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Annals of Biological Research, 2011, 2 (1): 60-75 (http://scholarsresearchlibrary.com/archive.html)



ISSN 0976-1233 CODEN (USA): ABRNBW

# Determination of Temperature and Light Optima for Seed Germination and Seedling Development of Spiderplant (*Cleome gynandra* L.) Morphotypes from western Kenya

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#### **ABSTRACT**

Spiderplant (Cleome gynandra) is an African indigenous vegetable (AIV) in many parts of sub-Saharan Africa, especially in most countries in eastern and southern Africa, where it is a semi-cultivated popular tropical species. In Kenya, it is among the most popular AIVs, particularly in the western and coastal regions. Seed supply systems for spiderplant is largely local and whatever material that is available is often of poor quality, mainly due to lack of development of seed quality testing protocols, thus hampering the correct assessment of the quality of seed used for sowing. The study aimed at determining temperature and light optima for seed germination and seedling development of spiderplant morphotypes from western Kenya. The original spiderplant seeds for the study were sourced from farmers in Kakamega District and from wildly growing plants within Chepkoilel Campus in Uasin Gishu District, both in western Kenya. Four morphotypes were identified and selected based on the presence or absence of anthocyanins on their stems and petioles, respectively. Tests on seed quality aspects were done at the Bioscience Business Unit at Plant Research International, Wageningen, The Netherlands. Determination of optimum temperature for seed germination was conducted by incubating three replicates of 100 seeds each of the four morphotypes at constant temperatures of 20, 25, 30, 35, 40 and 45°C, respectively. In order to determine the light regime for seed germination and optimum temperature for seedling development four replicates of 50 seeds each were incubated at constant temperatures of 30 and 35°C at 8-hr light day<sup>-1</sup> and 0-hr light day<sup>-1</sup> (in 24-hr darkness), respectively. Data analysis (ANOVA, statistical descriptors and t-tests) were done using statistical package SeedCalculator 3.0. Germination was strongly influenced by temperature showing declines at both extremes of 20 and  $45^{\circ}C$  and optimum of 35 and  $40^{\circ}C$ . Light had no influence on germination since seeds germinated equally well under light and in the dark. Seedling development was strongly influenced by temperature with higher percentage of normal seedlings at 30°C than at 35°C. It is concluded that while temperature had an effect on seed germination performance and seedling development, light on the other hand showed no effect on seed germination for the spiderplant morphotypes studied. However, white light is required in order to aid in seedling evaluation.

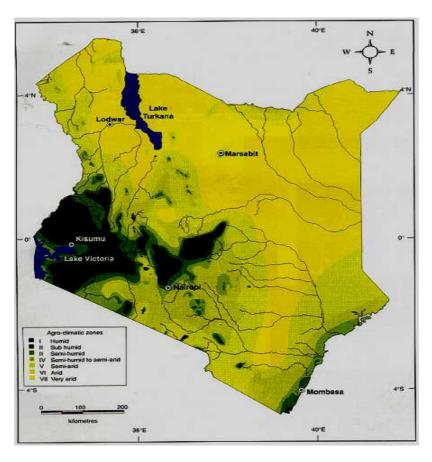
**Keywords**: *Cleome gynandra*, germination, morphotypes, seedling development, western Kenya.

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#### INTRODUCTION

Spiderplant (*Cleome gynandra*) as a  $C_4$ -species has high photosynthetic efficiency and for this it requires high radiation and temperature (18-25°C), in addition to sufficient soil moisture [1, 2, 3]. The  $C_4$  photosynthetic pathway is an adaptative mechanism for its survival in dry and hot environments [3].



Source: [6]

Plate 1. Map showing the agro-climatic zones of Kenya and the zones (I-VI) in which *C. gynandra* is distributed.

Kenya has been subdivided into seven agro-ecological zones (AEZ I-VII), based on temperatures and rainfall [4]. While AEZ I is the most humid, typified by the humid highlands such as the top of Mt. Kenya and parts of Kisii highlands, AEZ VII on the other hand is the most arid. AEZ I-V can support rain-fed agricultural activity, with reliability decreasing in the upper categories. AEZ V-VII form up to 80% of the country's land surface and are best utilized for game and pastoralism. The wide distribution of spiderplant in many agro-ecological zones of Kenya has been reported [4, 6], and this ranges from AEZ I to VI (Plate 1.). In terms of altitude, it is reported to occur from sea level up to 2400 m, although the crop requires warm conditions since its growth is hampered below 15°C [7].

In order to minimize the risk of a grower sowing the seed that does not have the capacity to germinate and produce a good crop yield of the required cultivar, it is important to carry out seed

quality test. Such a test aims at determining the planting value of a given seed lot. In the agronomic sense, high quality seed essentially encompasses the capacity of a seed lot to produce normal seedlings, have good field emergence, and show adequate storability [8, 9].

Farmers need to be protected against buying poor quality seed, by having in place certain quality criteria and control. The International Seed Testing Association (ISTA) has elaborated such quality criteria on a scientific basis; it has given standardized definitions of quality criteria and developed methods to determine them [10]. At present, no seed testing guidelines have been provided for under the ISTA rules for *C. gynandra*, except for *C. hassleriana*, which is an annual temperate flower [11]. Because of the continued prominence the vegetable is gaining in most tropical areas, and particularly in sub-Saharan Africa [5], it is important to develop optimum seed testing methods so that both seed producers and farmers would benefit from the set seed quality control criteria; the former for variety protection and the latter for seed quality guarantee. Such methods when developed would in addition aid in the improvement of the genetic and physical quality of seed [12], by ensuring that the tested seed meets the minimum quality standards.

When a dry, viable seed imbibes water, a chain of events are initiated and germination is signified to have successfully been completed when the emergence of the radicle results [13]. In a laboratory test, a seedling is observed till that stage when it has developed the essential structures that can be evaluated according to ISTA rules [10]. This will indicate whether or not it is able to develop further into a normal plant under favourable conditions in soil.

Seed germination and later seedling growth require proteins at increasing quantities [13]. Polysomes are absent in dry seeds, but rapidly increase on hydration [14]. Concurrent with an increase in polysomes is a decline in the number of single unattached or free ribosomes, and both these are required for protein-synthesis to sustain embryo growth [15, 14].

The germination process can be profoundly affected by both the external and internal seed factors. While external factors determine the environment for seed germination, the internal factors are related to the "history" of the individual seed [13]. These factors apply to seeds whose dormancy has been broken, as well as to those, which had no dormancy.

The optimum germination temperature is species-specific and needs to be determined by experimentation [16, 17]. The rate at which germination proceeds and the capacity of germination attained are temperature dependent, since temperature affects the rate of water absorption, the rate of diffusion of respiratory gases, and the rate of chemical reactions that are involved in seed metabolism [13]. While low temperature will bring about low germination rates, increase of temperature up to certain limits will increase the rate of germination; however, germination will be reduced or halted altogether due to further increase in temperature beyond certain limits [10].

As different species have different optimum temperatures for germination, seed testing is conducted at species-specific temperatures. For instance, a C<sub>4</sub> tropical and subtropical grass weed *Leptochloa sinensis*, had highest seed germination of 95% at temperature range of 25-35°C [18]. However, Proso millet (*Panicum miliaceum*, *Poaceae*), which is another C<sub>4</sub> plant,

germinated well at temperatures of 10-45°C, with highest germination occurring at 35 and 40°C and no germination at 5°C or 50°C [19]. This indicated that both the two C<sub>4</sub> plants had high temperature requirements for high germination performances.

According to their sensitivity to white light, seeds are often classified in three categories, called photoblastism [20], this being associated with recent advances in research on phytochrome forms and mechanisms of action. According to this classification the first category is termed positive photoblastic, in which seeds germinate only under white light; the second category is negative photoblastic, in which seeds germinate only in the dark and white light inhibits germination, and finally the third category is that of neutral photoblastic seeds, in which seeds germinate both in the dark and under white light. While light requirement is important for some species, other species, however will germinate whether light is present or not [13]. The germination of *Leptochloa sinensis* was reported to have been reduced by 80% when seeds were germinated in the dark, even under optimal temperature, thus highlighting a marked positive seed photoblastism [18]. However, with seed germination carried out in both light and dark incubation conditions, at the temperature range of 30-35°C, the results for the mean germination time were similar but quite low, which indicated neutral photoblastism [18].

In a study with *C. gynandra* seeds from ARC in South Africa, the germination done at alternating temperatures of 20/30°C resulted in a percentage germination of 57% and 41% in darkness and continuous light, respectively; however, at constant temperature of 20°C, it was 43% and 2% in darkness and continuous light, respectively [21]. Seeds from KSC in Kenya showed a similar germination pattern under the same conditions; at alternating temperatures 20/30°C, the KSC seeds had a germination of 90% in darkness and 74% in continuous light, while at constant temperature of 20°C the germination was 82% in darkness, but they failed to germinate in continuous white light [21]. The *C. gynandra* seeds used in this study exhibited negative photoblastism, but temperature and light had highly significant interaction [21].

Smaller seeds contain only small amounts of storage reserves for early embryo growth, therefore when they germinate too deep in the soil; these reserves may be exhausted before the seedlings emerge [10]. Whether it is essential for germination or not, light is normally provided in seed laboratories. This is done in order to prevent excessive etioliation of the seedlings, promote chlorophyll formation and aid seedling evaluation, which involves the examination and evaluation of each of the individual essential structures of a seedling that have developed during the prescribed test period as well as a whole seedling.

The objective of this study was to establish temperature and light optima for seed germination and seedling development of spiderplant morphotypes from western Kenya.

### MATERIALS AND METHODS

#### **Plant Materials**

The seeds of the four morphotypes used in this experiment were collected from small-scale farmers in Kakamega District and from wildly growing plants within Chepkoilel Campus, Moi University in Uasin Gishu District, both in Western Kenya. The morphotypes for a different study on morphological characterization were identified and selected from plants which were

raised in a plastic greenhouse at the Department of Seed, Crop and Horticultural Sciences in Moi University. From that study, seeds of each morphotype (GG- green stem/green petiole type of plants; GP- green stem/purple petiole type of plants; PG- purple stem/green petiole type of plants, and PP- purple stem/purple petiole type of plants) were saved and stored in hermatically-sealed aluminium foil packets at room temperature (about 20°C) until the time to undertake this study.

The tests on seed quality aspects of spiderplant were done in the research laboratory of the Bioscience Business Unit at Plant Research International, Wageningen, The Netherlands.

# **Determination of Optimum Temperature for Seed Germination**

The preliminary test on seed dormancy was conducted using potassium nitrate  $(KNO_3)$  as dormancy-breaking treatment and water as a control. The results showed no significant differences (Data not shown). Therefore all the subsequent tests were performed without dormancy-breaking treatment.

Three replicates of 100 seeds of each of the four morphotypes were incubated at constant temperatures of 20, 25, 30, 35, 40 and 40°C in the dark, in clear plastic germination boxes. The seeds were germinated on one layer of round filter paper (10 cm diameter), placed at random on one layer of thick filter paper (size 14.3cm x 20.2cm), which was moistened with 50 ml of tap water. A set of four boxes containing the four different morphotypes were stacked together and placed in germination cabinets at constant temperatures indicated above. A completely randomized design (CRD) was used in this study because the conditions in a laboratory situation are usually controlled and kept uniform [22].

Radicle protrusion was monitored between 8 and 120 hours, from the start of incubation. Germination rate (defined as time taken to reach 50% of maximum germination,  $T_{50}$ ), mean germination time, (defined as mean time taken to reach maximum germination, MGT ) and percentage maximum germination, Gmax, were calculated using the software package SeedCalculator 3.0 (Plant Research International B.V., Wageningen, The Netherlands).

# **Determination of Optimum Light Regime for Seed Germination and Optimum Temperature for Seedling Development**

Four replicates of 50 seeds of each of the four morphotypes were incubated in germination cabinets at constant temperatures of 30 and 35°C with light regime of 8-hr light/16 hr darkness and in the dark (0-hr light), respectively for each temperature. The germination boxes for dark incubation were first placed in black cellophane bags in stacks and the bags tightly fastened with clips to maintain total darkness inside. The boxes for light incubation were similarly stacked. Then all the germination boxes were put in the germination cabinets. CRD was also used in this study.

Radical protrusion was monitored between 6 and 32 hours from the start of incubation. After incubation in the cabinet for up to 32 hours, the seeds were transferred to 'Copenhagen' germination tables with temperature controls set at 30 and 35°C and 8-hour light per day for continued development of the seedlings without them getting etiolated. The samples were

covered with transparent plastic bell jars to prevent drying and to enable evaluation of seedlings. Radicle protrusion was further monitored after 48 hours from the start of incubation.

At 10 days after the start of incubation, final germination count and seedling evaluation were done. During seedling evaluation, seedlings of spiderplant were evaluated as those belonging to type E, represented by the genus Brassica [10]. The hypothesis of monophyly (i.e. derivation from a single stock) of the cabbage family Brassicaceae and the spiderplant family Capparaceae is strongly supported by [23] and [24], in their chloroplast and nuclear studies conducted on the two families.

The type E seedlings comprise of dicotyledons with epigeal germination; the seedling part that grows towards the light is the hypocotyl with two cotyledons attached and which turns green [10]. In the evaluation, spiderplant seedlings were thus considered normal if they had the root and the shoot systems intact. However, seedlings with slight defects, such as necrotic spots, cracks of minor depth with less than 50% of non-functional cotyledon tissue, with one cotyledon or primary leaf instead of two, or with three cotyledons, were counted as normal. Seedlings were classified as abnormal if they were deformed, fractured, yellow or white, spindly and decayed as a result of primary infection. Also seedlings with cotyledons emerged from the seed coat before the primary root appeared and those that were stunted or stubby or had a missing primary root were recorded as abnormal. T<sub>50</sub>, MGT, Gmax, percentage normal and abnormal seedlings, and percentage hard and dead seeds, were calculated using the software package SeedCalculator 3.0.

### **RESULTS**

# **Optimum Incubation Temperature for Seed Germination Germination rate**

A rise in incubation temperature from 20 to  $40^{\circ}\text{C}$  caused a general increase in the mean germination rate (represented by a lower  $T_{50}$  value) across the morphotypes (Figure 1a.). The highest germination rate for all morphotypes was attained at  $40^{\circ}\text{C}$  (Figure 1a.). However, the rate for morphotype PG was significantly ( $P \le 0.05$ ) lower than the rest at this incubation temperature. And while all the morphotypes had the lowest germination rate at  $20^{\circ}\text{C}$ , the rate recorded for morphotype GG was significantly ( $P \le 0.05$ ) higher than the rest at this temperature (Figure 1a.). Considering speed of germination, the optimum germination temperature was at  $40^{\circ}\text{C}$  since at this temperature range the lowest  $T_{50}$  value of about 0.5 was recorded. While it is only morphotype PG that significantly ( $P \le 0.05$ ) differed from the rest in speed of germination at  $40^{\circ}\text{C}$ ; however, at  $35^{\circ}\text{C}$  morphotypes did not differ. At  $45^{\circ}\text{C}$ , a decrease in germination rate occurred in all morphotypes, but morphotypes PG and PP showed significantly ( $P \le 0.05$ ) lower germination rates than GG and GP at this temperature (Figure 1a.).

# **Mean germination time**

Increasing incubation temperature from 20 to  $40^{\circ}\text{C}$  caused a general decrease in mean germination time (MGT) across the morphotypes, with the lowest value observed at  $40^{\circ}\text{C}$  (Figure 1b.). However, morphotype PG indicated a significantly ( $P \le 0.05$ ) higher MGT at this temperature. The highest mean germination time occurred at  $20^{\circ}\text{C}$  for all morphotypes, but GG recorded significantly lower time than the rest (Figure 1b.).

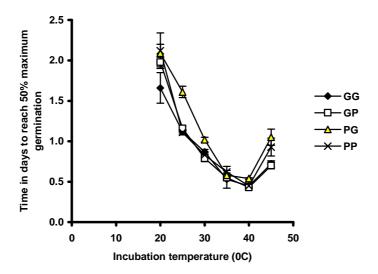


Figure 1a. Influence of constant temperatures (20, 25, 30, 35, 40 or 45°C) on time to reach 50% maximum germination (T<sub>50</sub>) of four spiderplant morphotypes during 4 days of incubation.

The vertical lines indicate S.E. for comparisons of significant ( $P \le 0.05$ ) differences between morphotypes.

The optimum germination temperature at 40°C, with MGT value of 0.5. There were no significant ( $P \le 0.05$ ) differences in MGT among morphotypes at 35°C (Figure 1b.). An increase in mean germination time occurred in all morphotypes at  $45^{\circ}$ C, and significantly ( $P \le 0.05$ ) highest time was recorded for morphotype PG. Morphotypes GG and GP were not different but had the lowest time (Figure 1b.).

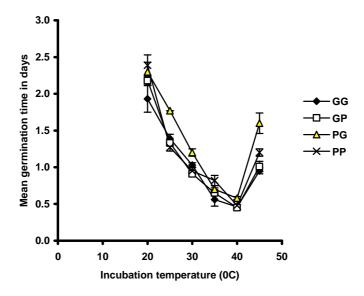


Figure 1b. Influence of constant incubation temperatures (20, 25, 30, 35, 40 or 45°C) on mean germination time (MGT) in days of four spiderplant morphotypes during 4 days of incubation.

The vertical lines indicate S.E. for comparisons of significant ( $P \le 0.05$ ) differences between morphotypes.

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#### Germination

A general increase in percentage maximum germination (Gmax) occurred with increasing incubation temperature from 20 to 35°C for morphotypes GG, GP and PP, and from 20 to 40°C for morphotype PG (Figure 1c.). Maximum germination for morphotypes GG, GP and PP was attained earlier at 35°C than for PG, whose maximum germination was attained later at 40°C (Figure 1c.). Significantly ( $P \le 0.05$ ) higher germination than the rest was observed for morphotype GG at 35 and 40°C (Figure 1c.). While morphotypes GP and PP were not different in germination at 40°C, they showed higher Gmax than PG; however at 35°C no differences in germination were recorded for the three morphotypes (Figure 1c.). At 45°C, significant ( $P \le 0.05$ ) drop in germinations occurred in all morphotypes, with GG recording the smallest drop while the greatest was indicated for morphotype PP (Figure 1c.).

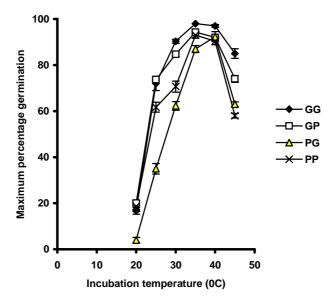


Figure 1c. Influence of constant incubation temperatures (20, 25, 30, 35, 40 or 45°C) on percentage maximum germination (Gmax) of four spiderplant morphotypes during 4 days of incubation.

The vertical lines indicate S.E. for comparisons of significant ( $P \le 0.05$ ) differences between morphotypes.

# Optimum Light Regime for Seed Germination Germination rate

Except for morphotype GP at incubation temperature of 35°C, light regime caused no significant ( $P \le 0.05$ ) difference in germination rate ( $T_{50}$ ) at temperature of either 30°C or 35°C (Table 1a.).

#### Mean germination time

No significant ( $P \le 0.05$ ) differences in mean germination time (MGT) were shown due to light regimes at incubation temperatures of either 30°C or 35°C (Table 1b.).

Table 1a. Influence of light regime at constant incubation temperatures of  $30^{\circ}\mathrm{C}$  and  $35^{\circ}\mathrm{C}$  on days to reach 50% maximum germination ( $T_{50}$ ) with four spiderplant morphotypes with seed incubation started under light duration of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under light duration of 8 hrs, and final germination count and seedling evaluation done on the  $10^{th}$  day.

	Incubation temperature <sup>O</sup> C				
Morphotype	30		35		
	Light re	egime	Light regime		
	8-hr light	In the dark	8-hr light	In the dark	
GG	$0.58 \pm 0.04a$	$0.67 \pm 0.02a$	$0.52 \pm 0.02a$	$0.49 \pm 0.05a$	
GP	$0.64 \pm 0.04a$	$0.66 \pm 0.04a$	$0.53 \pm 0.01a$	$0.57 \pm 0.19b$	
PG	$0.75 \pm 0.04a$	$0.81 \pm 0.03a$	$0.48 \pm 0.03a$	$0.59 \pm 0.02a$	
PP	$0.74 \pm 0.03a$	$0.77 \pm 0.03a$	$0.56 \pm 0.06a$	$0.52 \pm 0.04a$	

Figures followed by different letters for light regimes across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

Table 1b. Influence of light regime at constant incubation temperatures of  $30^{\rm O}{\rm C}$  and  $35^{\rm O}{\rm C}$  on mean number of days to reach maximum germination (MGT) with four spiderplant morphotypes with seed incubation started under daily light duration of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under daily light duration of 8 hrs, and final germination count and seedling evaluation done on the  $10^{\rm th}$  day.

	Incubation temperature <sup>O</sup> C			
Morphotype	30Light regime		35Light regime	
	GG	$0.64 \pm 0.04a$	$0.76 \pm 0.02a$	$0.58 \pm 0.02a$
GP	$0.71 \pm 0.03a$	$0.79 \pm 0.04a$	$0.61 \pm 0.03a$	$0.64 \pm 0.01a$
PG	$0.85 \pm 0.04a$	$0.89 \pm 0.05a$	$0.68 \pm 0.04a$	$0.65 \pm 0.02a$
PP	$0.82 \pm 0.01a$	$0.89 \pm 0.04a$	$0.60 \pm 0.04a$	$0.62 \pm 0.04a$

Figures followed by different letters for light regimes across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

#### Germination

There was no significant ( $P \le 0.05$ ) difference in percentage germination among morphotypes due to light regimes at incubation temperature of  $30^{\circ}$ C. At incubation temperature of  $35^{\circ}$ C significant differences in Gmax was only observed for morphotype GP (Table 1c.).

# Optimum Temperature for Seedling Development Percentage normal seedlings

Significantly ( $P \le 0.05$ ) higher percentage normal seedling was achieved at  $35^{\circ}$ C with germination started under light, for morphotype PG. When germination was initiated in the dark, significantly ( $P \le 0.05$ ) higher percentage normal seedlings was recorded for morphotypes PG and PP at  $30^{\circ}$ C, while at incubation temperature of  $35^{\circ}$ C, it was only observed for GP (Table 2a.).

Table 1c. Influence of light regime at constant incubation temperatures of  $30^{\rm O}{\rm C}$  and  $35^{\rm O}{\rm C}$  on maximum percentage germination (Gmax) with four spiderplant morphotypes with seed incubation started under daily light duration of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under daily light duration of 8 hrs, and final germination count and seedling evaluation done on the  $10^{\rm th}$  day.

	Incubation temperature <sup>O</sup> C				
34 1 4	30		35		
Morphotype	Light 1	ght regime Light reg		egime	
	8-hr light	In the dark	8-hr light	In the dark	
GG	$95.0 \pm 1.7a$	$90.0 \pm 2.5a$	$92.0 \pm 2.6a$	$99.5 \pm 0.5a$	
GP	$89.5 \pm 2.8a$	$94.0 \pm 2.2a$	$94.0 \pm 2.2a$	$99.5 \pm 0.5a$	
PG	$89.5 \pm 2.8a$	$89.0 \pm 2.1a$	$89.0 \pm 3.9a$	$91.5 \pm 1.0a$	
PP	$90.0 \pm 2.5a$	$92.0 \pm 2.6a$	$95.0 \pm 1.7a$	$93.0 \pm 1.3a$	

Figures followed by different letters for light regimes across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

Table 2a. Influence of constant incubation temperatures of 30°C and 35°C on percentage normal seedlings with four spiderplant morphotypes with seed incubation started under daily light regime of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under daily light regime of 8 hrs and final germination count and seedling evaluation done on the 10<sup>th</sup> day.

	Light regime				
M 1 .	8-hr light Incubation temperature		In the dark Incubation temperature		
Morphotype					
	30°C	35°C	30°C	35°C	
GG	$74.0 \pm 5.3a$	$75.5 \pm 2.1a$	$42.5 \pm 9.9a$	66.0 ±10.4a	
GP	$66.5 \pm 4.3a$	$71.0 \pm 3.1a$	$53.0 \pm 5.1a$	$42.0 \pm 5.5$ b	
PG	$42.0 \pm 3.3a$	$55.5 \pm 2.5b$	$31.5 \pm 4.1a$	$39.5 \pm 3.3b$	
PP	$64.0 \pm 2.9a$	$64.0 \pm 3.7a$	$47.5 \pm 3.7a$	$33.5 \pm 4.0b$	

Figures followed by different letters for temperatures across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

#### Percentage abnormal seedlings

Where germination process was initiated under light, significantly ( $P \le 0.05$ ) higher percentage abnormal seedlings was recorded at incubation temperature of 30°C than 35°C for morphotypes GP and PG (Table 2b.). With germination started in the dark, significantly ( $P \le 0.05$ ) higher percentage abnormal seedlings recorded at 30°C than at 35°C for only PG (Table 2b.).

### Percentage hard seeds

With germination process started under light, significantly higher percentage of hard seeds was recorded at incubation temperature 30°C than at 35°C for morphotypes GG and PG. Where the process was initiated in the dark higher percentage of hard seeds was observed at incubation temperature of 35°C than at 30°C for morphotypes GP and PP (Table 2c.).

Table 2b. Influence of constant incubation temperatures of  $30^{\circ}$ C and  $35^{\circ}$ C on percentage abnormal seedlings with four spiderplant morphotypes with seed incubation started under daily light regime of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under daily light regime of 8 hrs and final germination count and seedling evaluation done on the  $10^{th}$  day.

	Light regime			
26 1 .	8-hr light Incubation temperature		In the dark  Incubation temperature	
Morphotype				
	30°C	35°C	30°C	35°C
GG	$14.5 \pm 3.0a$	$13.5 \pm 1.3a$	$21.5 \pm 3.3a$	22.0 ±4.7a
GP	$20.5 \pm 2.5a$	$13.0 \pm 3.0b$	$31.0 \pm 1.7a$	$29.0 \pm 4.0a$
PG	$35.0 \pm 3.7a$	$24.5 \pm 1.7b$	$57.5 \pm 5.6a$	$46.0 \pm 2.9b$
PP	$23.5 \pm 1.9a$	$20.0 \pm 2.5a$	$38.0 \pm 6.5a$	$28.5 \pm 6.2a$

Figures followed by different letters for temperatures across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

Table 2c. Influence of constant incubation temperatures of 30°C and 35°C on percentage hard seeds with four spiderplant morphotypes from with seed incubation started under daily light regime of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under daily light regime of 8 hrs, and final germination count and seedling evaluation done on the 10<sup>th</sup> day.

	Light regime			
Morphotype	8-hr light		In the dark	
	Incubation	temperature	Incubation temperature	
	30°C	35°C	30°C	35°C
GG	$5.0 \pm 1.7a$	$2.0 \pm 0.8$ b	$0.5 \pm 0.5a$	0.5 ±0.5a
GP	$10.0 \pm 3.2a$	$6.0 \pm 2.2a$	$3.0 \pm 1.3a$	$7.5 \pm 1.5$ t
PG	$20.0 \pm 3.3a$	$11.0 \pm 2.1b$	$10.0 \pm 3.4a$	$8.5 \pm 1.0a$
PP	$9.0 \pm 1.7a$	$5.5 \pm 2.4a$	$3.5 \pm 1.5a$	$7.0 \pm 1.3b$

Figures followed by different letters for temperatures across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

Table 2d. Influence of constant incubation temperatures of  $30^{\rm O}$ C and  $35^{\rm O}$ C on percentage dead seeds of four spiderplant morphotypes with seed incubation started under daily light regime of 8 hrs or in the dark for up to a period of 32 hrs, seedling development continued under daily light regime of 8 hrs, and final germination count and seedling evaluation done on the  $10^{\rm th}$  day.

	Light regime			
34 1 .	8-hr light Incubation temperature		In the dark  Incubation temperature	
Morphotype				
	30°C	35°C	30°C	35°C
GG	$6.5 \pm 1.0a$	$9.0 \pm 1.7a$	$35.5 \pm 6.9a$	11.5 ±10.8b
GP	$3.0 \pm 0.6a$	$6.0 \pm 2.2b$	$13.0 \pm 5.1a$	$21.5 \pm 7.4a$
PG	$3.0 \pm 1.0a$	$9.0 \pm 2.7$ b	$1.0 \pm 0.6a$	$6.0 \pm 5.3a$
PP	$3.5 \pm 0.5a$	$10.5 \pm 2.6b$	$11.0 \pm 5.1a$	$31.0 \pm 5.1b$

Figures followed by different letters for temperatures across the rows are significantly ( $P \le 0.05$ ) different according to Student's t-Test. Data are means  $\pm$  S.E. of 4 replicates of 50 seeds each.

## Percentage dead seeds

With germination initiated under light, significantly ( $P \le 0.05$ ) a higher percentage dead seed was observed at incubation temperature of 35°C than at 30°C, for morphotypes GP, PG and PP out of the four studied (Table 2d.). Where the germination process was commenced in the dark, significantly ( $P \le 0.05$ ) higher percentage dead seeds was shown at incubation temperature of 35°C than 30°C for morphotype PP and, at 30°C than 35°C for GG, also for one morphotype out of the four (Table 2d.).

#### DISCUSSION

# **Optimum Temperature for Seed Germination**

Temperature had great influence on germination rate, mean germination time and final germination percentage (Figures 1a., 1b. and 1c.). From 20 to 40°C the mean germination time decreased, while the germination rate and percentage germination increased, with optimum value reached at temperatures of 40°C (Figures 1a., 1b. and 1c.). The reduced germination rate and percentage germination, and the increased mean germination time at low temperature of 20°C could probably be attributed to reduced absorption of water by the seed, accompanied by reduced diffusion of respiratory gases leading to reduced chemical reactions in the seed. The rate and capacity of seed germination were also observed by [13] to be temperature dependent through its effects on rates of water absorption, respiratory gases diffusion and chemical reactions. This is further in agreement with [10], where low temperatures are observed to bring about low germination rates, while increase of temperature up to certain limits tends to increase the rate of germination.

While germination occurred at 45°C, it did so at reduced levels than at optimum, and the radicles that protruded through the seed coats appeared shorter than what would be considered normal under the evaluation of type E seedlings [10]. It has also been reported by other researchers that further increase in temperature beyond the limits reduces germination or hinders it altogether [10], and this agrees with the observation that incubation temperature of 45°C, arrested growth of the radicles.

The optimum temperature when growth is maximum and the range of temperature at which growth is possible varies from species to species. The range of temperature optimum for the growth of tropical plants (10-45°C) is usually higher than for temperate plants (5-35°C) [25]. From its wide distribution in Kenya (Plate 1.), spiderplant seems to tolerate wide range of environment conditions, as was also evident from our study. Germination rate is said to be invariably low at low temperature but increases gradually as temperature rises, similar to chemical rate-reactions curve [26]. There occurs an optimum level where the germination rate is most rapid and above which a decline in rate occurs as the temperature approaches a lethal limit when the seed is injured.

Proso millet and *Leptochloa sinensis* which both have  $C_4$  physiological pathways, had their highest seed germinations at temperatures of 35 and  $40^{\circ}$ C [19] and 25-35°C [18], respectively, which suggests that the temperature requirements for seed germination of these two species are high. Since spiderplant is a  $C_4$  plant [1, 2], this may partly explain why temperatures of 30, 35 and  $40^{\circ}$ C resulted in better germination than lower ones. The observation tends to be supported

by reportedly wide distribution of *Cleome* genus in the drier parts of the tropics and subtropics, where temperatures are usually high [5, 6]. The wide distribution of the species in Kenya has also been documented, both in terms of altitude and agro-climate [5, 6]. Optimum germination takes place at higher temperatures for tropical species than for temperate ones.

# **Optimum Light Regime for Seed Germination**

Germination response to light was not affected by spiderplant morphotypes studied. The difference between light and dark germination was generally not significant in the morphotypes at 30 and 35°C (Tables 6.1a, 6.2a and 6.3a). Although it has been reported that very small seeds require light to ensure that germination occurs in seeds lying near the surface [10], it however apparent in this study that despite spiderplant being small-seeded, germination will still occur both in seeds lying near the surface and those buried deep in the soil.

In a study with *Leptochloa sinensis*, light played a role in inducing seed germination [17]. However, when seeds were incubated at temperature range of 30-35°C under both light and dark conditions, similar and quite low mean germination times (MGT) were attained. These observations agree with the results obtained in our study where there were no differences in MGT when germination process was initiated under light or in the dark at 30 and 35°C. There were no significant differences in germinations noted between the morphotypes, at incubation temperatures of 30 and 35°C, either with germination process started under light duration or in the dark. This characterized the seeds of the spiderplant morphotypes to have neutral photoblastism. This is contrast other reports [18], where germination performed at incubation temperature range of 30-35°C in the dark reduced germination by 80%, thus signifying a marked seed positive photoblastism in *L. sinensis*. Further contrasts are reported from studies carried with *C. gynandra* seed seedlots from ARC in South Africa and KSC in Kenya, whereby both seedlots exhibited negative photoblastism, when they were tested at either alternating temperatures (20/30°C) or constant temperature (20°C), in darkness or under continuous white light [21].

The independence of light stimulus to seed germination of spiderplant could be a factor necessary for seedling survival, which is also crucial for successful plant establishment by the species.

Morphotypes however, showed some differences from one another, in the way light and temperature regimes combined to influence their germination performances (Tables 6.1b, 6.2b and 6.3b). Generally, better performance in terms of germination rate, mean germination time and percentage germination was observed to occur in spiderplant morphotype GG. Morphotypes GP and PP hade intermediate performances, while the least in germination performance recorded in PG. The indicated good germination performance in the spiderplant morphotype GG, with regard to different combinations of light and temperature regimes, could be a pointer for its capability to successfully establish itself and survive in more ecological niches than the others.

# **Optimum Temperature For Seedling Development**

For percentage normal seedlings, response to temperature was equally balanced between the two incubation temperatures (30 or 35°C) under test, and combinations light. Morphotype GG generally produced higher percentage of normal seedlings than the other morphotypes at any of

the two temperatures tested, and also under any light combinations. Incubation temperature of 30°C tended to influence the development of more abnormal seedlings. Morphotype GG generally produced lower percentage of abnormal seedlings compared to others, while more abnormal seedlings with morphotype PG, at the two temperature regimes and combinations of light thereof. The effect of temperature on percentage hard seeds was equally exhibited by the two incubation temperatures. Morphotype GG performed better by producing lower percentage of seeds that remained hard than the other morphotypes, while PG produced the highest percentage. In terms of percentage seeds, incubation temperature of 35°C generally caused the death of more seeds than 30°C. More death also generally resulted with seeds of morphotype GG compared to others, and PG recorded lower percentage death in seeds.

The optimum temperature may shift after germination begins because seedling growth tends to have different temperature requirements than seed germination [26], for instance in the nursery or laboratory the usual practice is to shift the seedlings to a somewhat lower temperature regime following germination so as to prepare the plants for transplanting and reduce disease problems in the seed bed.

Prolonged high temperatures may be injurious to plant life [25]. At above-optimum temperatures, growth and other development are affected adversely and ultimately the vital processes of the plant cease and death results. Seed germination and seedling growth require increasing quantities of proteins [13]. Polysomes are reported to be required for protein-synthesis to sustain embryo growth rapidly and that they increase in hydrated cells of seed, accompanied by a decline in the number of single unattached or free ribosomes [15, 14]. Therefore, any factor such as prolonged high temperature during germination and seedling growth that could interfere with protein synthesis thus affecting the development of seedlings in the negative.

Temperature affects biochemical reactions and the metabolism of plants. When the temperature increases beyond the optimum, the rate of the processes decreases and finally the processes cease at a temperature of, e.g.  $60^{\circ}$ C and above. This happens as most of the enzymes are inactivated and protoplasmic proteins coagulate, which leads to injury or death of the plant [25].

Light is needed for seedling growth to produce sturdy and vigorous plants with green expanded leaves [25]. Chlorophyll pigment does not develop and the seedlings remain chlorotic, and soon die [25]. Light is essential for the supply of energy that is necessary for photosynthesis, which will not take place in darkness, regardless of what the other environmental conditions may be.

The higher percentage of hard seeds with incubation temperature of 35°C and under light could possibly be attributed to the development of secondary dormancy, coupled with prolonged exposure to light (i.e. photodormancy). Other researchers observed inhibition of germination of spiderplant seeds at 20°C in the presence of light, while in darkness the germination improved [21]. However, there was no observed improvement of germination when the process was undertaken at alternating temperature 20/30°C [21]. The phenomenon of secondary dormancy is one of the observed of the conditions involved in the seasonal rhythms and prolonged survival of certain kind of weed seeds in soil [13]. Since spiderplant is a weedy species, there is likelihood of it still having such characteristics.

Differences in seed germination and seedling development were evident among the four morphotypes, indicating that there are some differences, which could be due to their genotype. Since the germination process can be profoundly affected both by the external and internal seed factors [13], it is thus possible that the internal plant factors pertaining to the individual spiderplant morphotypes could have contributed to some of the observed differences among them.

#### **CONCLUSION**

Results from the present laboratory studies have demonstrated that seed germination in spiderplant is strongly influenced by temperature, with optimum germination occurring at constant incubation temperatures of 40°C. Incubation temperatures of 30 and 35°C generally influenced seedling development in equal measure for normal seedlings and seeds that remained hard. At 30°C more abnormal seedlings resulted compared to 35°C, while temperature of 35°C caused more seed death than 30°C. Morphotype GG had better performing seedlings except when temperature and light combinations caused more seed death than in the other morphotypes. Morphotype PG performed poorest in seedling development except where it had lower seed death than others.

#### Recommendations

A number of African indigenous vegetables (AIVs) have been confirmed through various scientific studies to possess superior nutritional attributes compared to some of the exotic vegetables, apart from some of them having important medicinal values. In addition, they have definite role to play in the food and nutritional security of the African households, this in addition to their income generation potentials when they are put under good cultivation. There is huge demand in Kenya at the moment for the various AIVs, amongst them being spiderplant. To enhance the capability of the producers meet this huge demand, both in terms of quantity and quality vegetables supplied to the consumers, easy access by the producers to seeds of high quality must be prioritized. It is our humble opinion that the results of this study would therefore form part of the development of any seed testing protocol that is quite necessary in order to ascertain the quality of any spiderplant seeds made available to producers, for them to realize the value for their money, through increased yield and profitability.

# Acknowledgements

The authors acknowledge the financial support by MHO-MU Seed Technology Phase II funded by the Royal Netherlands Government and Moi University for granting the opportunity to carry out this study away at Plant Research International in the Netherlands.

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