathways in between plots.

5.2.3 Agronomic practices

Before planting, the fallow land was ploughed, disked and rotovated. From planting to emergence, plots were irrigated to facilitate intial establishment. Irrigation was applied three times a week at 12 mm per application event. Prior to planting, soil samples were analysed for fertility. Based on the analysis, phosphorus (212 kg ha^{-1}) was applied as solid NPK 2:3:2 (22) and the balance of nitrogen (125 kg/ha^{-1}) was applied as lime-stone ammonium nitrate LAN (28% N).

5.2.4 Observations

Data were collected on the middle row of the plot while the boarder rows were excluded from data observations. Recorded data included leaf number per plant, plant height, and leaf area index (LAI) at various time intervals (66, 75, and 120 days after planting) during the growing season from five representative plants per plot. Leaf number was observed as the number of fully formed, fully unfolded leaves with at least 50% green area. Plant height was measured

using a tape measure from the soil surface to the tip of the youngest leaf. Leaf area index (LAI) was measured using the AccuPAR LP80 Ceptometer (Decagon Devices, USA) at midday (12 noon - 2 pm).

Harvesting chicory roots is usually carried out from 20 to 28 weeks after planting. Harvest maturity was defined as the time when plants were 20 weeks old. Upon harvest maturity, fresh roots in the experimental plot were harvested and the total fresh biomass was determined. Thereafter, five plants per plot were sampled for the determination of root fresh and dry weight. Roots and shoots were separated and individual fresh mass was determined. Root diameter was measured on the shoulder of the root. Roots and shoots were then oven-dried for four days at 85°C. Total biomass, root and dry root yield per plot were calculated as a function of planting density.

5.2.5 Statistical analysis

Recorded crop parameters were subjected to analyses of variance (ANOVA) using GenStat® 18th edition (VSN International, UK) to observe the difference between treatments and across time period. Means were separated using least significant differences (LSD, $P = 0.05$).

5.3 Results

The interaction of planting density, seed coat colour and weeding method (overall interaction) was only significant ($P \leq 0.05$) for biomass plot yield, and not significant ($P > 0.05$) for all the other data parameters collected. Despite the overall interaction not being significant for most parameters, individual factors and two-way interactions were significantly different for selected parameters.

5.3.1 Canopy characteristics

Leaf number was significantly ($P < 0.05$) affected by the interaction of seed coat colour and weed control method (Figure 5.1). Hand-weeded chicory had a higher leaf number than when herbicide plus hand-weeding was used. Under the latter treatment, leaf number for dark-seeded chicory had the lowest number of leaves followed by plants from light-coloured seeds, while mixed-coloured seed produced plants with the highest leaf number. Under hand-weeding, dark coloured seed had a tendency to produce the lowest leaf numbers followed by plants from mixed-coloured seeds, while light-coloured seed had the highest leaf number (Figure 5.1). On

the other hand, LAI was significantly affected by the interaction of seed coat colour, plant populations and weeding for the two observed time intervals (Figure 5.2). Overall, chicory grown under medium plant population had the highest LAI relative to low and high plant populations when the treatment of herbicide plus weeding was applied. On the other hand, and contrary to herbicide plus hand weeding, the highest LAI under hand weeding was observed under low plant populations (Figure 5.2). Mixed-coloured seed had the highest LAI across both weeding treatments. At 66 days after planting, chicory grown under hand-weeding had the highest LAI. At 75 days after planting, high plant population had the highest LAI under herbicide plus hand-weeding while the lowest LAI was observed under low planting density. When herbicide plus hand-weeding was applied, dark seeds produced the shortest plants (Figure 5.3), while light-coloured seeds produced taller plants and mixed-coloured seed had the tallest ones. Under hand-weeding, darkcoloured seeds resulted in the tallest plants followed by plants from light-coloured seeds, while mixed-coloured seeds produced the shortest plants.

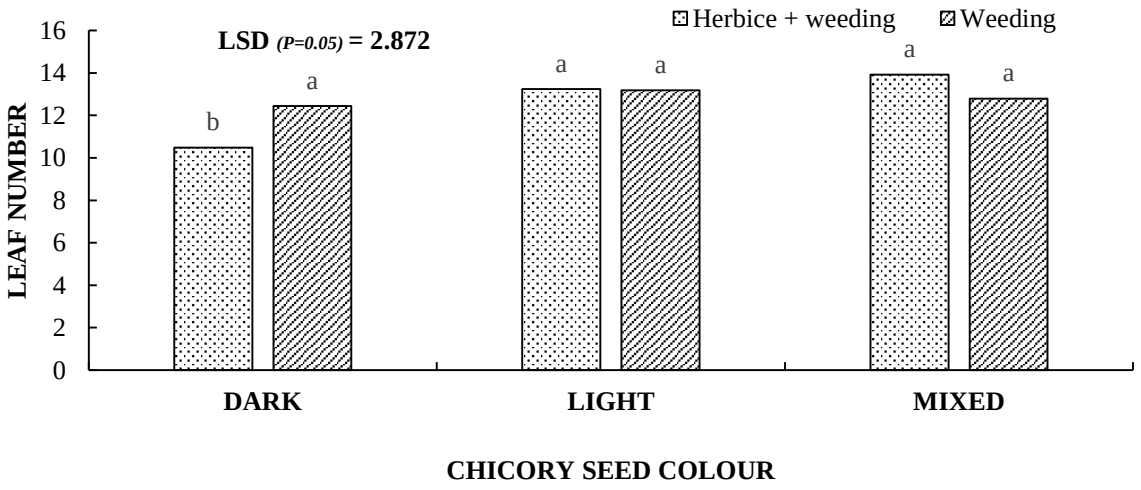


Figure 5.1: Comparison of chicory leaf number in response to the interaction of weeding method (herbicide plus hand-weeding, and hand-weeding only) and seed colour (dark-, light- and mixed-coloured seeds)

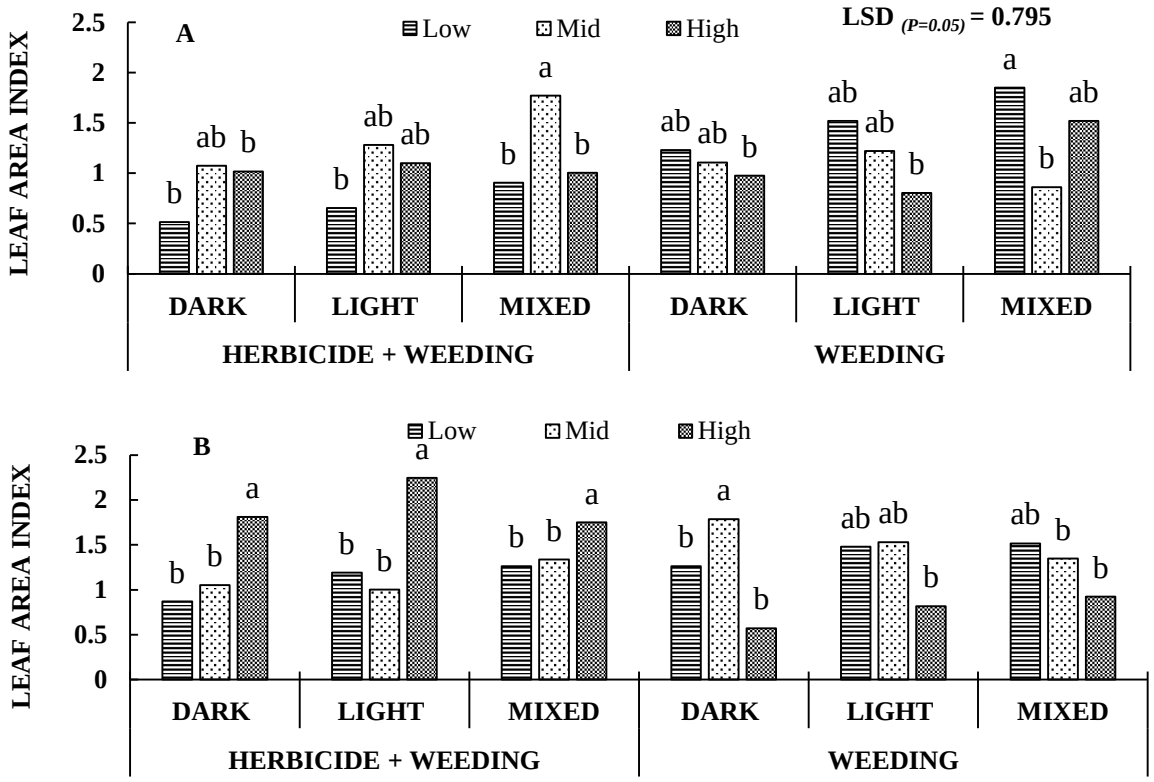


Figure 5.2: Comparison of chicory leaf area index in response to the interaction of weeding method (herbicide plus hand weeding, and hand-weeding only), seed colour (dark-, light- and mixed-coloured seeds) and planting density (low, mid, and high) measured 66 (A) and 75 (B) days after planting

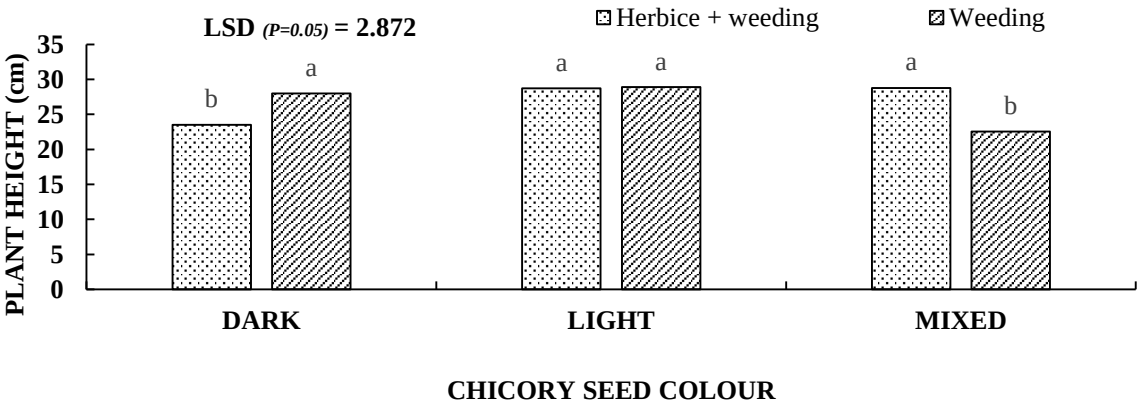


Figure 5.3: Comparison of chicory plant height in response to the interaction of weeding method (herbicide plus hand weeding, and hand-weeding only) and seed colour (dark-, light- and mixed-coloured seeds)

5.3.2 Chicory biomass and biomass plot yield

Total biomass per plant was not significantly (P > 0.05) affected by the overall interaction of weeding method, seed coat colour and planting density (Figure 5.4a); however, total biomass per plant was highly and significantly (P < 0.001) affected by the interaction of weeding method and seed coat colour (Figure 5.4b). Biomass plot yield was significantly affected by the overall interaction of weeding method, seed coat colour, and planting density. High planting density had relatively high yields, while low yields were recorded under low planting density.

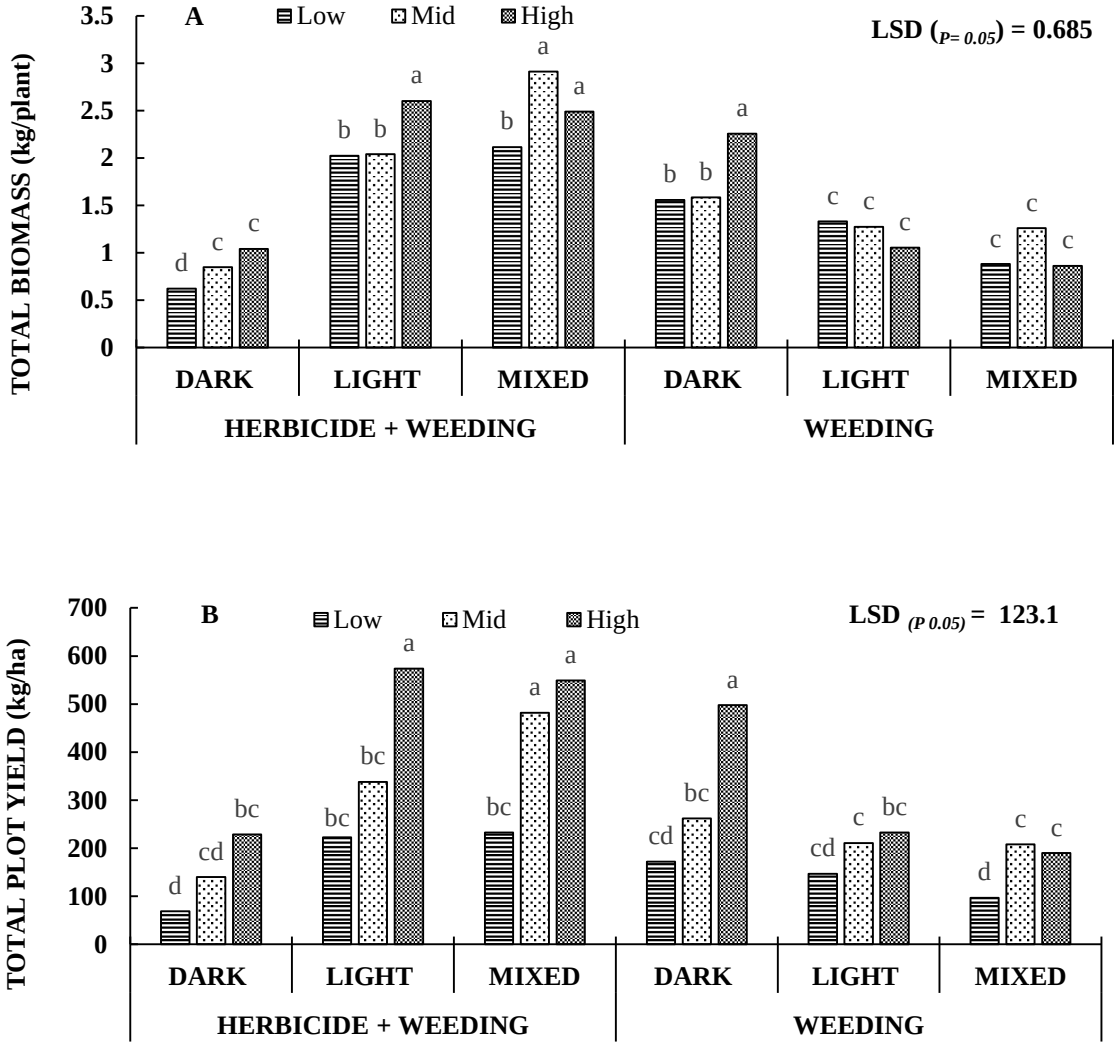
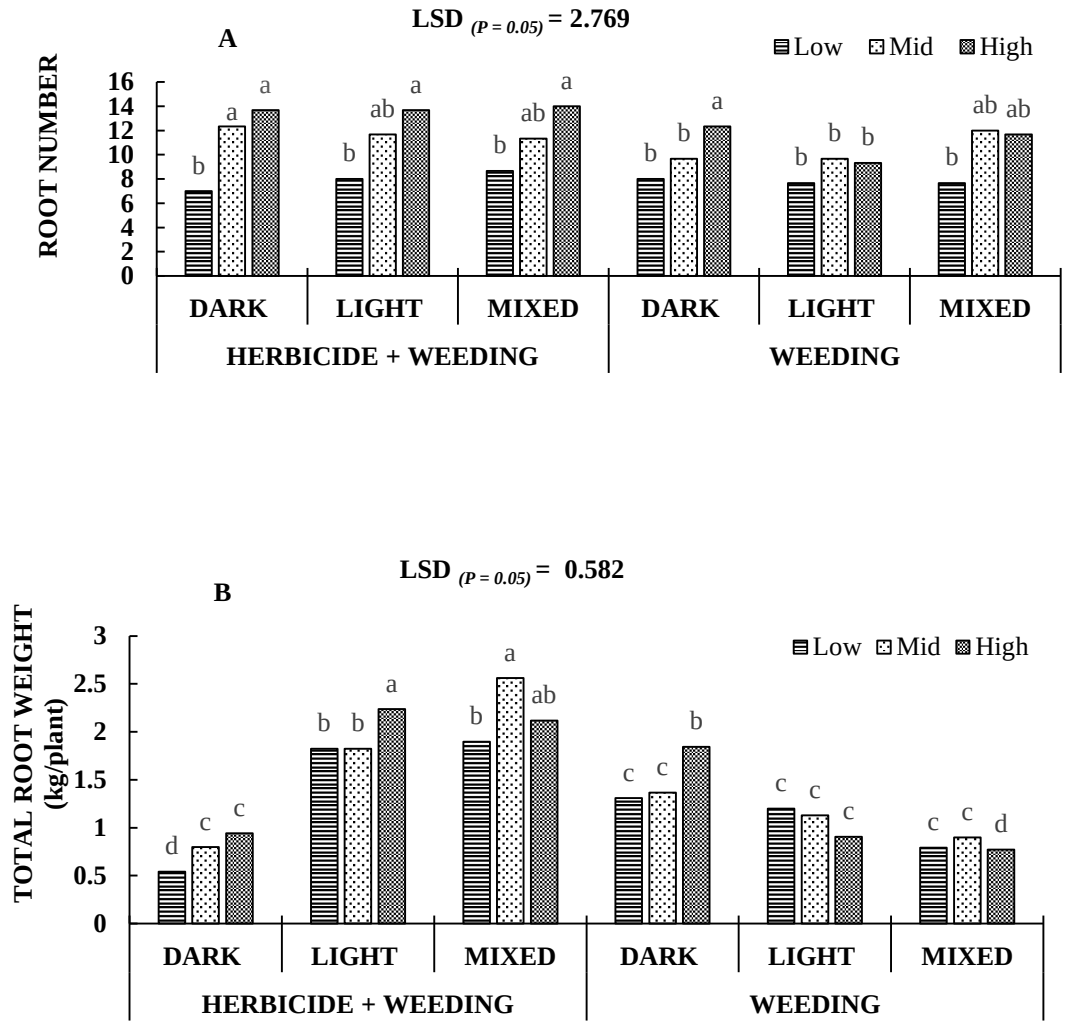
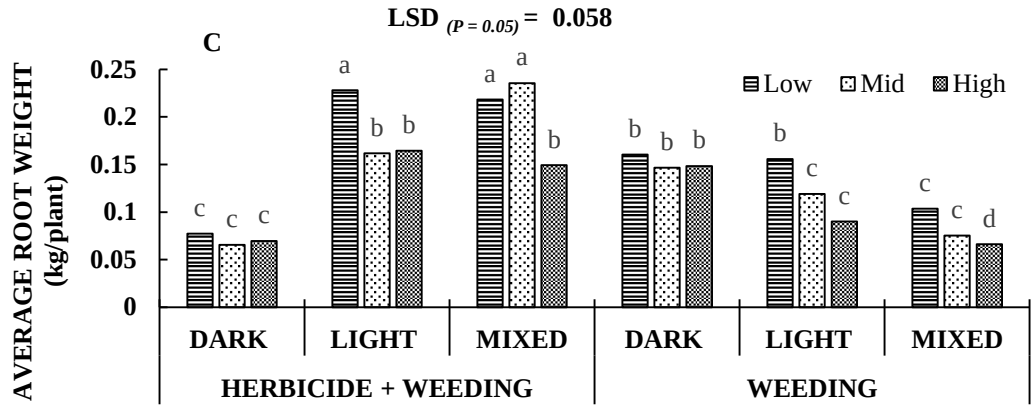


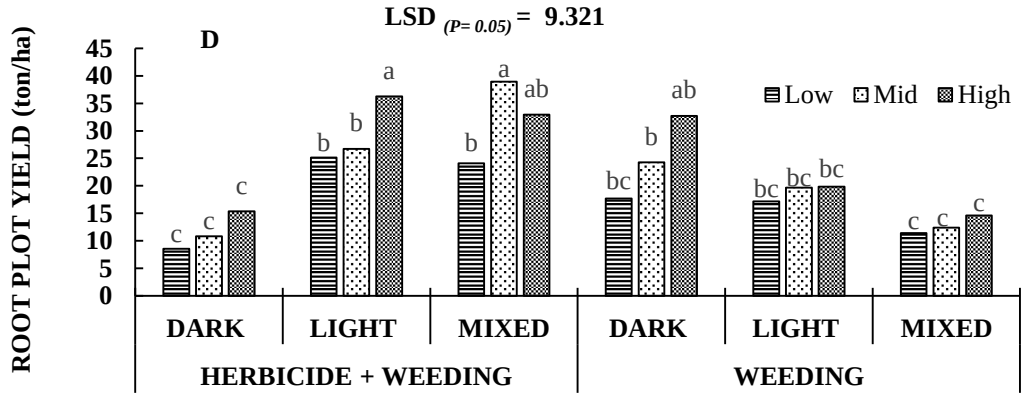
Figure 5.4: Total biomass (A) and total plot yield (B) of chicory under different weeding method (herbicide plus hand-weeding, and hand-weeding only), seed colour (dark-, light- and mixed- coloured seeds), and planting density (low, mid, and high) interaction

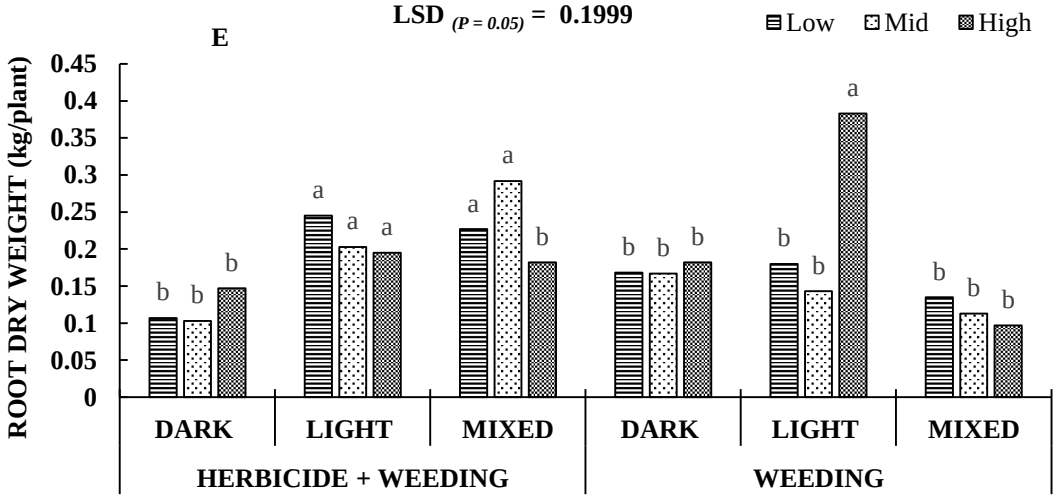
5.3.3 Root yield related parameters

Root number, average root diameter, root dry weight and fresh root plot yield and dry root plot yield were not significantly ($P > 0.05$) affected by the overall interaction of weeding method, seed coat colour, and planting density (Figure 5.5 a–f); however, the interaction of weeding method and seed coat colour significantly ($P < 0.05$) influenced average root weight, average root diameter and dry root plot yield; and significantly ($P < 0.001$) affected root number, total root weight and root plot yield.




LSD (P = 0.05) = 0.058
C
AVERAGE ROOT WEIGHT (kg/plant)
0.25
0.2
0.15
0.1
0.05
0
Low Mid High
DARK LIGHT MIXED DARK LIGHT MIXED
HERBICIDE + WEEDING
WEEDING


LSD (P= 0.05) = 9.321
D
ROOT PLOT YIELD (ton/ha)
45
40
35
30
25
20
15
10
5
0
Low Mid High
DARK LIGHT MIXED DARK LIGHT MIXED
HERBICIDE + WEEDING
WEEDING


LSD (P = 0.05) = 0.1999
E
ROOT DRY WEIGHT (kg/plant)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Low Mid High
DARK LIGHT MIXED DARK LIGHT MIXED
HERBICIDE + WEEDING
WEEDING

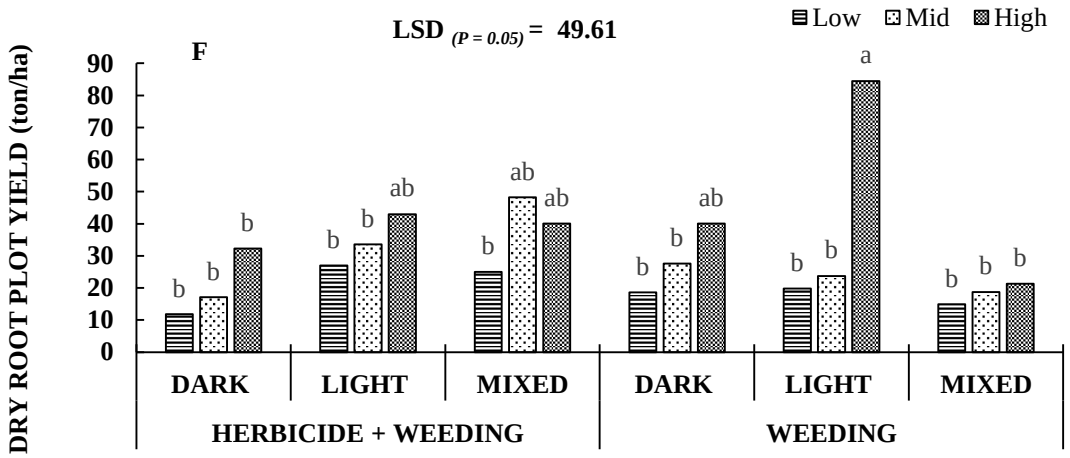


Figure 5.5: Root number (A), total root mass (B), average root mass (C), root plot yield (D), root dry mass (E), and dry root plot yield of chicory under different weeding methods (herbicide plus hand-weeding, and hand-weeding only), seed colour (dark-, light- and mixed-coloured seeds), and planting density (low, mid, and high) interaction

5.4 Discussion

Commercial herbicides are synthetic compounds that act on specific biochemical reactions or cause specific biochemical reaction of leafy green plant matter. While these may be crop-or species-specific, it has been observed that there are some reactions that can affect optimum functioning of non-target crop species. This could explain why leaves of chicory grown under herbicide plus hand-weeding had fewer leaves. Similar results of growth retardation have been observed by Ahuja et al. (2015) for *Avena fatua* growth rate and by Park et al. (2015) on *Lactuca sativa* (lettuce).

Biomass was significantly affected by seed coat colour and weeding (Figure 3.4). Seeds of different colours can be found among fruit in the same developmental stage or due to differences in the developmental stage. This may result in differences in seed quality and vigour. Darker seeds of chicory produced plants of the smallest canopy size based on leaf number (Figure 3.1), LAI (Figure 3.2) and plant height (Figure 3.3); suggesting that these seeds had less vigour. When dark seed colour was coupled with herbicide application, an even smaller canopy, in comparison with light- or mixed-coloured seed resulted. These observations are similar to those reported by Chibarabada et al. (2014) for dark-seeded bambara in comparison with the light-seeded ones. The inflorescence structure of chicory (capitual) could possibly

contribute to differences in seed maturity and could be aligned with the seed coat colour; some seeds of the inflorescence are already while other seeds continue to develop within the same inflorescence structure (McDonald and Copeland 1997).

The observed results of chicory biomass could be attributed to the observed plant canopy characteristics under the different treatments, as treatment combinations that resulted in larger canopy structures also produced the highest root biomass. A large canopy suggests that a plant can capture more light energy and is able to assimilate more carbohydrates and is able to translocate them from the leaves to the sink organ, the root. A large canopy can intercept solar radiation well, allowing photo-assimilates to be produced. These results imply that the use of either light- or mixed-colour seed in combination with hand-weeding can significantly improve biomass yield of chicory.

Despite plant biomass not being significantly affected by the overall interaction of weeding method, seed coat colour and plant density, increasing the number of plants per given space significantly improved chicory total yield. This would suggest that the recommended optimum plant densities of chicory may be low for the environment in which it was grown. Dark-coloured seeds performed poorly with regard to total biomass and total plot yield, when herbicide plus hand-weeding was applied; however, these seeds outperformed the light- and mixed-coloured ones when herbicide application was not included as part of the weeding strategy. This suggests that herbicide application has differential effects on chicory based on seed colour, and further research is required to gain insight into the mechanisms of this interaction. In general, total biomass and biomass plot yield were higher, when herbicide plus hand-weeding were applied compared with the weeding only treatment.

Although the application of herbicide retarded growth, it was apparent that it significantly improved chicory yield components (Figure 3.5). Regardless of seed coat colour and planting densities, it could be that herbicide application increased the overall harvest index (HI) by reducing partitioning of biomass to vegetative organs and directing carbohydrates to the root as the main storage organ. Harvest index is the ratio of reproductive yield to total plant biomass which measures the efficiency of photosynthate partitioning to harvestable product (Gur et al. 2010). The current drive for most agricultural crops is to increase the harvest index to increase productivity. The effect of herbicide on harvest index has been observed by Beigzadeh et al. (2013) who observed an increase in HI for pea.

5.5 Conclusion

The interaction of planting density, seed coat colour, and weeding method was only significant for total plot yield, and insignificant for the tested canopy, biomass and root yield characteristics. This suggests that no optimal crop stand exists with regard to the tested weeding methods and seed coat colour, with the exception of biomass plot yield. If the agronomic parameter of interest is biomass plot yield, however, the optimal plant density would be 200 000 plants ha^{-1}. Herbicide application tended to reduce agronomic performance in dark-coloured seeds, suggesting that further research is required to determine the effect of herbicide application on different chicory seed coat colours.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The availability of low quality seed is one of the major problems to chicory growing, often resulting in poor stand establishment and low yields. In addition, benfluralin, a herbicide controlling grass weed species is currently the only product registered in South Africa for weed control in chicory, no herbicides are available for broadleaf weed control therefore, economic yields are often not achieved. Hence, this study was aimed at investigating the association of seed colour variation and seed quality. The study was also aimed at assessing the response chicory seeds varying in seed coat colour to seed priming and to examine the influence of seed coat colour on the optimal plant population of chicory under two weed management practices.

6.1 Seed quality

Dark-coloured seeds are possibly more mature than light-coloured seeds as shown by the germination and GVI. However, shorter time taken by light-coloured seeds to reach 50% germination following the AA test could be linked to high levels of antioxidant properties for seed storability (Selvi et al. 2014). Greater water uptake of dark-coloured seeds than light-coloured seeds could be possibly associated with the permeability of the seed coat to water and this could be linked to thinner seed coats, seed coat cracking and scarifying which in turn influenced water uptake Lui et al. (2007).

6.2 Seed priming

The study showed that priming chicory seeds improves seed quality. The choice of priming solution depends on the availability of resources. Osmo-priming was more effective in improving seed quality of chicory however where resources are limiting hydro-priming is also effective.

6.3 Optimal plant population

Crop establishment in chicory was erratic and for yield maximisation, this study recommends optimal plant density as 200 000 plants ha^{-1}. With respect to weed control method, it was observed that the registered herbicide affects the performance of dark coloured seeds.

6.4 Conclusions

To improve the overall seed quality of chicory there is need to develop cultivars that produce seeds that mature at the same time. In addition, there may be need to improve and introduce seed production strategies that ensure uniformity during chicory seed production. Future research should determine whether chicory seeds varying in seed colour differ in phenolic composition as well as determine whether variations in seed performance may be linked to differences in the phenolic concentrations. Differences in seed colour could suggest differences in seed proteins and soluble sugar composition. Future research should also determine whether chicory seeds varying in seed colour differ in genetic composition and whether such a potential genetic variation could be aligned with seed quality. With respect to priming duration, results were inconclusive suggesting the need for further research. In addition, it is important to conduct field trial experiments to conclusively determine effects of seed priming on chicory growth, development and yield. With regards to the effect of herbicide application on seeds differing in seed coat colour, results suggest the need for further research on this effect.

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