Effect of light and temperature on seed germination of selected African leafy vegetables

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ABSTRACT

Using laboratory incubation, the response of seed germination and emergence to variability in temperature and light was examined for spider flower (*Cleome gynandra* L.), amaranth (*Amaranthus cruentus* L.), non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*), nightshade (*Solanum retroflexum* Dun.), pumpkin (*Cucurbita maxima* Duchesne), tsamma melon (*Citrullus lanatus* Thunb.), Jew's mallow (*Corchorus olitorius* L.) and cowpea (*Vigna unguiculata* (L.) Walp.). Effect of temperature on seed germination and emergence was monitored under continuous darkness and at constant temperatures that ranged from 4 °C to 44 °C with 4 °C increments. The effect of light on seed germination was measured at 25 °C. Temperature affected germination rate and final germination percentage of all species tested in ways that were characteristic for each of the species tested. Generally, optimum germination occurred at temperatures ranging between 29 °C and 32 °C but at higher temperatures for *V. unguiculata* (36 °C) and *C. olitorius* (35 °C). The minimum temperature for germination ranged between 8 °C and 15 °C, and the maximum between 36 °C and 44 °C. Optimum temperatures for seedling emergence ranged from 25 °C to 31 °C, the maximum between 32 °C and 40 °C and minimum between 2 °C and 13 °C. Light positively ($p < 0.01$) affected onset of germination in *A. cruentus*, *B. rapa* subsp. *chinensis* and *C. olitorius*, and final germination percentage of *B. rapa* subsp. *chinensis*, *C. lanatus* and *S. retroflexum*. The results suggested that under South African conditions, seeds of the eight species will typically germinate optimally as temperatures rise during spring before the occurrence of very hot temperatures in summer. Due to their positive response to light, germination of *B. rapa* subsp. *chinensis*, *C. lanatus* and *S. retroflexum* seeds is expected to be optimal when sown at or close to the soil surface.

1. Introduction

Leafy vegetables are plant species of which the leafy parts, which may include young succulent stems, flowers and fruits, are used as vegetables (Jansen van Rensburg et al., 2007). African leafy vegetables (ALVs), also called 'traditional leafy vegetables', comprise "the collective of leafy vegetable species that form part of the culinary repertoire of particular contemporary African communities" (Jansen van Rensburg et al., 2007; Van Averbeke et al., 2012). Consumption of leafy vegetables is traditional practice in many African communities (Abukutsa-Onyango, 2007; Odhav et al., 2007; Faber et al., 2010). Dark-green leafy vegetables are high in micronutrients (Schönfeldt and Pretorius, 2011; van Jaarsveld et al., 2014), and their consumption can improve the nutritional balance of cereal-based, nutrient-poor human diets, which are

characteristic of many poor rural communities across the globe (Faber et al., 2010; Uusiku et al., 2010). The leafy vegetables that are consumed by rural African people in South Africa consist primarily of weedy species, which grow as wild plants, or as weeds in fields planted to other crops. They are usually obtained locally by gathering, and availability tends to be limited quantitatively and temporally (Abukutsa-Onyango, 2007; Faber et al., 2010). Cultivation and commercialisation of African leafy vegetables increase their consumption by broadening and prolonging access (Diouf et al., 2007; van Averbeke et al., 2007). Cultivation of a wider range of species than is the case at present could make a significant contribution to nutritional security in the rural areas of South Africa, but information on the agronomic requirements of many of these potential crops is scant (Oelofse and van Averbeke, 2012). Such knowledge is needed to guide their effective cultivation. The current study is concerned with the process of germination and emergence of a selection of these traditional leafy vegetable species, and in particular the effect of temperature and light on the germination and emergence process.

Among the various germination factors (Ghaderi et al., 2008), temperature is the most prominent environmental factor regulating

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growth and development of plants (Koger et al., 2004). The temperature (optimum) at which the maximum germination and emergence percentage are recorded tends to differ among crops. Clear understanding of the germination and emergence response of crop seeds to temperature, obtained by determining cardinal temperatures, is important, because it enables identification of tolerance to low and high temperatures, and climatic conditions under which particular crops can germinate and establish successfully. It also assists in the construction of models that predict crop development processes (Ghaderi et al., 2008).

The requirement of light for the germination of seeds of certain plant species prevents germination in places and times not favourable for seedling establishment (Fenner and Thompson, 2005). The light requirement of such seed acts as a mechanism that determines where and when germination takes place, and is important for survival of the plant species concerned, as it prevents stored seed reserves from being depleted. Some seeds germinate equally well in light and darkness, whilst others germinate better under only light or darkness (Chanyenga et al., 2012).

Existing knowledge of the effects of light and temperature on seed germination and emergence of traditional South African leafy vegetables appears to be limited. Therefore, the objective of this study was to investigate the effect of temperature on the germination and emergence and the effect of light on seed germination of a selection of these vegetable species. Pigweed (*Amaranthus cruentus* L.), nightshade (*Solanum retroflexum* Dun.), non-heading Chinese cabbage (*Brassica rapa* L. subsp. *chinensis*), spider flower (*Cleome gynandra* L.), tsamma melon (*Citrullus lanatus* Thunb.), Jew's mallow (*Corchorus olitorius* L.), pumpkin (*Cucurbita maxima* Duchesne) and *Vigna unguiculata* (L.) Walp. were selected for the study, because they are already being cultivated or have considerable potential to be developed into cultivated species (Oelofse and Van Averbeke, 2012).

2. Materials and methods

2.1. Seed sources and treatment

Seeds of pigweed (*A. cruentus*), spider flower (*C. gynandra*), tsamma melon (*C. lanatus*), Jew's mallow (*C. olitorius*), pumpkin (*C. maxima*) and *V. unguiculata* Walp. were obtained from the Vegetable and Ornamental Plant Institute of the Agricultural Research Council at Roodeplaat (VOPI). Seeds of the non-heading Chinese cabbage (*B. rapa* subsp. *chinensis*) (land race *dabadaba*, van Averbeke et al., 2007) and black nightshade (*S. retroflexum*) were obtained from Mr. Mabulannga, a smallholder farmer at Dzindi Irrigation Scheme (Itsani village) in Limpopo Province of South Africa (23°01′45″S and 30°26′30″E). Seeds were surface-sterilised in a warm water bath at 50 °C for 20 min (Labcon™ shaking water bath, 5070 U, model WBM-SPL 25) to reduce the risk of fungal growth. Thereafter, seeds were chilled in cold distilled water, evenly spread on a piece of germination paper and dried overnight at 20 °C (Floyd, 2005).

2.2. Seed germination and temperature

Small seeds (*A. cruentus*, *B. rapa* subsp. *chinensis*, *C. gynandra*, *C. olitorius*, and *S. retroflexum*) were placed on four layers of brown anchor germination paper (115 mm × 125 mm) and moistened with 10 ml distilled water. Large seeds (*C. maxima*, *C. lanatus* and *V. unguiculata*) were placed on four layers of rolled germination paper (260 mm × 380 mm) moistened with 50 ml distilled water (ISTA, 2008). Seed weights of 100 seeds of the ALVs are shown in Table 2. Germination was monitored every 6 h during the first 10 days (240 h), and every 12 h throughout the remainder of experiments. Seeds were considered to have germinated once the radicle had protruded at least 2 mm from the testa.

2.3. Seedling emergence and temperature

Seeds were sown in containers (280 mm × 190 mm) filled with germination sand [Rolfes Silica, 0.4–0.85 grading; dry graded silica sand (SiO_2, 98% $Fe_2 O_3$ 0.18%)] moistened with distilled water for small seeds (0.0675 l kg^{-1} sand) and large seeds (0.4675 l kg^{-1} sand). Water was allowed to redistribute in the sand before incubation for 24 h at the designated treatment temperatures to allow the sand to attain the desired temperature. Small seeds were broadcasted evenly on top of moistened sand and firmly pressed into the substratum to allow contact with sand. Large seeds were sown at a depth of 1 cm. Spacer sticks were used to separate sample containers inside the incubators to allow sufficient circulation of air around the containers. Water was replenished as needed. Emergence was recorded every 24 h. Seeds were considered to have emerged once the cotyledons were visible above the surface of the sand (Koger et al., 2004; Maraghni et al., 2010).

2.4. Seed germination and light

To investigate the effect of light on germination, seeds were incubated at a constant temperature of 25 °C, and exposed to alternating light (8 h dark and 16 h light) and continuous darkness in an environmentally controlled Labcon™ (220V, 50 Hz) low-temperature incubator and growth chamber. Light was provided by six OSRAM DULUXSTAR light bulbs (14 W/840, 220–240 V, 116 mA, 50/60 Hz). Seed germination was recorded every 24 h. In the light effect experiment, samples incubated under darkness received small quantities of light during daily evaluations, and this could have triggered germination and affected the final germination percentage. For this reason, a second experiment was undertaken to determine the effect on light on final germination percentage in which the short exposure to light of the continuous darkness treatment as part of daily germination counts was eliminated. Accordingly, seeds were kept in the respective incubators for 10 days (240 h) without daily evaluation and germination counts were done after 240 h. The reason for incubating the seeds for 240 h was that in the first light-effect experiment final germination had been reached in all treatments and for all species before 240 h had expired.

2.5. Temperature regimes

Germination and emergence experiments were conducted in incubators set at constant temperatures which ranged from 4 °C to 44 °C with 4 °C increments, under continuous darkness. All experiments were incubated over a period of 14 days (336 h). In all experiments seeds were exposed to normal light during observations. All treatments were replicated four times with 50 seeds per treatment. Replicates were arranged in a completely randomized design in controlled incubators/growth chambers. Seeds that showed signs of fungal growth were removed from the population. Germinated or emerged seeds were counted, removed and expressed as a percentage of the total number of tested seeds.

2.6. Data analysis

The non-intercept sigmoid function as described in TableCurve® 2D (2002) was fitted on the cumulative germination/emergence percentage to determine the time to 50% germination/emergence (T_{50}) (Jami Al-Ahmadi and Kafi, 2007): $y = \frac{a}{1+e^{\left(-\frac{x-b}{c}\right)}}$, where a is the maximum germination/emergence percentage, b is the turning point, c is slope of the line, x is the time (h) and y is the germination/emergence %. T_{50} germination/emergence was calculated and subjected to an appropriate analysis of variance (ANOVA) using SAS® statistical software version 9.2 (SAS, 1999).

The rate of germination/emergence was defined as the reciprocal of the time taken for half the population to germinate/emerge ($1/T_{50}$). The

optimum temperature (T_{opt}) was determined by fitting a simple piece-wise linear model (broken-stick regression) using a non-linear (NLIN) procedure with SAS between temperature and rate of germination/ emergence ($1/T_{50}$) for each species separately. The rate of germination/ emergence increased linearly with temperature from a minimum (T_{min}) to a sharply defined optimum (T_{opt}) beyond which the rate decreased linearly with temperature. The maximum temperature (T_{max}) was taken as the highest temperature where 50% germination/emergence was reached.

Analysis of variance (ANOVA) was used to test for temperature and light treatment effects. Treatment means were separated using Fisher's protected least significant difference test at the 1% instead of the 5% level of significance (Snedecor and Cochran, 1980), because of hetero-geneity of variances. For light experiment 2, treatment means were separated at the 5% level of significance. The data were analysed using the statistical program GenStat® (Payne et al., 2007).

3. Results

3.1. Seed germination and seedling emergence

Rate of germination/emergence ($1/T_{50}$) of the different ALVs in-creased linearly as temperature was raised up to the optimum at which maximum germination/emergence percentage was recorded and then decreased linearly as temperature was elevated further. The ALVs for which the $1/T_{50}$ for the two processes were calculated and their estimated cardinal temperatures are shown in Table 1. Optimum germination temperatures (T_{opt}) ranged from 29 to 36 °C (Fig. 1), with *C. olitorius* (35 °C) and *V. unguiculata* (36 °C) recording higher optima than *B. rapa* subsp. *chinensis* (29 °C), *A. cruentus* (31 °C), *C. lanatus* (30 °C), *C. gynandra* (31 °C), and *C. maxima* (32 °C). Estimated T_{min} values ranged from 8 °C for *B. rapa* subsp. *chinensis* and *C. lanatus* to 15 °C for *C. gynandra*.

The $1/T_{50}$ values for emergence indicated optima ranging from 25 to 31 °C (Fig 2). *B. rapa* subsp. *chinensis* and *V. unguiculata* (25 °C) had the lowest optimum temperatures and *C. olitorius* and *C. maxima* (31 °C) the highest. Noteworthy was that *B. rapa* subsp. *chinensis*, *V. unguiculata* and *C. olitorius* demonstrated a relatively wide optimum temperature range, whilst for the other three species in Fig 2 the optimum temperature range was narrow.

Estimated T_{opt} for emergence compared well T_{opt} for germination for most species except with *B. rapa* subsp. *chinensis* and *V. unguiculata*, for which the optimum temperature for seedling emergence was lower than that for seed germination as the two exceptions. Estimated $1/T_{50}$ T_{min} values for seedling emergence were higher than those for germina-tion for *C. lanatus*, *C. olitorius* and *V. unguiculata*, lower for *A. cruentus* and *B. rapa* subsp. *chinensis*, and the same for *C. maxima*.

Important was that most species germinated and emerged well at high temperatures. Using $1/T_{50}$ germination (Fig. 1) and $1/T_{50}$ emer-gence (Fig. 2) as a theoretical model to predict cardinal temperatures,

Table 1
Cardinal temperatures for time to 50% germination and emergence of selected African leafy vegetables.

African leafy vegetables	Temperature (°C)					
	Minimum		Optimum		Maximum	
	G	E	G	E	G	E
A. cruentus	12	8	31	29	40	40
B. rapa subsp. *chinensis*	8	2	29	25	36	32
C. gynandra	15	–	31	–	36	–
C. lanatus	8	13	30	30	40	40
C. olitorius	12	13	35	31	44	40
C. maxima	12	12	32	31	40	40
V. unguiculata	10	12	36	25	40	36
S. retroflexum	Did not reach 50% germination and emergence					

G = germination; E = emergence.

the T_{min}, T_{opt} and T_{max} germination of the ALVs ranged between 8–15 °C, 29–36 °C and 36–44 °C, respectively. The T_{min}, T_{opt} and T_{max} for ALVs seedling emergence was 2–13 °C, 25–31 °C and 32–40 °C, respectively (Table 1).

3.2. Light

Sensitivity to light in relation to onset of germination and final ger-mination percentage of the eight vegetable species is presented in Table 2, which also lists their 100 seed weight. Positive effects of light on the onset of germination were recorded for *B. rapa* subsp. *chinensis*, *A. cruentus* and *C. olitorius*, and negative effects for *C. lanatus* and *V. unguiculata*. For *B. rapa* subsp. *chinensis* the positive effect of light was significant (p 0.01) throughout the incubation period, and in *C. lanatus* and *S. retroflexum* exposure of the seed to light increased final percentage germination significantly (p 0.01). Light did not have a significant effect on the final germination percentage of *A. cruentus*, *C. gynandra*, *C. olitorius*, *C. maxima* and *V. unguiculata*, but this effect tended to be positive for all of these species except *V. unguiculata*.

4. Discussion

4.1. Seed germination and seedling emergence

Whilst the cardinal temperatures (T_{min} and T_{opt}) for germination of the ALVs tended to be higher than those for seedling emergence this did not apply to all the ALV species. For example, in *C. lanatus* and *C. maxima* no material differences between the cardinal tempera-tures of these two processes were observed. Differences in cardinal tempera-tures for seed germination and seedling growth are not anomalous and were also reported by Cochrane et al. (2011). Saeidnejad et al. (2012) indicated that such differences could be due to genetic variability among the seeds used or to differences in latitude at which accessions of seeds were collected. The fairly high optimal temperature (T_{opt}) for both germination and seedling growth indicated that most ALV species, with the exception of *B. rapa* subsp. *chinensis* and *V. unguiculata*, were adapted to high day-time temperatures, which are characteristic of tropical and subtropical regions. This trait is considered to be an impor-tant pre-adaptation for the weedy or wild habit of species (Cristaudo et al., 2007) and helps to prevent the seeds of these plants from germi-nating too early or too late. For most species the T_{max} could not be esti-mated because they still germinated and emerged well at the highest temperature of 40 °C. This supports the notion that most of these plant species have a high tolerance to high temperatures during germi-nation, when high rates of respiration and failure of metabolic activity in the seed could cause reduced emergence by inhibiting hypocotyl elon-gation (Ndunguru and Summerfield, 1975). The T_{min} estimated indi-cates tolerance of ALV to low temperatures during germination. Conversely, longer exposure at such temperature range may cause sub-stantial reduction in the rate of germination and subsequent growth of the crop (improper development of seedlings). This could evidently cause increased seedling exposure to pathogens in the soil, leading to decreased emergence due to seed and seedling diseases (which cause rotting of the cotyledons) (Soltani et al., 2006; Souza and Fagundes, 2014). Poor germination at very low and high temperatures reported in legume species, such as *V. unguiculata* (Balkaya, 2004), and in *Brassica* species (Tokumasu et al., 1985) may indicate that little or no germina-tion will take place during winter or mid-summer and could be seen as a protection mechanism against excessive seedling mortality (Chanyenga et al., 2012).

Although the cardinal temperatures of some of the ALV species were fairly specific and constant, indicating potential for modelling of emer-gence, it has been pointed out that most cardinal temperatures for non-crop species are unstable due to genetic variability, positional effects, environmental factors and seed size (Wang, 2005; Souza and Fagundes, 2014). Germination of smaller seeded species may be
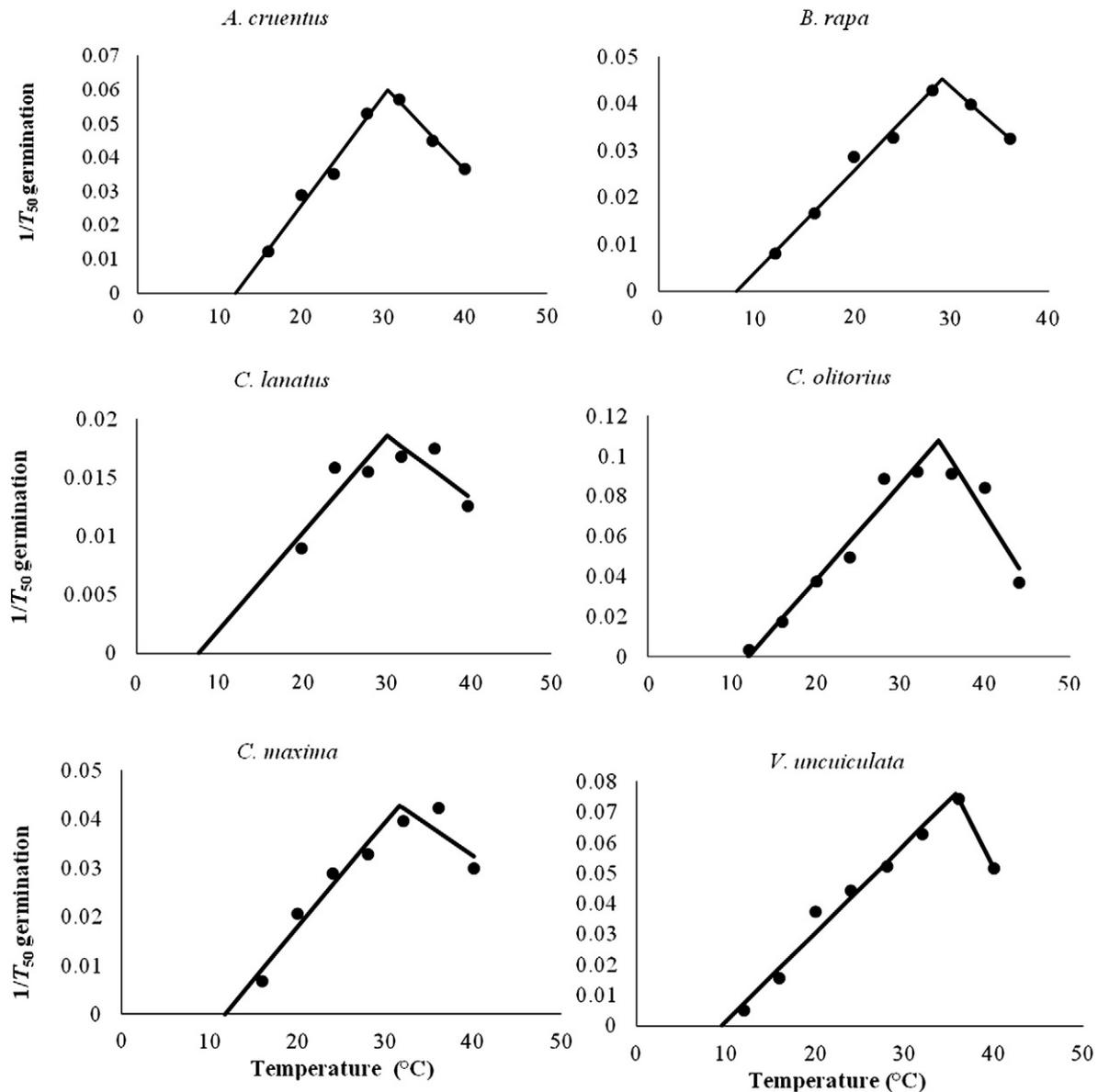
Fig. 1. Effect of temperature on the inverse of time to 50% germination of selected traditional South African leafy vegetables.

expected to be faster providing a greater competitive advantage in early successional stages compared to that of larger seeds since larger seeds have higher amounts of reserves in their cotyledons and require extended periods to incorporate these nutrients in seedling tissues (Souza and Fagundes, 2014). In our study, only three ALVs would be classified as having larger seeds based on their average seed weights; these being *C. lanatus*, *C. maxima* and *V. unguiculata* (Table 2). However, rate of germination/emergence was not found to be that much different to that of smaller seeds. Although our study did not investigate effects of seed size on seed germination and seedling emergence, this seed trait is expected to vary among species in relation to their ecological strategies for seed dispersal and seedling establishment (Soriano et al., 2011).

Cardinal temperatures for seed of *C. gynandra* and *S. retroflexum* could not be estimated due to poor germination and emergence. However, it appears as though seed germination for *C. gynandra*, although greater than 50% percentage germination was only attained at 28 °C (65%), 32 °C (75.5%) and 36 °C (56.5%) could be favourable at 31 °C. Compared to Böhringer et al. (1999), the germination of *C. gynandra* seed observed in this study was in fact more complete. This could be due to the seed lots used in the current study had been in storage for a longer period, favouring immature embryos to reach maturity, than

those used by Böhringer et al. (1999), which were only 6 months old (Ochuodho and Modi, 2005; Ekpong, 2009).

The particularly low germination percentage recorded for *S. retroflexum* could be due to the inherent dormancy problems of nightshades, which are exacerbated by harvesting and processing methods that fail to adequately remove the sugars and germination inhibitors present in the fruit during the extraction of the seed (Abukutsa-Onyango, 2007). In this study, *S. retroflexum* emerged optimally at 20 °C, which was within the optimal growth range of most Solanaceae (Edmonds and Chweya, 1997). Use of pre-sowing treatments and/or extended storage periods, aimed at releasing seed from dormancy, could assist the development of a better understanding of the germination of this species.

4.2. Light

The seed of *B. rapa* subsp. *chinensis*, *C. lanatus* and *S. retroflexum* demonstrated positive photosensitivity, suggesting that seed germination of these species is a photochrome-mediated response, which is an attribute commonly observed among weeds (Kettenring et al., 2006). Environmentally induced photosensitivity of seed is usually interpreted
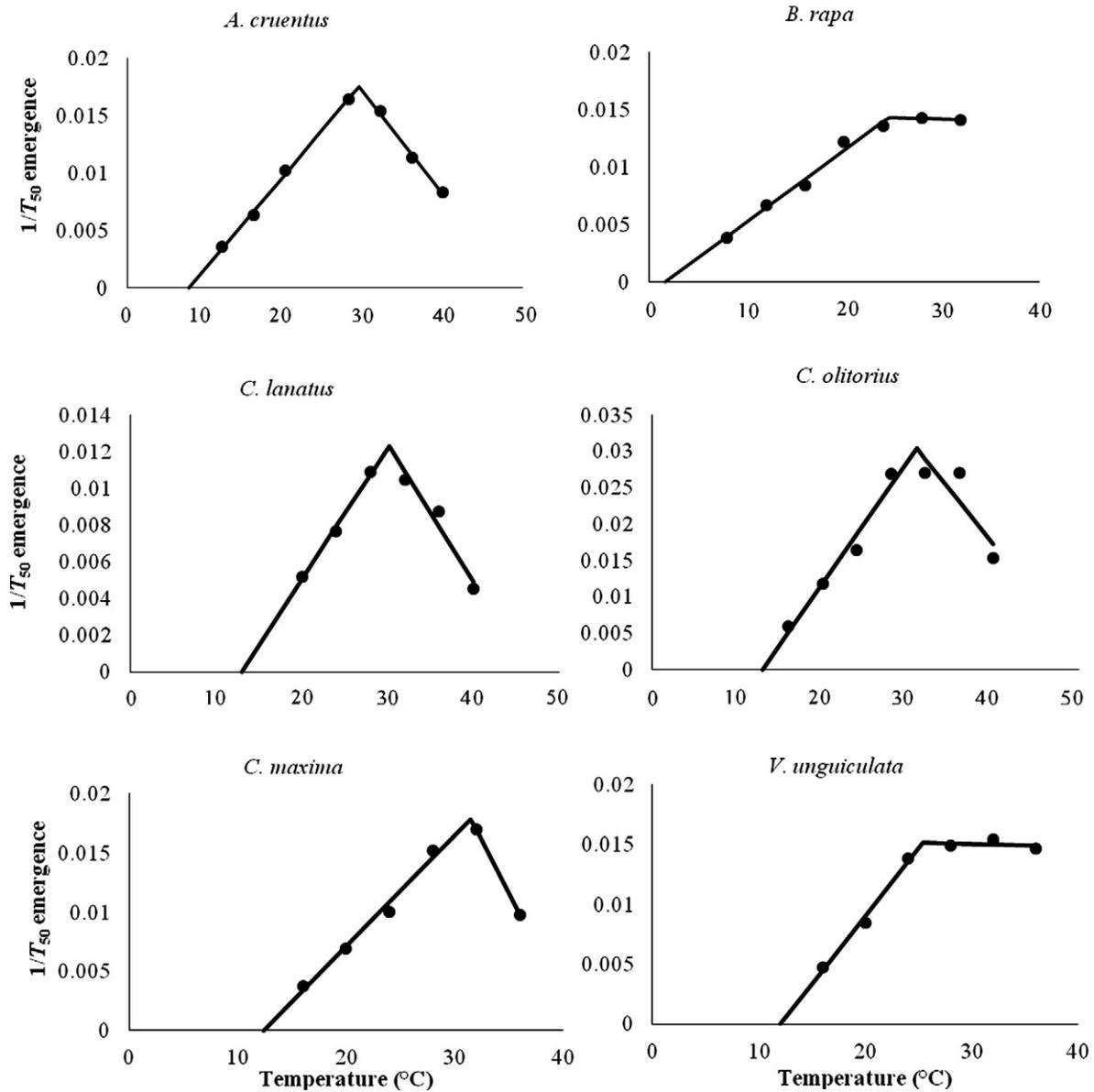
A. cruentus

Fig. 2. Inverse of time to 50% emergence of the different African leafy vegetables in response to different temperatures.

Table 2
Seed weight and final germination percentages of selected African leafy vegetables incubated at a constant temperature of 25 °C in alternating light and continuous darkness.

Species	Weight of 100 seeds (g)	Final germination percentage (%)			
		Light treatments			
		336 h		240 h	
		Light	Dark	Light	Dark
A. cruentus	0.05	81.5ab	99.5a	91.5bcd	89.0cd
B. rapa subsp. chinensis	0.21	93.0ab	53.5c	87.5d	55.0g
C. gynandra	0.13	43.5cd	43.5cd	40.5h	37.5h
C. lanatus	20.10	97.0ab	97.0ab	89.0cd	79.0e
C. olitorius	0.20	96.5ab	96.0ab	97.5ab	96.5abc
C. maxima	10.25	100.0a	99.0ab	100.0a	100.0a
S. retroflexum	0.07	49.5cd	32.5d	57.5g	0.5i
V. unguiculata	12.25	94.0ab	95.0ab	89.0cd	92.0abcd

Different letters indicate significant difference between values of pairs of species (p < 0.01) for daily evaluations (336 h).
Different letters indicate significant difference between values of pairs of species (p < 0.05) without daily evaluations (240 h).

as an adaptation that ensures that seed germinates in places where there is a high probability of seedling establishment (Mpati, 2006). The seed of these three species is likely to prefer germinating at or near the soil surface. In the case of the 'dabadaba' landrace of B. rapa subsp. chinensis, this behaviour has been captured in indigenous knowledge. In Tshivenda, the word 'dabadaba' describes someone (or something) who is doing his or her own thing and does not follow the rules. According to Tshikalange (2006) people in Venda named this land race of B. rapa subsp. chinensis 'dabadaba', because it germinates like a weed, i.e. following soil disturbance. Soil disturbance practices, such as cultivation, expose buried seeds to light inducing germination of species that are light-sensitive (Kettenring et al., 2006). Löwe and Dillenburg (2011) argued that light reliant species usually have small seeds that have faster photosynthetic cotyledons and growth, as appears to be the case for B. rapa subsp. chinensis and S. retroflexum, and shorter use of seed reserves than non-light reliant species. Aud and Ferraz (2012) agreed by pointing out that the limited resources contained in small seeds were compensated for by light dependency of the germination of such seeds. Conversely, in large-seeded species

the seed reserves are greater and this tends to reduce their dependence on photosynthesis during the early stages of establishment (Khan, 2004).

The germination of the other five species was essentially indifferent to light, even though there was some evidence of a minor negative effect of light on the onset of germination of *C. lanatus* and *V. unguiculata*. Negative photosensitivity has been attributed to light inhibiting cell elongation by suppressing the expression of selected proteins that enhance germination (Ochuodho et al., 2008). Mpati (2006), citing Mayer and Poljakoff-Mayber, noted that germination of the seed of cultivated species was usually indifferent to light, but Fenner and Thompson (2005) pointed out that even in species of which seed germination was known to be indifferent to light, there are usually a few individual seeds that are light-sensitive. This could explain the observed minor effect of light on the onset of germination in *C. lanatus*, and *V. unguiculata*.

5. Conclusion

Seed of *A. cruentus*, *B. rapa* subsp. *chinensis*, *C. gynandra*, *C. lanatus* and *C. maxima* germinated optimally at 29–32 °C, but *V. unguiculata* (36 °C) and *C. olitorius* (35 °C) had higher optima. Accordingly, seed of these species is expected to germinate optimally when planted during spring before the occurrence of hot mid-summer temperatures. Delaying planting to summer appears most appropriate for *C. olitorius* and *V. unguiculata* because their seeds germinated optimally at 35 °C. However, seed of *V. unguiculata* together with that of *B. rapa* subsp. *chinensis* and *S. retroflexum* emerged best when the temperature ranged between 20 °C and 25 °C, indicating their preference for cool climates or cool periods for seedling emergence during the year in areas where the climate is hot. The ability to emerge at low temperatures, which applied to most crop species tested, indicated that seed could be planted when the temperatures are still relatively low, knowing that the process of emergence will accelerate as temperatures rise towards the optimum. The cardinal temperatures that were determined for the different African leafy vegetables could help to predict their suitability for new localities, but germination and emergence are function of multiple environmental factors. In this study, the experiments were conducted under controlled environmental conditions of constant temperatures, whereas under natural conditions temperature is subject to diurnal fluctuations. Consequently, the cardinal temperatures that were presented could differ from those applying in nature. Emergence has not been studied in sufficient detail to permit reliable predictions for the African leafy vegetable species covered by this study. Future studies should focus on the interactive effects of responsible factors to refine the results obtained in this study.

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