South African Journal of Botany 110 (2017) 68–74

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Germination characteristics of dimorphic honeybush (*Cyclopia* spp.) seed

J. Koen [a,b,*], M.M. Slabbert [a], C. Bester [b], F. Bierman [c]

[a] Department of Horticulture, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa
[b] Agricultural Research Council Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa
[c] Department of Biomedical Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

ARTICLE INFO

Available online 9 April 2016

Edited by E Joubert

Keywords:
Cyclopia
Honeybush
Seed
Anatomy
Germination

ABSTRACT

Cyclopia is a genus of leguminous shrubs endemic to the fynbos biome of South Africa. *Cyclopia* spp. are used to make honeybush tea for which a high market demand has led to the overharvesting of *Cyclopia* species in the wild. Consequently, it has become important to study the cultivation requirements of honeybush in order to support farmers with the cultivation of this crop. Honeybush seeds are known to have either physical or combinational dormancy upon maturity, depending on the species. The plants also produce colour-dimorphic (green and brown), mature seeds in the same pod. A study of honeybush (*Cyclopia maculata*, *Cyclopia genistoides* and *Cyclopia subternata*) seed anatomy was carried out using light and dissection microscopy. It is hypothesized that seed physical dormancy is attributable to a relatively thick (3.5 μm) cuticular layer of the integument, a layer of macrosclereid cells, and the presence of a hygroscopically activated hilar valve. There is an absence of other structural openings by which moisture may enter the seed. No consistent structural differences were found amongst the three species or their colour-dimorphic seed. Seeds of the same three honeybush species were divided by colour and their germination response to selected variables (scarification, stratification, seed age and germination temperature) was evaluated. Three dormancy-breaking treatments (wet heat, dry heat and microwave energy) were identified as potential alternatives to conventional sulphuric acid scarification. Highest germination percentage of scarified seed for the three species tested was obtained after 3 weeks of cold stratification at 2 °C and incubated at 15 °C. Brown *C. subternata* seeds stored for three years gave a significantly higher germination percentage than seed stored for one year only. In non-treated seed, brown seeds had a higher germination percentage than green seeds. In treated seed (i.e. scarified and/or stratified), green seeds had a better germination percentage than brown seeds.

1. Introduction

"Honeybush" is the shared English language common name for all 23 known species of *Cyclopia*, a genus in the Fabaceae (legume) family and of the Podalyrieae tribe. Members of the Podalyrieae tribe are long-lived perennials that vary extensively in growth habit from small, multi-stemmed shrubs to tall, single-stemmed trees (Schutte and Van Wyk, 1996). *Cyclopia* includes the woody, perennial shrub species that are used to prepare honeybush tea (Schutte and Van Wyk, 1996; Joubert et al., 2011). Demand for honeybush tea exceeds supply and most of the annual harvest is taken directly from the wild, a destructive and unsustainable practice (Bester, 2013; Department of Agriculture, Forestry and Fisheries, 2013). In the mid 1990s, various honeybush cultivation programs were initiated to meet this need (Joubert et al., 2011). Honeybush seed does not have a good germination percentage even under ideal environmental conditions and is therefore acknowledged to possess one or more forms of dormancy (Baskin and Baskin, 2004). Honeybush also produces dimorphic seed with both green and brown mature seeds in the same pod (M. Motsa, pers. comm., 2015). Seed dimorphism has been known to affect germination and may sometimes be an indication of divergent survival strategies (Dechang et al., 2012; Wang et al., 2012; Baskin and Baskin, 2014). The study of seed anatomy can assist in the interpretation of the data obtained through germination trials (Cutler et al., 2008). Treatments of smoke–water, stratification, heat shock, hot water and H_2SO_4 cold scarification have previously been investigated to improve germination of honeybush seeds, with mixed results (Hanley et al., 2001; Mbangcolo, 2008). Seeds scarified with sulphuric acid outperformed any other scarification treatment in terms of germination rate and percentage, but greener alternatives to sulphuric acid are needed. Stratification and smoke–water stimulation (octanoic acid) were shown to increase seed sensitivity to ethylene, which Whitehead and Sutcliffe (1995) and Sutcliffe and Whitehead (1995) proposed as the most important hormonal aid in the germination of honeybush. The age and species of the seed as well as the source were also found to have an effect on germination response (Mbangcolo et al., 2013). The aims of this study were to 1) study the anatomy of honeybush seeds to better understand germination response and 2) to investigate differences in germination

* Corresponding author at: Private Bag X5026, Stellenbosch 7599, South Africa. Tel.: +27 21 804 3495; fax: +27 21 809 3002.
E-mail address: koenj@arc.agric.za (J. Koen).

http://dx.doi.org/10.1016/j.sajb.2016.03.006
0254-6299/

response of selected *Cyclopia* species with regard to seed colour (dimorphism), scarification, stratification, seed age and germination temperature.

2. Materials and methods

Images of honeybush seeds (*Cyclopia maculata*, *Cyclopia genistoides* and *Cyclopia subternata*) were taken using dissection and light microscopy. Seeds were softened by soaking in water overnight (12 h). For fixation these seeds were placed in a 10% neutral buffered formalin solution containing 105 g sodium hydroxide, 465 g di-sodium orthophosphate and 2.5 L of 37% formaldehyde dissolved in 25 L of distilled water. After formalin fixation, seeds were prepared for wax infiltration. The penetration of paraffin wax into the seed was found to be impeded in intact seeds. In order to improve results, the seeds were cut into halves, the location of the cut depending on the angle of the desired final section, and folded in permeable paper after fixation, before being placed into the Sakura Tissue Tek VIP 5 Junior Vacuum Infiltration Processor™ for automatic preparation. The processor carried out dehydration with ethanol, xylene was used as a clearing agent and paraffin wax was used as the infiltration media. The following day, the seeds were embedded in paraffin wax to make wax blocks with the Sakura Tissue Tek Embedding/Cryo console. Once the paraffin wax had hardened, the blocks containing the seeds were sectioned to a thickness of 5 μm using a Leica RM 2125 rotary microtome. The prepared sections were then stained with certified biological stains (Toluidine Blue, Safranin O or Masson Trichrome) and mounted onto slides. Histological images and measurements were taken using a Nikon Digital System DS-L3.

The germination study was carried out by using a randomized complete block design. Seed sources were sorted into green and brown seeds, equal numbers of each seed colour were used in the experiments. Sterile Petri dishes were lined with two sheets of Grade 292 Munktell™ filter paper and moistened with purified water. LTIE20 Labcon™ Low temperature incubators were used throughout. The Petri dishes were placed in individual polyethylene pockets to minimize moisture loss. Seeds were incubated at 22 °C in interrupted darkness, with the exception of those used in the germination temperature experiment. Data collection took place every 24 h for 14 days from the day germination was first observed. Seeds were considered germinated as soon as the radicle visibly emerged from the testa.

2.1. Scarification

Five methods of scarification, two chemical and three mechanical, were tested on *C. subternata* seeds from Elsenburg Research Farm near Stellenbosch in the Western Cape of South Africa (−33.923797, 18.872996). For the chemical scarification study, seeds were either soaked in naturally fermented white vinegar (5% acetic acid) for 6, 12, 18, 24, 30 and 36 h or hydrogen peroxide at 10, 20 and 30% concentration for 1, 6 and 12 h. For the mechanical scarification study, seeds were either exposed to dry heat scarification using an oven set at 80, 90 and 100 °C for 2, 4 and 6 min, wet heat scarification by immersion in boiling water for 20, 40, 60, 80, 100 and 120 s or microwave energy scarification at 180, 540 and 900 W for 10, 20 and 30 s. Positive control was sulphuric acid scarification according to guidelines set out by the Agricultural Research Council (Bester and Cronje, 2013). Negative control was a 12 hr soak in purified water at room temperature.

2.2. Stratification

Stratification periods of 1, 2, 3, 4 and 5 weeks at 2 °C were tested on three species of honeybush. *C. subternata* from the area Kanetberg near Barrydale (−33.924648, 21.028496), harvested during 2011, *C. maculata* seeds from Genadendal (−34.031375, 19.516152), harvested during 2012 and *C. genistoides* seeds from the area Koksrivier near Pearly Beach (−34.660699, 19.501336), harvest year unknown, were used in this study. Seeds were scarified in sulphuric acid, according to guidelines set out by the Agricultural Research Council (Bester and Cronje, 2013), in order to eliminate the influence of physical dormancy on the outcome of the study.

2.3. Seed age

Seeds of three cultivated *C. subternata* clones (SGD-7, SHL-2, SKB-11) were sourced for three consecutive years (2011 to 2013) from Elsenburg Research Farm near Stellenbosch in the Western Cape of South Africa (−33.923797, 18.872996). No scarification or stratification was done in order to identify potential changes in seed coat permeability with age.

2.4. Germination temperature

Germination temperatures of 10, 15, 20, 25 and 30 °C were tested on three species of *Cyclopia*. *C. subternata* from Elsenburg Research Farm near Stellenbosch, 2012, *C. maculata* from Nietvoorbij Research Farm near Stellenbosch (−33.923797, 18.872996), 2012 and *C. genistoides* from the farm Toekomst near Bredasdorp (−34.530669, 20.047804), harvest year unknown, were used in this study.

3. Results and discussion

3.1. Honeybush seed anatomy

Honeybush seeds are dicotyledonous and share many anatomical features with other legume species (Ma et al., 2004). Their external anatomy consists of an elaiosomic aril attached around the hilum, a sealed raphe and an extrahilar region, also known as the seed-coat or integument. There was no visible lens or micropyle on any of the seed samples investigated. It is however possible that the micropyle can be observed at greater magnifications, or that it exists beneath the cuticle layer and is thus hidden from view (Dübbern de Souza and Marcos-Filho, 2001). The anatomy of the seed was investigated using light microscopy and was found to consist of four seed coat layers and a substantial albuminous endosperm which completely surrounds the unattached embryo (Fig. 1). The hilar tissues and layers of the seed coat are thought to prevent water uptake, resulting in seed physical dormancy.

3.1.1. The hilum

The hilum is a scar which remains on the seed at the point where the funiculus attached to the body of the ovule, connecting the ovule to the placenta (Mauseth, 2014). The hilar slit (or hilar fissure) serves as a natural opening for water and gas exchange for many species, but members of the Papilionoideae subfamily, including *Cyclopia*, have a specialized adaptation of the hilar slit and associated funicular tissue which is called the hilar valve (Figs. 2 and 4) (Gumula, 2014). The type and arrangement of cells in this structure causes it to function as a hygroscopically-activated, one-way valve which is known to contribute towards physical dormancy in other species (Kozlowski, 1972). The valve opens when relative humidity outside the seed is lower than that within the seed, allowing moisture to escape. The valve seals when the relative humidity outside the seed is higher than within the seed, effectively preventing moisture from entering the seed. The structure of the valve is such that, when closed, even vapours are unable to penetrate the seed (Kozlowski, 1972). The hilar valve is composed of funicular tissue, which is made up of two layers of palisade cells, and a tracheid bar (Fig. 4).

The tracheid bar structure occurs only in papilionoid legume seeds and extends from the micropylar side of the hilum to the opposite end (Lersten, 1982; Tran and Cavanagh, 2013). The tracheid bar contains large cells that are lignified and pitted to the point of perforation

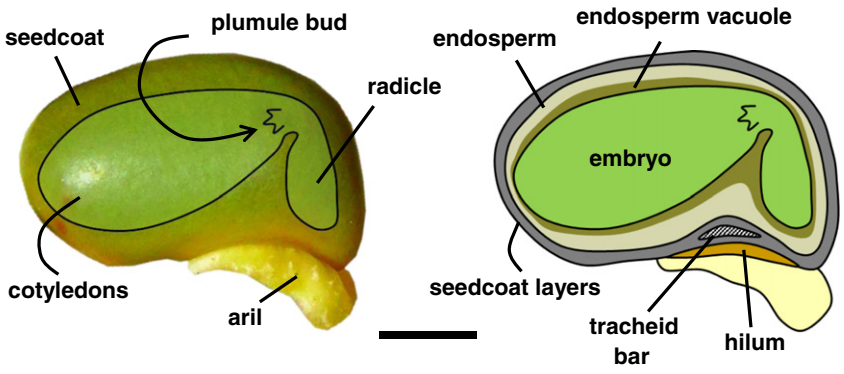

Fig. 1. A *Cyclopia* seed in longitudinal section; scale bar = 1 mm.

(Fig. 4). Lersten (1982) found that the tracheid bar is relatively uniform in structure across different tribes of papilionoid legumes and suggested that it may serve to enhance the efficiency of gas exchange.

3.1.2. The seed coat

The seed coat or testa is the protective outer covering of a mature seed. The seed coat consists of layers called integuments that develop from maternal tissue (sacs of the ovule) and is therefore determined by maternal genotype (Mauseth, 2014). Dübbern de Souza and Marcos-Filho (2001) mentioned that the seed coat functions in the preservation of the integrity of the seed parts against injury by mechanical damage and/or attack by pests and disease. The seed coat is also a modulator of seed–environment relationships, regulating gaseous exchange and imbibition (Dübbern de Souza and Marcos-Filho, 2001; Ma et al., 2004).

The honeybush seed coat, in accordance with many other legume species, was found to consist of four layers: 1) the outer, waxy cuticle layer, 2) the epidermis or macrosclereid layer, 3) the hypodermis or lagenosclereid layer, and 4) the inner parenchyma layer (Fig. 3). The macrosclereid layer lacks both a clear "light line" and uniformity in cellular structure. The layer was observed to occur as both polycellular and unicellular in random distribution on a single seed for all species and seed colours investigated (Fig. 3). The light line is a pseudo-structure which appears in the seed macrosclereid layer of many species, including *Glycine max* and *Canna indica* (Harris, 1987; Geneve, 2010). The light line is thought to appear as a result of differences in refraction due to variations in chemical composition in the upper and lower parts of the macrosclereid cell layer, differences in cellulose microfibril orientation, or the simply the tight compression of the cell walls at the same level in adjacent cells (Ma et al., 2004; Baskin and Baskin, 2014). The macrosclereid layer is present in the seed coats of many species and does not necessarily prevent imbibition, but a tight cellular arrangement and a prominent, unbroken light line may be visible indications that water uptake through the macroscleried layer is inhibited (Harris, 1987; Maiti, 2012). In *Cyclopia* the light line was observed to be faint or absent altogether. Furthermore, whereas the macrosclereid cell lumen of other species, such as canna, are severely constricted due to the tight compaction of these columnar cells, the cell lumen of the honeybush seed macrosclereids seem to be relatively open (Fig. 3) (Geneve, 2010). The spacious cellular arrangement of the cells suggests that the macrosclereid layer of honeybush seeds may be relatively non-dense (Maiti, 2012). It is not known whether the macrosclereid layer causes physical dormancy in honeybush or merely plays a role in regulating the rate of water uptake, thereby preventing imbibition damage (Asiedu et al., 2000). Because the seeds were imbibed prior to sectioning, the possibility that the macrosclereid layer has greater density/uniformity when dry cannot be eliminated. The presence of hydrophobic substances within the macrosclereid layer would also indicate that the layer is a significant water barrier, but this could not be verified in the present study. Future studies may investigate these possibilities.

The seed cuticle is a waxy, water-repellant layer which serves as the seed's outermost barrier to water uptake and effectively prevents imbibition when intact (Ma et al., 2004; Shao et al., 2007; Geneve, 2010;

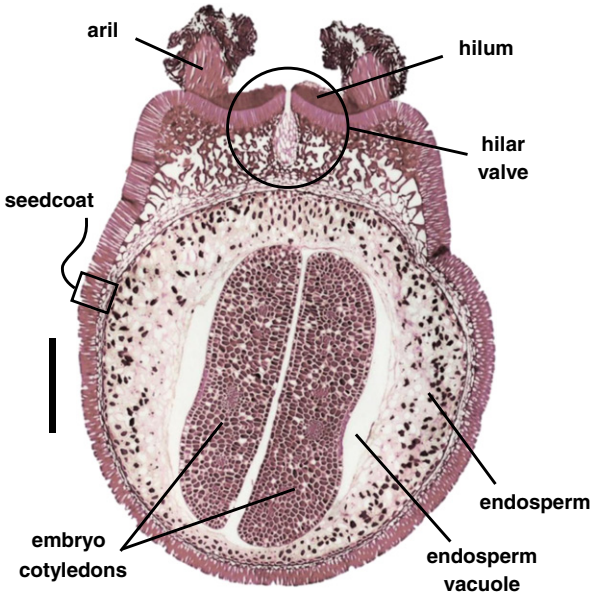

Fig. 2. Cross section through the hilum, perpendicular to the longitudinal axis, of an imbibed *Cyclopia* seed, scarified by a 60-min exposure to 98% sulphuric acid, under a light microscope; scale bar = 500 μm.

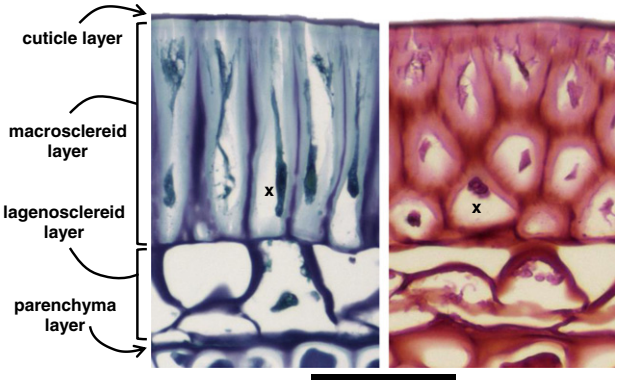

Fig. 3. *Cyclopia* seed coat layers seen under a light microscope; left = section showing a unicellular macrosclereid layer, right = section showing a polycellular macroscleried layer, x = cell lumina, scale bar = 50 μm.

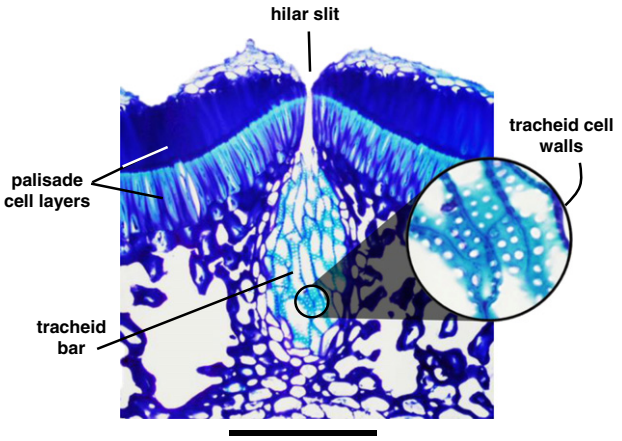

Fig. 4. *Cyclopia* hilar valve in section, perpendicular to the longitudinal axis, under a light microscope; scale bar = 500 μm.

Smýkal et al., 2014). Ma et al. (2004) found that microscopic cracks in the cuticle of soybeans were the primary factor affecting seed coat permeability. During our study, the cuticle layers of *C. maculata*, *C. genistoides* and *C. subternata* were measured at an average thickness of three micrometers (3 μm) for both seed colours. The cuticle thicknesses of other cultivated legume species such *Phaseolus lunatus* (Aniszewski et al., 2006) and *G. max* (Ma et al., 2004) have been measured at <1 μm, signifying that *Cyclopia* species have a relatively thick cuticle layer when compared to that of other legume species. It thus seems that the cuticle layer may play a major role in the impediment to water-uptake, but further research is needed.

No consistent anatomical differences were found when comparing the species and dimorphic seed in this study. Differences in seed morphology and germination response could not be explained by investigation of the seeds' cellular structure. These differences may be biochemical but were not investigated in this study, which only serves as an introduction to the anatomy and histology of *Cyclopia* seeds. Future studies may look more closely at this subject using more specialized microscopy techniques.

3.2. Honeybush seed germination

3.2.1. Scarification

Neither vinegar nor hydrogen peroxide scarification were effective as alternative scarification methods; germination percentage (GP) was

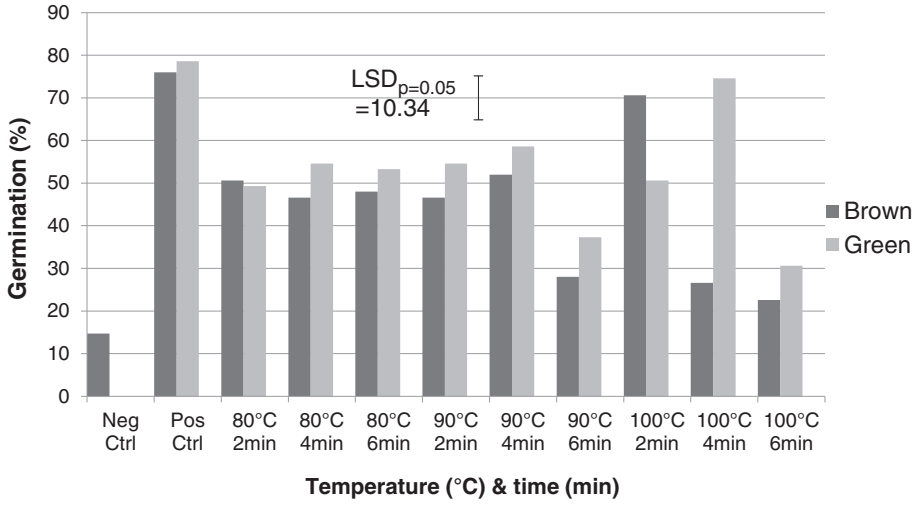

Fig. 5. The effect of dry heat scarification and seed colour on germination percentage of *Cyclopia subternata* after 14 days of incubation at 22 °C in interrupted darkness; pos ctrl = positive control, neg ctrl = negative control.

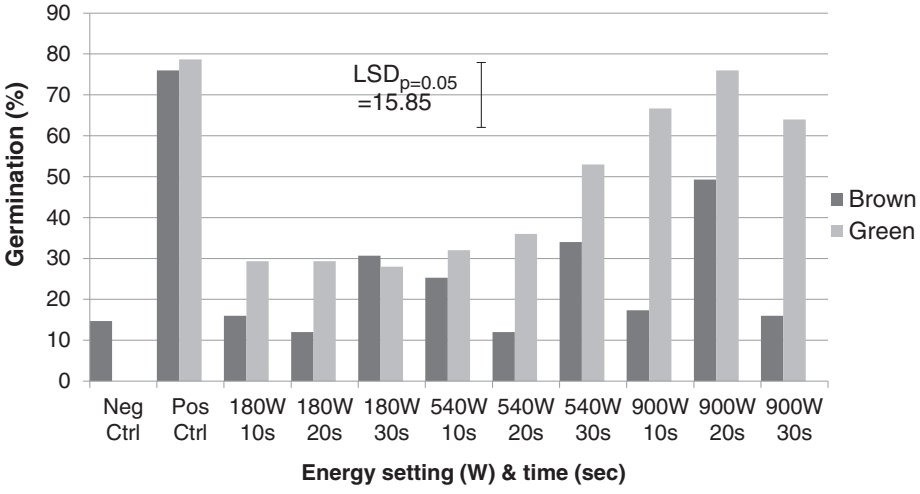

Fig. 6. The effect microwave energy scarification and seed colour on the germination percentage of *Cyclopia subternata* after 14 days of incubation at 22 °C in interrupted darkness. Pos ctrl: positive control; Neg ctrl: negative control

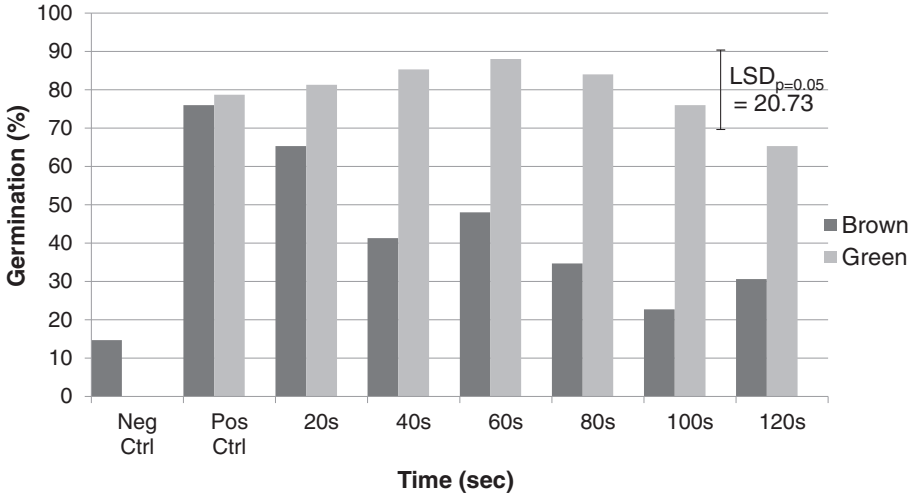

Fig. 7. The effect of wet heat scarification and seed colour on germination percentage of *Cyclopia subternata* after 14 days of incubation at 22 °C in interrupted darkness; pos ctrl = positive control, neg ctrl = negative control.

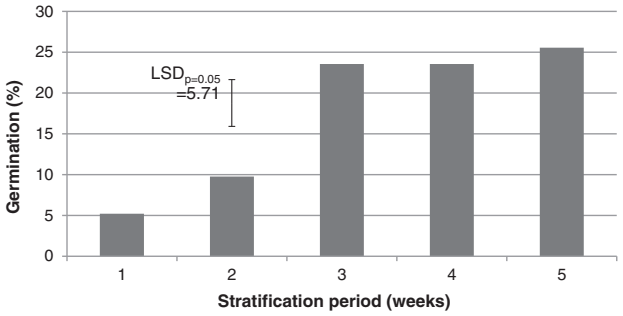

Fig. 8. The effect of stratification period at 2 °C on the germination percentage of dimorphic *Cyclopia* spp. seed after 14 days of incubation at 22 °C in interrupted darkness (data pooled).

less than or equal to the negative control (data not shown). Dry heat scarification improved GP to the level of the positive control at 4 min/100 °C for green seed (74.6%) and 2 min/100 °C for brown seed (70.6%) (Fig. 5). Microwave energy scarification improved GP to the level of the positive control at 10, 20 and 30 s scarification at 900 W for green seed (65–76%) (Fig. 6). Wet heat scarification improved GP to the level of the positive control at all treatment lengths for green seeds (65–88%) and at ≤20 s for brown seeds (65.3%) (Fig. 7).

3.2.2. Stratification

Maximum GP of 24–26% was obtained at ≥3 weeks of stratification for all three species and their dimorphic seed (Fig. 8). There was no significant difference in GP response to stratification length amongst the three species. A significant difference in GP was observed between the two seed morphologies of *C. subternata* (green 33% and brown 16%) and *C. maculata* (green 26.1% and brown 6.6%), this difference was not correlated to stratification period (Fig. 9).

3.2.3. Seed age

GP was low as expected of honeybush seeds that were neither scarified nor stratified. The GP of brown *C. subternata* seeds improved significantly with seed age (8.4% for 2011 and 1.7% for 2013). There was a significant difference in GP between the seed morphologies for seeds harvested in 2011 (green 1.7% and brown 8.4%). Although GP also increased for green seeds over the three years (0.4–1.7%), the increase was not significant (Fig. 10).

3.2.4. Germination temperature

The highest GP for all three species and their dimorphic seed was obtained at 15 °C (32%) (Fig. 11). There was no significant difference in GP response to germination temperature amongst the three species. A significant difference in GP was observed between the two seed morphologies (green 41.3% and brown 24.8%) of *C. subternata*, but this

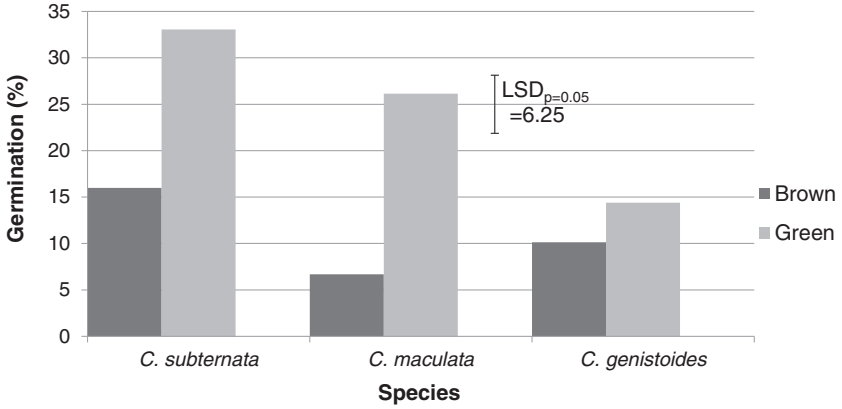

Fig. 9. The effect of species and seed colour on the germination percentage of *Cyclopia subternata*, *Cyclopia maculata* and *Cyclopia genistoides* seed stratified at 2 °C for 1–5 weeks (data pooled) after 14 days of incubation at 22 °C in interrupted darkness.

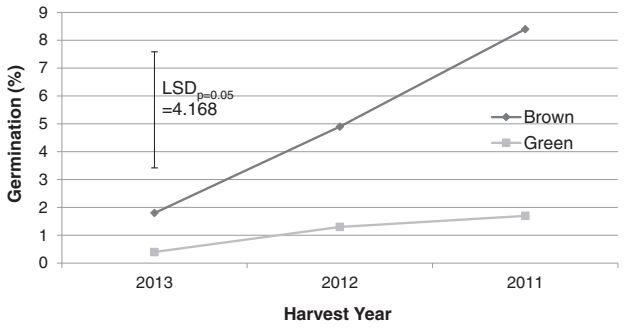

Fig. 10. The effect of seed age and seed colour on the germination percentage of *Cyclopia subternata* after 14 days of incubation at 22 °C in interrupted darkness.

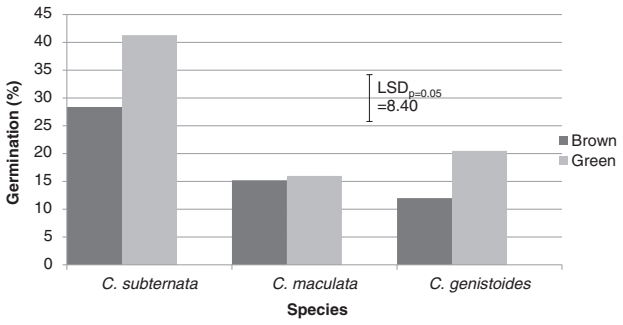

Fig. 12. The effect of species and colour on germination percentage of *Cyclopia* spp. after 14 days of incubation at 22 °C in interrupted darkness.

difference was not correlated to variation in germination temperature (Fig. 12).

Dry heat, wet heat and microwave energy seed scarification were found to be as successful as traditional sulphuric acid scarification for *C. subternata* under certain conditions, and could be employed as alternative scarification techniques in order to retain organic labelling. Future studies may refine the techniques to maximize results. There was a difference in GP response to the scarification treatments between the dimorphic seed, with green seed generally having a higher GP. The latter is attributable to high seed mortality rates in brown seed following treatment, indicating that brown seeds may be more vulnerable to damage than green seeds. The dramatic fluctuations in GP observed in dry heat scarification at 2 and 4 min for 100 °C may signify changes to the hilar valve aperture. It is possible that the variation in temperature/humidity conditions created as a result of dry heat exposure may have triggered the hilar valves to open (increasing permeability and GP) or close (decreasing permeability and GP). Microwave energy scarification was effective at 900 W for improving GP in green seeds but the GP of brown seeds remained lower than the positive control. Wet heat scarification was found to be particularly effective to improve GP in *Cyclopia* spp., complementing the study by Mbangcolo et al. (2013). It was found that green seeds could better survive long exposure times to wet heat than brown seeds, which became rapidly damaged resulting in seed mortality.

The optimum stratification period was found to be a minimum of 3 weeks for all three species, confirming the results of an earlier study by Whitehead and Sutcliffe (1995). Seed mortality during stratification was high for brown seeds, resulting in a significantly higher GP for green seeds than brown in *C. subternata* and *C. maculata.* The *C. genistoides* seeds used in this experiment were not as clearly colour distinct as the other two species, making accurate separation by colour (green and brown) difficult. The mixed ages and sources of the seed may influenced results.

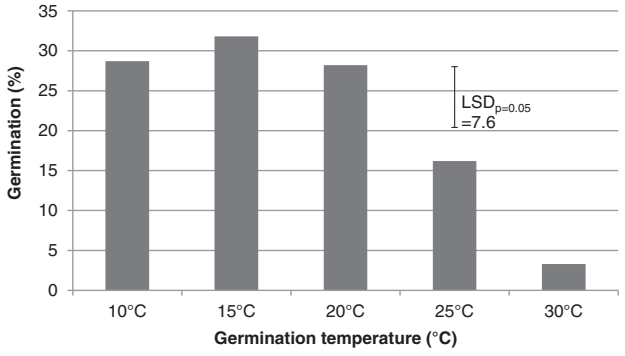

Fig. 11. The effect of germination temperature on germination percentage of dimorphic *Cyclopia* spp. after 14 days of incubation at 10, 15, 20, 25 and 30 °C in interrupted darkness (data pooled).

Germination percentage was found to increase with seed age over the three seasons tested, presumably due to increasing seed permeability. The improvement was significant for brown seed but not for green. Seed colour has been known to be an indicator of permeability in other species, such as *Ononis sicula* Guss (Gutterman and Evenari, 1972). It is probable that microscopic cracks form in seed coat over time due to loss of seed coat lipids as suggested by Zeng et al. (2005) or mechanical stress caused by fluctuations in environmental conditions such as temperature and humidity (Ma et al., 2004). The observed improvement in GP over three years was insufficient to obviate scarification, but the peak was not found. Future studies may measure the permeability vs. viability of seeds older than 3 years.

With regard to germination temperature, GP was found to be highest around 15 °C and decrease significantly between 15 °C and 25 °C. Previous studies of *Cyclopia* germination, such as those conducted by Whitehead and Sutcliffe (1995) and Mbangcolo et al. (2013), used incubation temperatures of 22–28 °C, this practice will have to be re-evaluated. The mixed ages and sources of the seed could also have had an influence on the results.

Seed dimorphism was found to play a role in regulation of germination response in the *Cyclopia* spp. tested. Green seeds were observed to generally be more "hardseeded" (i.e. more impermeable) than brown, while brown seeds were more susceptible to degradation during storage and damage during pre-treatments such as scarification and stratification. A correlation between level of colour distinction and level of divergence in germination response was observed: the more colour-distinct the seeds, the more divergent their germination response.

Acknowledgements

Thanks go to the Tshwane University of Technology and the Agricultural Research Council, as well as the Department of Science and Technology (DST) and the Monetary Treasury Economic Fund (MTEF) (DST/CON 0023/2015), for providing funding for this study. We would also like to acknowledge Prof Ben-Erik van Wyk for his assistance in the identification of seed cell layers.

References

Aniszewski, T., Karttunen, A.L., Hyvarinen, H., 2006. Structure of *Phaseolus lunatustesta* at its central point. Acta Biologica Cracoviensia 48 (1), 69–76.
Asiedu, E.A., Powell, A.A., Stuchbury, T., 2000. Cowpea seed coat chemical analysis in relation to storage seed quality. African Crop Science Journal 8 (3), 283–294.
Baskin, C.C., Baskin, J.M., 2004. A classification system for seed dormancy. Seed Science Research 14, 1–16.
Baskin, C.C., Baskin, J.M., 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, USA.
Bester, C., 2013. A model for commercialisation of honeybush tea, an indigenous crop. Acta Horticulturae 1007, 889–894.
Bester, C., Cronje, C., 2013. Manual for Propagation of Honeybush Seedlings and Cuttings. Agricultural Research Council, Stellenbosch (Training material).
Cutler, D.F., Botha, C.E.J., Stevenson, D.W., 2008. Plant Anatomy: An Applied Approach. Blackwell Publishing, USA.

Dechang, C., Baskin, C., Baskin, J., Yang, F., Huang, Z., 2012. Comparison of germination and seed bank dynamics of dimorphic seeds of the cold desert halophyte *Suaeda corniculata* subsp. *mongolica*. Annals of Botany 110, 1545–1558.
Department of Agriculture, Forestry and Fisheries, 2013. The Agri Handbook for South Africa, 2013/2014. Rainbow SA Publishing, Pretoria.
Dübbern De Souza, F.H., Marcos-Filho, J., 2001. The seed coat as a modulator of seed–environment relationships in Fabaceae. Brazilian Journal of Botany 24 (4), 365–375.
Geneve, R.L., 2010. Morphological characteristics of seeds with physical dormancy. Meeting of the International Plant Propagators' Society, North Carolina. USA.
Gumula, I., 2014. Phytochemical Investigation of Three Leguminosae Plants for Cancer Chemopreventive Agents (PhD thesis) University of Nairobi, Kenya.
Gutterman, Y., Evenari, M., 1972. The influence of day length on seed coat colour, an index of water permeability, of the desert annual *Ononis sicula* Guss. Journal of Ecology 60 (3), 713–719.
Hanley, M.E., Fenner, M., Ne'eman, G., 2001. Pregermination heat shock and seedling growth of fire-following Fabaceae from four Mediterranean-climate regions. Acta Oecologica 22, 315–320.
Harris, W.M., 1987. Comparative ultrastructure of developing seed coats of "hard-seeded" and "soft-seeded" varieties of soybean, *Glycine max* (L.) Merr. Botanical Gazette 148 (3), 324–331.
Joubert, E., Joubert, M.E., Bester, C., De Beer, D., De Lange, J.H., 2011. Honeybush (*Cyclopia* spp.): from local cottage industry to global markets — the catalytic and supporting role of research. South African Journal of Botany 77, 887–907.
Kozlowski, T.T. (Ed.)1972. Germination control. Metabolism, and Pathology volume 2. Academic Press, USA.
Lersten, N.R., 1982. Tracheid bar and vestured pits in legume seeds (Leguminosae: Papilionoideae). American Journal of Botany 69 (1), 98–107.
Ma, F., Cholewa, E.W.A., Mohamed, T., Peterson, C.A., Gijzen, M., 2004. Cracks in the palisade cuticle of soybean seed coats correlate with their permeability to water. Annals of Botany 94, 213–228.
Maiti, R., 2012. Crop Plant Anatomy. Centre for Agriculture and Bioscience International (CABI), UK.
Mauseth, J.D., 2014. Botany: An Introduction to Plant Biology. Jones & Bartlett Publishers, USA.
Mbangcolo, M.M., 2008. Aspects of Honeybush Tea (*Cyclopia* species) Propagation (M.Sc. dissertation) University of Stellenbosch.
Mbangcolo, M.M., Reinten, E.Y., Agenbag, G.A., 2013. Effect of different seed treatments on germination of *Cyclopia* spp. seeds. Elsenburg 10, 22–24.
Schutte, A.L., Van Wyk, B.E., 1996. Evolutionary relationships in the Podalyrieae and Liparieae (Fabaceae) based on morphological, cytological, and chemical evidence. Plant Systematics and Evolution 209, 1–31.
Shao, S., Meyer, C.J., Ma, F., Peterson, C.A., Bernards, M.A., 2007. The outermost cuticle of soybean seeds: chemical composition and function during imbibition. Journal of Experimental Botany 58 (5), 1071–1082.
Smýkal, P., Vernoud, V., Blair, M.W., Soukup, A., Thompson, R.D., 2014. The role of the testa during development and in establishment of dormancy of the legume seed. Frontiers in Plant Science 5, 351. http://dx.doi.org/10.3389/fpls.2014.00351.
Sutcliffe, M.A., Whitehead, C.S., 1995. Role of ethylene and short-chain saturated fatty acids in the smoke-stimulated germination of *Cyclopia* seed. Journal of Plant Physiology 145, 271–276.
Tran, V.N., Cavanagh, A.K., 2013. Structural aspects of dormancy. In: Murray, D.R. (Ed.), Germination and Reserve MobilizationSeed Physiology volume 2. Academic Press, USA.
Wang, H.L., Wang, L., Tian, C.Y., Huang, Z.Y., 2012. Germination dimorphism in *Suaeda acuminata*: a new combination of dormancy types for heteromorphic seeds. South African Journal of Botany 78, 270–275.
Whitehead, C.S., Sutcliffe, M.A., 1995. Effect of low temperatures and different growth regulators on seed germination in *Cyclopia* spp. Journal of Plant Physiology 147, 107–112.
Zeng, L.W., Cocks, P.S., Kailis, S.G., Kuo, J., 2005. The role of fractures and lipids in the seed coat in the loss of hardseededness of six Mediterranean legume species. Journal of Agricultural Science 143, 43–55.