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EFFECT OF TEMPERATURE, LIGHT AND PRE-GERMINATION TREATMENTS ON SEED GERMINATION AND VIABILITY OF SPIDER PLANT (*Cleome gynandra*)

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1.INTRODUCTION

Cleome gynandra L. syn. *Gynandropsis gynandra* (L.) Briq. from the Capparaceae family is an indigenous, semi-domesticated leafy vegetable. It is referred to as the African spider flower, cat's whiskers, and bastard mustard, *Cleome* has over 150–200 species, of which 50 are native to Africa (Schippers, 2000). The exact origin of *Cleome* is unknown; however, reports indicate that it is a native plant of the African Islands, tropical Africa, Asia, and the Middle East (Chweya & Mnzava, 1997); (Fletcher, 1999). It spread to tropical and subtropical nations in both the southern and northern hemispheres, (Kokwaro, 1976).

It is among the major native leafy vegetables in Africa (HCDA, 2014), and is highly consumed in the rural areas of many African communities. Tender shoots, leaves, and occasionally inflorescences are eaten (Chweya & Mnzava, 1997). *C. gynandra* have a high nutritional value and medicinal properties. Chweya and Mnzava (1997); Jinazali et al. (2017);Nesamvuni et al. (2001) and Odhav et al. (2007) reported a rich source of minerals, such as iron, zinc, magnesium and calcium, vitamins A and C, and proteins in spider plants.

Furthermore, they have been reported to have higher levels of dietary phytochemicals and antioxidant activity than exotic and commercial vegetables (Moyo et al., 2013); (Chepkwony et al., 2020). Opole (1995) found that women commonly eat it before and after giving birth, and boys are fed it shortly after circumcision due to its medical benefits of restoring blood circulation. Additionally, spider plants have been traditionally used in various communities to treat a variety of illnesses, such as bacterial infections, snake bites, food poisoning, rheumatism, inflammation, toothaches, headaches, and bacterial infections (Chweya & Mnzava, 1997; (Mishra et al., 2011).

In rural areas, this native leafy vegetable is an important part of household diets, (Schippers, 2000; Jansen Van Rensburg et al., 2004). It plays a major role in household food and nutrition security of rural communities (Dansi et al., 2012);(Silue, 2009); (Onyango et al., 2013);(Ekpong, 2009). In most areas, this vegetable is harvested during the rainy season and is also preserved to be consumed in the dry period and sold in local markets (Carr et al., 2012). It provides a source of income for the poor and unemployed people in those areas (Onyango et al., 2013). It primarily grows and is collected from the wild or farmers' fields as weedy crops or volunteer plants that spread on their own throughout the wet season (AOCC, 2017);(Carr et al., 2012);(DAFF, 2010).

Alternatively, farmers that want to cultivate and improve the crop stands of spider plants in the home gardens and fields tend to source seeds from nearby markets, neighbors, or wild plants (Maundu et al., 1993); (Muasya et al., 2009). Some farmers wait for self-dispersal of seed shattering. In some countries, seeds are sold along with other vegetable seeds by seed producers and dealers in South Africa, Kenya, and Tanzania (Muasya et al., 2009); (Shango, 2015). Abukutsa-Onyango, (2007), however, noted that one of the main productivity constraints is low-quality seed and underperforming farmer cultivars. Seed quality is vital for successful crop production and high yields.

Among these, several constraints owing to the underutilization and limited production or commercialization of spider plants in Africa are their low seed germination potential. Geneve (1998), reported significant non-deep physiological dormancy in *Cleome* seeds. Seed dormancy diminishes the quality of seeds used in commercial seed production, which lowers germination and crop stands (Ochuodho & Modi, 2005).

Thus far, various studies have focused on enhancing the germination rate and overcoming seed dormancy. Muasya et al. (2009) showed the association of *C. gynandra* seeds with physiological dormancy, as seed dormancy was overcome by chilling, exogenous GA3 use, and exposure to darkness. Studies by Ochuodho and Modi (2005), showed that in relation to temperature, light, and pre-germination treatments, spider plant germination can be best performed at alternating temperatures of 20/30°C or 30°C in constant darkness. Therefore, this study aimed to investigate the effects of temperature, light, and pre-germination treatments on the seed germination and viability of spider plants (*Cleome gynandra*).

2.OBJECTIVE OF THE STUDY

Main objectives

The overall objective of the study is to determine the effects of alternating temperatures, light and seed pre-treatment on germination and viability of *Cleome gynandra*.

Specific objectives

1. To determine the effects of pre-germination treatments on the germination of spider plant.

2. To examine the effects of alternating temperatures and light variations on the germination.

3. To examine the seed viability of *Cleome* seeds.

2.LITERATURE REVIEW

2.1 Introduction of *Cleome gynandra*

Spider plant (*Cleome gynandra L.*) belong to the botanical family Capparaceae, subfamily Cleomoideae. This family comprises approximately 700-800 species across 45 genera, as noted by (Kuhn, 1988) & (Kokwaro, 1994). The genus *Cleome* have over 200 species, as documented by Iltis (1967) and Bruinsma (1985) consists of highly diverse herbaceous plants. The *Cleome* genus is phylogenetically closely related to the Cruciferae (Brassicaceae) family (Bremer & Wannorp, 1978). These species are primarily found in tropical and subtropical regions, with a significant presence in Africa.

C. gynandra is an annual, erect herbaceous and branched plant that can grow up to 1.5 m tall (Vorster et al., 2002) depending on environmental conditions but is usually 0.5 to 1.0 m tall. The leaves are compound and palmate with three to seven leaflets that radiate from the tip of the petiole (Mishra et al, 2011). The leaves exhibit a circadian movement, where they orient themselves in the direction of the sun (Kwarteng et al., 2018).The stems and petioles are thickly granular and hardly hairless, with longitudinal parallel lines on the stems. It contains numerous branches which becomes woody as the plant ages (DAFF, 2010); (Shilla et al., 2016). The pigment on the stems varies from green to pink and purple (Van Rensburg et al., 2007); (Mishra et al, 2011). It has a racemoid inflorescence, that bears many flowers and elongates when fruit develops (Mishra et al., 2011). (Figure 1. 2).

The terminal inflorescences have distinct small white, purple or pink-coloured flowers (Schippers, 2000). The flowers are attached to the stem by short, equal stalks at equivalent distances (DAFF, 2010). The flowers are bisexual. Chweya and Mnzava (1997) noted that the pollination characteristics of *C. gynandra* had not been established, yet observed that the species exhibited both self- and cross-pollination.

The fruits of *C. gynandra* are tiny and siliques, while the seeds are round black or brown and circular with rough seed coat and parallel longitudinal lines (Van Wyk & Gericke, 2000; DAFF, 2010). The plant has a long taproot and a small number of secondary roots covered with root hairs (DAFF, 2010; Shilla et al., 2019).

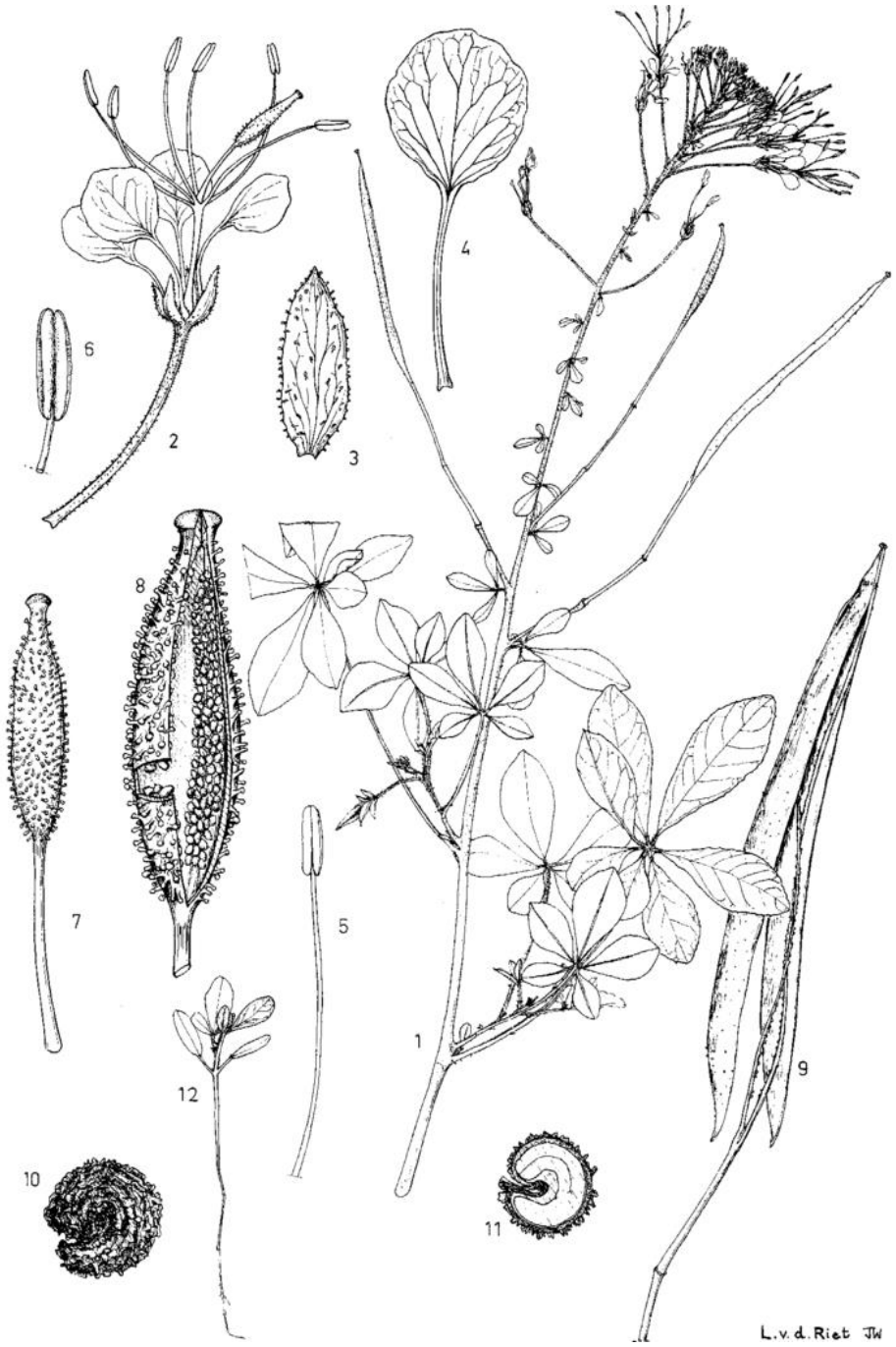


Figure 1 Cleome gynandra L.: 1-flowering and fruiting branch (x0.6); 2-flower (x2); 3-sepal, external view (x8); 4-petal, internal view (x4); 5-stamen (x4); 6-anther (x6); 7-gynoecium (x6); 8-ovary, longitudinal section (x10); 9-dehiscent fruit (x1.2); 10-seed (x8); 11-seed, opened with embryo (x8). Chweya and Mnzava (1997)

Source: Web (43) Pinterest)

C. gynandra is propagated through its seeds. It has the ability to thrive in harsh environments such as dry lands, degraded lands, and wetlands (Baldermann et al., 2016). Commonly, these plants grow in fertile soil, particularly in areas where animal manure or homestead refuse has been incorporated. They can grow on a wide range of soils as long as they are deep and well drained, with a pH range of 5.5-7.0, and can vary from sandy loam to clay loam Chweya & Mnzava, 1997).

Cleome gynandra is well adapted to a wide range of environmental conditions because of its C4 photosynthetic mechanism, which allows efficient water utilization and high photosynthetic capacity at high temperatures (Raju & Rani, 2016). This leafy vegetable plant thrives in areas with high light intensity and can grow well from sea level up to 2400 meters. It can tolerate both high and low temperatures but prefers a temperature range of 18-25°C. However, it does not tolerate shade well and requires a high light intensity to grow. The plant can grow in areas with short periods of rainfall, but is not particularly resistant to prolonged drought. While it can tolerate some drought conditions, water stress hastens the maturity and senescence of plants, leading to stunted growth under drought conditions (Chweya & Mnzava 1997).

Cleome gynandra is a vital leafy vegetable that ensures food security in many rural areas of Africa (Van Wyk & Gericke, 2000; Vorster et al., 2002; Mabhaudhi et al., 2017). During times of abundance, leaves and tender shoots are sold in both rural and urban markets by mostly female rural gatherers and growers. This vegetable provides a source of income for rural areas, particularly for economically disadvantaged and unemployed individuals (Chweya & Mnzava 1997). In India, it is consumed as a pot herb and flavoring in sauces, while in Thailand, it is eaten in the form of a fermented product called 'paksian-dong' (FAO, 1990).

The plant has a variety of uses, providing food and medicinal benefits for humans and animal feed, and has potential as a plant protector. According to a study by Moyo et al., (2018), the leaves of *C. gynandra* contain higher levels of calcium, iron, phosphorus, potassium, and vitamin C than common vegetables such as *Brassica oleracea* var. capitata (cabbage), and *Beta vulgaris L.* (Swiss chard). It is a rich and easily accessible source of essential vitamins (A and C), proteins (23.4%), fibers (8.3%), and minerals (Uusiku et al., 2010; Aworh, 2014). Leaves contain dietary polyphenolic phytochemicals that support good health, including flavonoids, essential ions, polyphenols, and terpenoids (Chataika et al. 2021; Moyo & Aremu, 2022).

Spider plant is traditionally used to treat a range of medical conditions in different countries, such as anemia, cancer, arthritis, diabetes, cardiovascular diseases, chest pain, earaches, constipation, epilepsy, malaria, piles, rheumatism, scurvy, stomachaches, tumors, and relieving eyewash (Faber et al., 2010; (Mishra et al., 2011; Moyo & Aremu, 2022). Studies have shown that compounds found in *C. gynandra* possess high medicinal value and exhibit anti-inflammatory, antibacterial, antimicrobial, anticancer, antioxidant, antiallergenic, antispasmodic, antihyperglycemic, and cytotoxic properties (Bala et al., 2011; Lawal et al., 2015; (Chand et al., 2022).

Despite its potential benefits, spider plants are currently underutilized and neglected globally. The cultivation of spider plants has not yet been commercialized. It is generally considered a wild, weedy, and volunteer plant, and is only semi-domesticated in-home gardens or on fertile land near homesteads in select African countries, such as Kenya, Uganda, Botswana, Zambia, South Africa, Zimbabwe, Malawi, Nigeria, Cameroon, Namibia, Swaziland, Tanzania, and Ghana (Chweya & Mnzava 1997). However, it has the potential for cultivation.

2.2 Seed Morphology of Spider plant

Cleome gynandra seeds are typically brown or black in color. These seeds are generally round or nearly round and may feature a pointed tip at the apical region where the radicle resides. The hilum is situated at the seed's center. Seed surfaces can be rough or slightly rough, featuring small, rounded, or ocellated depressions and ridges covering the entire seed surface (Blalogoe et al.,2020). The embryo comprises a hypocotyl-radicle axis and two cotyledons, with an endosperm present in the micropylar region (Figure 1. 11). Few studies have examined seed morphological variations among accessions. Spider plants produce a substantial number of seeds, which are harvested when the pods are fully ripe and yellow but before they naturally open to avoid shattering (DAFF, 2010). However, the low germination and dormancy exhibited by seeds pose a constraint that hinders species productivity.

2.2.1 Seed dormancy of *Cleome gynandra*

Seed dormancy is a critical phase in the plant life cycle. *Cleome gynandra* seeds are characterized by their dormancy. This is a natural phenomenon that incapacitates the embryo to germinate, even under ideal conditions (Bewley & Black, 1994). Geneve (1998) grouped the *Cleome* genus among vegetable and floral genera that have primary non-deep endogenous

physiological seed dormancy, whose germination is light-dependent. This implies that endogenous dormancy occurs because of chemical changes within the seed embryo. Harper classification clearly shows this type of dormancy and the methods to break it (Appendix 3).

A study by Muasya et al. (2009) showed that the seeds of *C. gynandra* exhibit physiological dormancy because they were able to overcome their dormancy through exposure to darkness, chilling, and the introduction of exogenous GA3. Another report indicated that seeds possess both physiological and physical dormancy, which is eliminated by a combination of GA3 imbibition and seed aging (Saifullah et al., 2023). The research conducted by Khan et al. (2019) focused on dormancy mechanisms in *Cleome gynandra* seeds, and their findings revealed that physiological dormancy, caused by embryo immaturity, has a significant impact. This study showed that seeds with underdeveloped embryos experienced prolonged dormancy, indicating a connection between embryo development and the release of dormancy.

Seed dormancy allows seeds of weedy species to germinate over an extended period, thereby ensuring long-term survival (Ochuodho & Modi, 2005).This permits germination to be distributed both temporally and spatially. Such temporal distribution may be beneficial for the survival and spread of this species (Bewley & Black, 1994). This also means that there will be less competition among the seedlings that germinate over time. Consequently, dormancy provides a time distribution for germination, because it breaks dormancy according to environmental factors that also have a temporal distribution (Bewley & Black, 1994). Examples include seeds that are chilled for weeks or months at 1 to 5℃ temperatures to break dormancy, which must wait for the cold season to pass before they can germinate, as these temperatures are only experienced during the winter.

In addition, studies have demonstrated that environmental factors, like light and temperature changes, can lead to the breakage of dormancy (Hilhorst & Karssen, 1992). Researchers have found that varying temperatures can create conditions similar to those in natural settings and activate physiological reactions associated with the end of dormancy.

Dormancy is a complex quantitative trait influenced by numerous genes, some of which are regulated by environmental factors, as observed by (Caboche et al., 1998). Moreover, researchers proposed that the most effective model for explaining the maintenance and breakage of dormancy involves equilibrium between ABA and GA. A combination of dormancy-breaking

treatments not only transitions the seed from dormancy to a quiescent state but also triggers germination.

In addition, several pretreatment methods have been used to overcome the dormancy of *C. gynandra* seeds. However, these findings were contradictory and inconclusive (Shilla et al., 2016). However, Muasya et al. (2009) found that gibberellic acid was the most effective treatment for breaking dormancy in *C. gynandra* seeds when investigating the effects of different pretreatments including light, chilling, gibberellic acid, potassium nitrate, and leaching

Pre-heating the seeds at 40°C for 24 h effectively broke seed dormancy and increased germination to approximately 66% compared to chilling for 3, 5, and 7 days (Ekpong, 2009). Pre-chilling releases dormancy due to various metabolic processes that occur during these treatments, such as increasing the level and endogenous gibberellin responsiveness, but significantly reducing the level of abscisic acid (Bewley & Black, 1994).

To enhance the cultivation of spider plants, it is crucial to explore more effective ways to overcome dormancy in the seeds of *Cleome gynandra* using advanced technologies. In cultivated species, seed dormancy often delays the germination and emergence of seedlings.

2.2.2 Seed germination of *Cleome gynandra*

Germination is a vital phase in the life cycle of plants, which begins with the uptake of water by the dry seed (imbibition) and continues with biochemical and physical processes. During this stage, the embryonic axis elongates, and the radicle eventually emerges from the seed (Bewley & Black, 1994). There are several requirements that should be met for germination to occur; typically, there must be an adequate amount of aerobic respiration and temperature to allow different processes to proceed at a satisfactory rate (Bewley & Black, 1994). Environmental conditions affect seed germination, and understanding this effect is crucial for successful plant establishment in both ecology and agriculture (Ghaleb et al., 2022). Optimal germination is essential to ensure the growth and establishment of plants and crops.

In agriculture, seed germination is important to ensure successful and continuous plant production. It is a crucial process that affects seed quality, plant stands, and crop yield. Bewley and Black (1994) explained the purpose of a seedlot germination test is to assess the germination capacity, rate of germination, and overall homogeneity. Hence, seed testing standards, such as those set by the International Seed Testing Authority (ISTA) through their Rules for Seed Testing (ISTA, 2012), are necessary and strive to offer testing procedures for seeds.

Several factors can impede seed germination, even under favorable environmental conditions, including adequate levels of water, light, oxygen, and temperature. Temperature is the most critical environmental factor that regulates seed germination timing and rate under adequate water and oxygen availability. It affects seed physiology through deterioration and dormancy release, as well as the rates of various bio-physical processes involved in germination itself (Bewley & Black, 1994;Baskin & Baskin, 2014;Brändel, 2004;Ghaleb et al., 2021;McGinnies, 1960).

Cleome gynandra seeds are characterized by low germination and complex physiological processes influenced by various internal and external factors. The germination percentage under field conditions has been reported to be as low as 37 and 46% (Almekinders & Louwaars, 2000); (Ndinya, 2003). Additionally, the germination rate for gene bank accessions from different Kenyan research organizations was reported to be between 25% and 37% (Oseko, 2007). The low and erratic germination rate of *C. gynandra* is due to several factors. The conditions at harvest maturity, seed moisture content, storage conditions, environmental conditions, and dormancy state of seeds are among the factors reported. Simiyu, et al. (2004) and Kamotho (2004) reported that low germination was indeed due to the considerable dormancy exhibited by seeds. According to Ochuodho and Modi (2005), the primary reasons behind the poor seed germination observed in *Cleome* were the presence of a hard seed coat, immature embryos, or the occurrence of induced secondary dormancy.

Spider plant seeds require natural after-ripening before dormancy is released (Chweya et al., 1997; Geneve 1998; Kamotho et al. 2014). Similar findings have been observed in *Arabidopsis thaliana*, a closely related species to the spider plan (Ali-Rachedi et al., 2004). However, Ochuodho and Modi (2005) and (Motsa et al. (2015) reported that seeds that were after ripened for 3 months and 1 year, respectively, exhibited low initial germination but increased after applying certain mechanisms to break dormancy. Several studies have investigated possible treatments to break dormancy and enhance the germination of spider plant seeds.

Seed germination has been investigated under both alternating and constant temperature conditions In seed germination research, alternating diurnal temperatures have been used to mimic field conditions and have been reported to enhance germinability in various species (Baskin & Baskin, 2014).

Several studies have investigated the effects of light and temperature on germination of *C. gynandra* seeds. K'Opondo (2011); Muasya et al. (2009); (Ochuodho (2005); Ochuodho and Modi (2005); Sowunmi and Afolayan (2015); Kamara et al. 2017; (Zharare, 2012) found that the optimal temperature range for seed germination was between 25°C and 40°C, whereas Ochuodho & Modi (2005;2007) achieved the highest germination percentage at 20-30°C with constant darkness and scarification by puncturing the seeds at the radicle end. In contrast, Zharare (2012) discovered a new finding for this species with a tropical origin, where alternating 4°C/27°C for 16/8 h was optimal for seed germination of *C. gynandra*.

On the other hand, the results of the light conditions demonstrated a negative response of germination rate decline in *C. gynandra* when the seeds were subjected to constant light for more than 12 h. Research has shown that seeds exhibit greater germination rates under light conditions and display photoblastic germination behavior. As result, the optimal conditions for seed germination in *C. gynandra* involve continuous darkness and alternating periods of darkness and light for 8 h. This phenomenon, as described by Geneve (1998), is characterized by seeds being termed photo dormant, which is observed in spider plant seeds because of their small seed size of less than one milligram (Bewley et al., 2012).

A study by Ekpong (2009) demonstrated that heating freshly harvested seeds of *C. gynandra* at 40°C for 1 to 5 days was the most effective method, achieving up to 90% germination capacity compared to other dormancy-breaking techniques such as heating, soaking, leaching, potassium nitrate (KNO3), and gibberellic acid (GA3). Ekpong (2009) observed the lowest germination percentage in seeds treated with gibberellic acid (34%) and potassium nitrate (16%). However, (Muasya et al. (2009) found that gibberellic acid was the most effective treatment for breaking dormancy in *C. gynandra* seeds when investigating the effects of different pretreatments including light, chilling, gibberellic acid, potassium nitrate, and leaching.

Ochuodho's (2005) research examined the effects of pre-germination treatments, including scarification, hydration, chilling, and germination with potassium nitrate and gibberellic acid, on seeds of different origins stored for one year and two years. The study found that preheating at 40°C for 15 d and scarification effectively broke *C. gynandra* seed dormancy, whereas pretreating photo-inhibited seeds with GA3 improved germination. Despite these findings, different seed lots exhibit different germination rates (Ochuodho, 2005).

Farmers typically acknowledge the lack of high-quality seeds as a significant hindrance to attaining optimal plant growth (Abukutsa-Onyango, 2007). Poor seed germination negatively impacts seed quality and hinders crop improvement and seed production, which can ultimately lead to the domestication and cultivation of spider plants.

Certainly, temperature has a significant influence on the physiological state of seeds by impacting seed deterioration and the release of dormancy, as well as the rates of numerous biological and physical processes that are essential for germination

2.2.3 Seed viability of *C. gynandra*

The viability of seeds is a critical determinant of whether the seed embryo is still alive, which is crucial for plant propagation and conservation efforts.

Few studies have evaluated seed viability in *Cleome gynandra*, but they have mainly focused on seed longevity and storage behavior. For example, (Kamotho et al., 2014) investigated seed longevity and storage behavior in *Cleome gynandra* and reported that seeds maintained high viability levels under proper storage conditions. The study identified optimal storage conditions, including low humidity and temperature, that could preserve seed viability over an extended period.

Assessments of seed viability have revealed the effects of aging and physiological deterioration on germination capacity. Ekpong (2009) examined the impact of seed aging on the viability of *Cleome gynandra* and found a decrease in the performance of germination over time. This highlights the significance of regularly conducting seed viability tests and implementing quality control measures to guarantee seed health and vitality. Seeds with high viability and vigor can account for around 30% of the total crop production (Ngoze and Okoko, 2003).

According to (Ochuodho and Modi, 2007) the tetrazolium test's inability to provide indications of *Cleome* seed viability may be due to its negative results, where the seeds failed to be stained. This finding may be attributed to the impermeability of the seed coat membrane to the tetrazolium solution, as explained for Brassica by (Debeaujon & Koornneef, 2000). When viable seed tissues absorb tetrazolium chloride solution, it accurately indicates the activity level of the dehydrogenase enzyme system, which is closely connected to seed respiration and viability (Blalogoe et al., 2020;Marcos-Filho, 2015). These enzymes are crucial for seed respiration and, hence, seed viability.

This study argues that the Tetrazolium Test (TZ) test can be performed on *Cleome* seeds following the procedures of the plant species. The results of the TZ and germination tests are typically in close agreement, with differences between them being smaller when using high-quality seeds compared to low-quality ones. The TZ test was successfully conducted on various crop seeds and the results were positive. A strong correlation was observed between the germination percentage and viability percentage determined through TZ in wheat, rice, and maize (DigitalAgrifarm, 2024). Regression equations were also developed and utilized to predict the germination percentage, which was found to be extremely close to the actual germination percentage. Kamotho et al. (2014) indicated that seed viability has a direct relation and is accurately reflected by the percentage of germinated seeds. Thus, the TZ test is particularly useful when seeds are dormant or slow to germinate.

3. MATERIALS AND METHODS

3.1 MATERIALS

The study was conducted at the Department of Vegetable and Mushroom Growing, Hungarian University of Agriculture and Life Science, MATE (47.5930° N, 19.3615° E), from January to March 2024.

Three accessions from two different geographical origins, that is. Western Africa and Asia used in the experiment were acquired from the University of Namibia (UNAM). These accessions were TOT7196 from the World Vegetable Center, Malaysia; ODS-15-038 from GBioS, Benin; and ODS-15-053 from GBioS, Togo, (Chataika et al., 2021). These accessions have been included in ongoing breeding and research programs. The findings will be useful for improving the quality of seeds of these species.

The analytical chemicals used in the experiment were sulfuric acid, gibberellic acid, Ethanol, Hydrochloric acid, and 2,3,5 triphenyl tetrazolium chloride (TTC). They were sourced from the Hungarian University of Agriculture and Life Science, with TTC obtained from the HACH Company, Germany.

3.2 METHODS

3.2.1 Experimental design

The experimental design was a split-split-plot laid out in a completely randomized block design (CRBD) with four replications for each treatment (four treatments) and two blocking factors, resulting in a total of 96 experimental units (as illustrated in Figure 2).

3.2.2 General Seed Germination Procedure

Healthy and uniform (black colored) seeds from the accessions were selected and surface-sterilized by shaking in a solution of hydrochloric acid (37%) for 2 min, then triple-rinsed with distilled water and dried before being used in the germination studies.

Germination treatments were conducted by placing 25 seeds evenly on 11 cm diameter glass Petri dishes with a double layer of Whatman No. 1 filter paper moistened with 5 ml of distilled water. Petri dishes were placed in a growth chamber (SANYO, Versatile Environmental Test Chamber, MLR-351H). Seed germination was observed every 3 days for 15 days. Subsequent moistening of the seeds was performed during the counting day or as needed. Germinated seeds were removed from the Petri dishes during the first observation. Seeds were considered

germinated when the radicle was at least 2 mm in length. The germination test was performed according to ISTA (ISTA, 2012).The calculations were made using the following equations:

$$\text{Final germination percentage (FGP)} = \frac{\text{Total number of seeds germinated}}{\text{initial number of seeds used}} \times 100$$

(1)

$$\text{Mean germination Time (MGT)} = \frac{\Sigma nidi}{\Sigma N}$$

(2)

Where ni = the number of germinated seeds at day i, di = incubation period in days, and N = the number of germinated seeds in the test (Baskin & Baskin, 2014). The MGT units are days.

$$\text{Mean Germination Rate, (MGR)} = \sum(n \times d)N$$

(3)

Where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = the total number of seeds germinated at the end of the experiment (Ellis & Roberts, 1981). The unit for MGR is per day (d^{-1}).

3.2.3 Experiment Procedures

3.2.3.1 Germination condition treatment

To investigate the effect of temperatures and light on germination, seeds from each accession subjected to different germination treatments were incubated in a growth chamber at an alternating temperature of 30℃ for 12 h in the light at 20℃ for 12 h in dark. Another set of seeds was subjected to 30℃ in constant darkness. Relative humidity (RH) was maintained at 50%. A slight interruption occurred during counting.

3.2.3.2 Pre-germination treatments

Four replicates of 25 seeds from each accession were subjected to pre-germination treatment. For every accession, untreated seeds served as controls for all other treatments. The following physical and chemical treatments were applied to the seeds:

Gibberellic acid (GA3): Seeds from each accession were placed evenly on a double filter paper in the petri dish and moistened with 5 ml of 500 ppm GA3 (89%) solution. Petri dishes were

incubated according to previously described procedures. The planted seeds were subsequently watered with distilled water.

Scarification (liquid-sulfuric acid): Seeds from the three accessions were soaked in a 1 M Sulfuric Acid (96%) solution for 10 min and triple rinsed by swirling in distilled water for 5 min. Seeds were germinated in the growth chamber at an alternating temperature of 30℃ for 12 h in the light and at 20℃ for 12 h in dark. Another set of seeds was subjected to 30℃ in constant darkness.

Warm stratification (preheating): Seeds of each accession were heated at 40℃ in an oven (SANYO Convection Oven MOV-212F) for 24 h before incubation. Non-stratified seeds were used as controls.

3.2.4 Seed viability testing (TZ)

The International Seed Testing Association (ISTA) book (ISTA, 2012) specifies the tetrazolium (TZ) viability test, which was conducted to confirm seed viability after the germination test. TZ testing is a rapid and effective method for assessing seed viability in a seed lot, i.e., the portion of a seed lot that is alive.

After 15 d, ungerminated seeds were tested for seed viability using a tetrazolium test (TZ). The test was performed by randomly selecting 25 ungerminated seeds from the germination treatments. They were then allowed to imbibe distilled water for 18 h. The seeds were poked with a needle around their central region to weaken the seed coat and allow tetrazolium solution to access the embryo. The pH of the solution was measured at 6.7. The seeds were then heated in an oven for 24 h at 30°C in 0.5% (w/v) tetrazolium chloride solution. Tetrazolium-stained seeds, which indicate metabolically active tissues, were assessed under a dissecting microscope at the Mushroom Laboratory.

To view the stains clearly, the seeds were crushed between steel tweezers to remove the seed coats. Seeds that exhibited a significant amount of red staining in the root and shoot meristems were counted viable and those that remained white/yellow as non-viable.

The number of seeds that germinated and the number of dormant seeds that stained red in the tetrazolium test were used to determine the treatment's final seed viability.

$$\text{Final seed viability (FSV)} = \frac{\text{Total number of seeds germinated+dormant stained seeds}}{\text{initial number of seeds used}} \times 100 \qquad (4)$$

3.2.4 Data analysis

Analysis of variance was performed using the Statistix software version 8.0. Differences between treatments were determined by the least significant difference at the 5% level (LSD0.05).

4. RESULTS

4.1 Effects of pre-germination treatments on seed germination of *C.gynandra*.

The results showed a significant effect of pre-germination treatments on the seed germination of spider plant accessions (**Figure 2**). The application of GA3 500 ppm varied significantly among accessions, with ODS-15-038 having the highest germination and ODS-15-053 the lowest germination rate of 50% and 36.5%, respectively. Pre-heating of seeds at 40°C for 24 h slightly increased the germination of TOT7196 accession and had no significant effect on the germination of the other two accessions. Generally, seed soaking in sulfuric acid (H2SO4) significantly reduced seed germination in all three accessions.

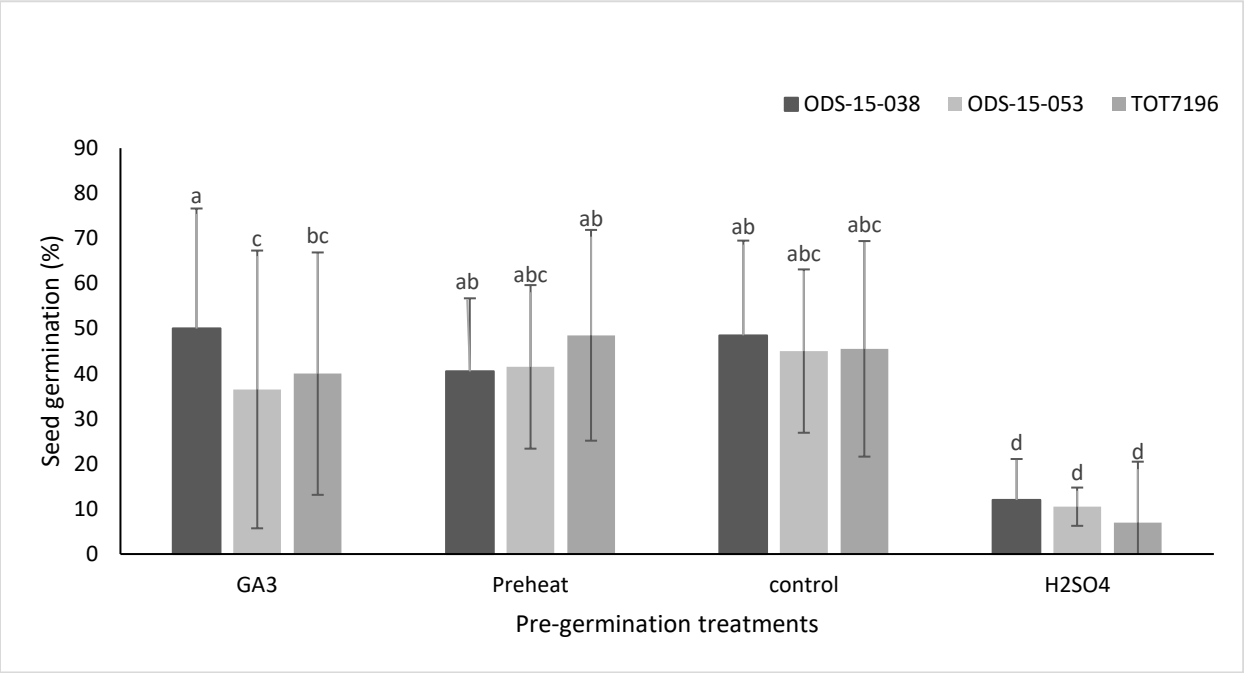


Figure 2 Effects of pre-germination treatments on seed germination of three accessions

4.2 Effects of alternating temperatures and light variations on the seed germination

The results showed that temperature and light had a strong effect on the seed germination of *C. gynandra* accessions (**Figure 3**). Subjecting the seeds to 30°C for 24 h in constant darkness improved seed germination compared to an alternating temperature of 30°C for 12 h in the light and 20°C for 12 h in the dark. However, there was no significant difference among the accessions.

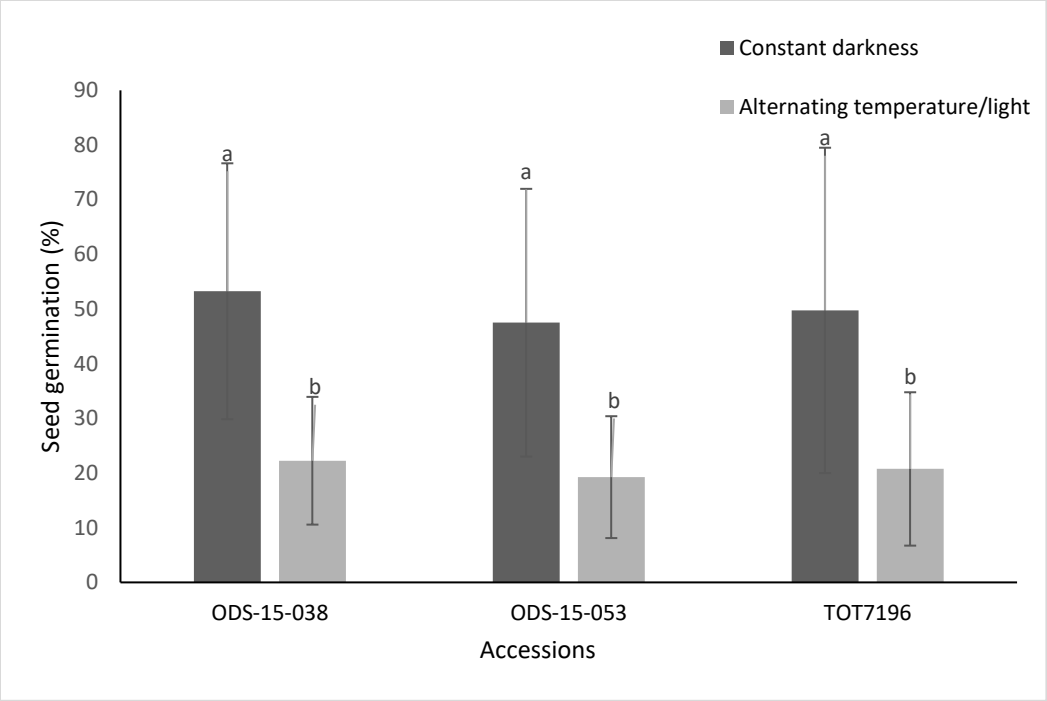
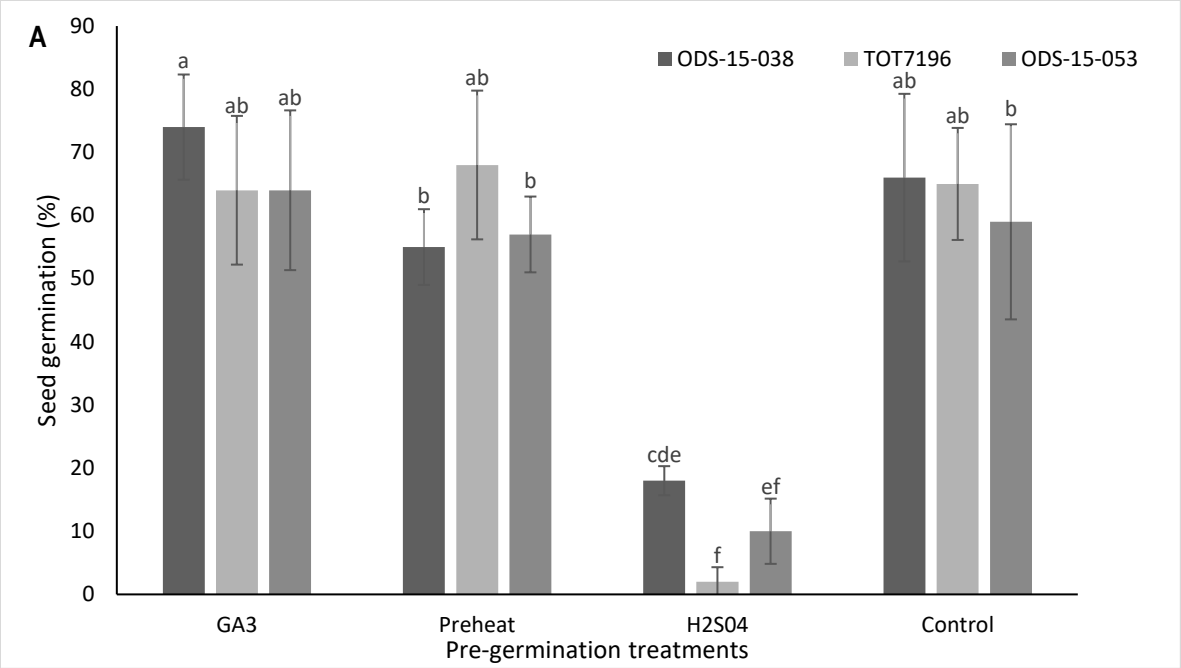


Figure 3 Effect of temperature and light on the germination of three accessions under constant temperature of 30 °C under darkness and alternating temperature of 30 °C 12 h in the light and 20 °C 12 h in the dark.

There are significant interactions between pre-germination treatments, temperature, and light on the germination of spider plants (**Figure 4**). Higher germination was observed at 30°C for 24 h in constant darkness **(Figure 4A)** than at an alternating temperature of 30°C for 12 h in the light and 20°C 12 h in the dark **(Figure 4B).** The pre-germination treatments showed no significant effects on seed germination, except for accession ODS-15-038 and ODS-15-053, which showed relative increase in germination of 8 % and 5 %, respectively, with the application gibberellin acid (GA3) **(Figure 4A). Figure 4B** shows a decrease in germination of all accessions under pre-germination, except accession TOT7196, with a 3% increase when the seeds were pre-heated at 40°C for 24 h. Soaking the seeds in sulfuric acid (H2SO4) significantly decreased germination for all three accessions under both temperature and light conditions **(Figure 4)**.



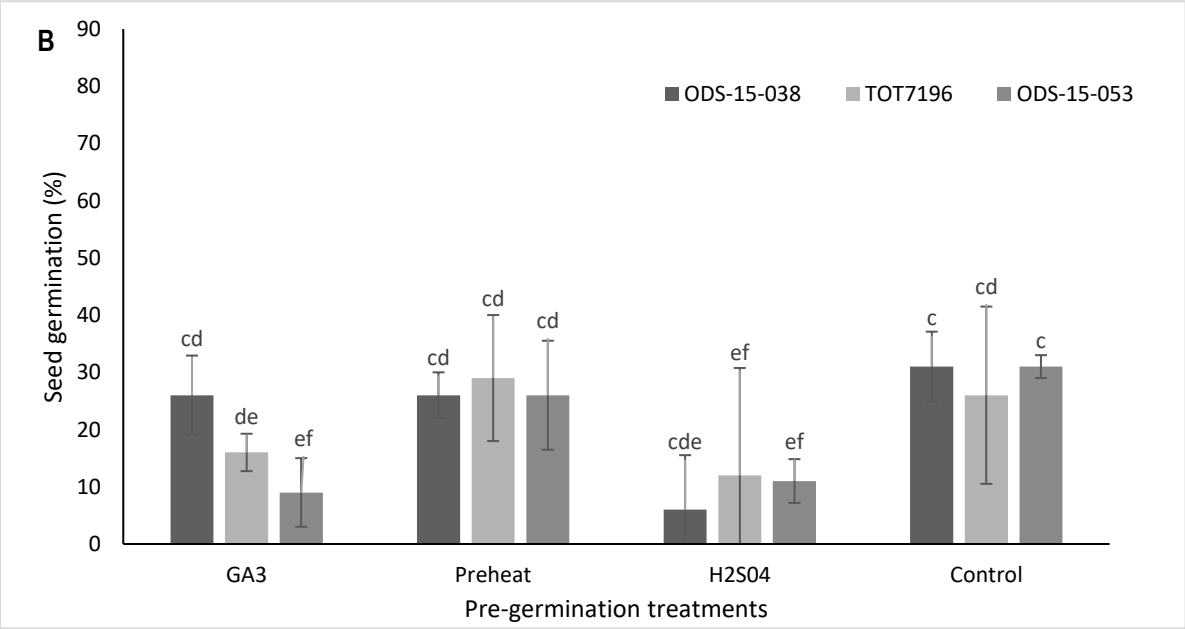


Figure 4 Interactions of pre-germination treatments, temperature and light on seed germination; **(A)** constant temperature of 30°C under darkness; (**B)** alternating temperature of 30°C for 12 h in the light and 20°C for 12 h in the dark on seed germination of the three accessions.

4.3 Seed viability of *C.gynandra*

Seed viability and germination differed significantly across the accessions. Seed viability was higher than seed germination across accessions, pre-germination, and under temperature and light conditions. Accession ODS-15-038 showed the highest seed viability of 98.7 % with gibberellin acid (GA3) application at 30°C under darkness, and the lowest viability of 22.7% in sulfuric acid (H2SO4) at 30°C 12 h in the light and 20°C 12 h in the dark **(Figure 5).**

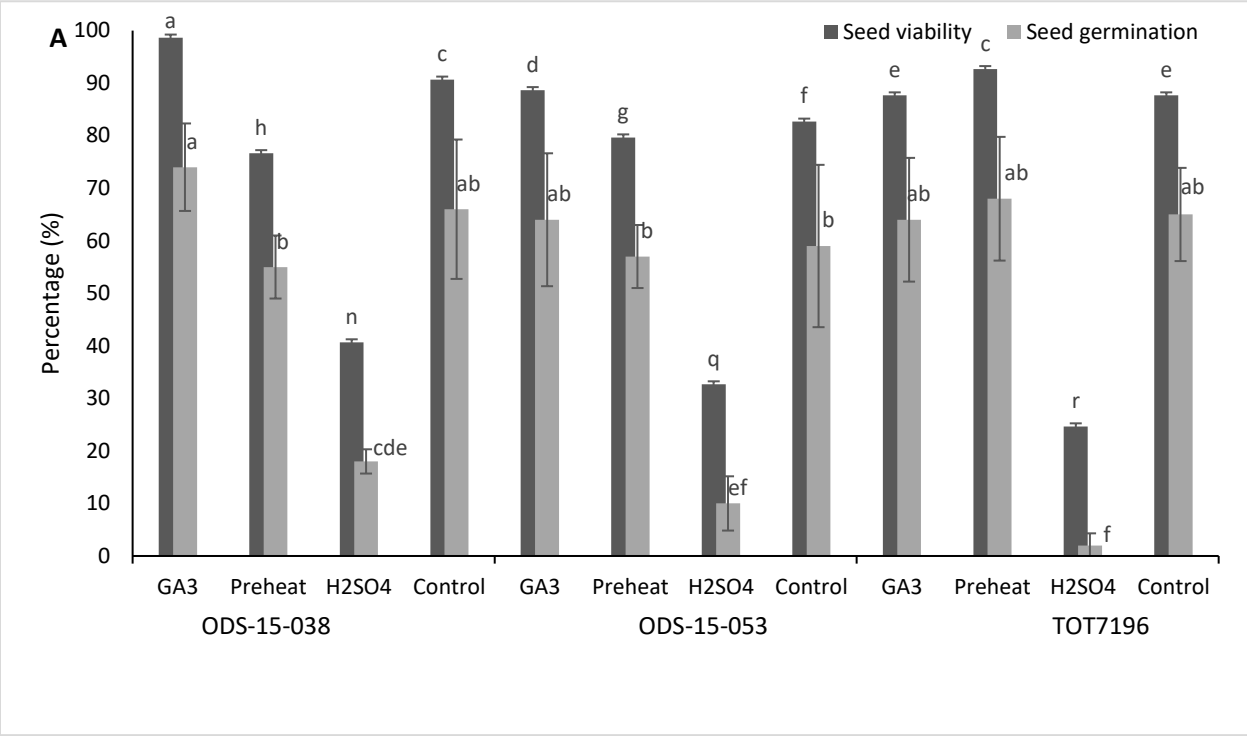


Figure 5 Comparison of seed viability and germination across accessions and treatments. **(A)** constant temperature of 30 °C under darkness **(B)** Alternating temperature of 30 °C 12 h in the light and 20 °C 12 h in the dark after the seeds had been subjected to different pre- germination treatments for 15 days and ungerminated seeds tested for viability with Tetrazolium (TZ) test.

The final germination percentage (FGP) and final seed viability (FSV) varied significantly among the accessions and between temperature and light conditions **(Tables 1).** Seed germination was significantly affected by temperature and light, decreasing the MGR to 0.32 per day (day^{-1}). The MGT depicted low reduction at 30°C under constant darkness than at an alternating temperature of 30°C for 12 h in the light and 20°C for 12 h in the dark. Accession ODS-15-038 showed the overall highest germination characteristics in both temperature and light conditions amongst other accessions while ODS-15-053 had the least **(Table 1).**

Table 1 Germination characteristics of the accessions under alternating and constant temperature and light

	Accessions	FGP (%)	FSV (%)	MGR (day-1)	MGT (days)
30°C under constant darkness	**ODS-15-038**	53.25A	76.67A	0.89A	3.73D
	ODS-15-053	47.50A	70.92C	0.79B	4.61C
	TOT7196	49.75A	73.17B	0.83A	4.17C
30°C/20°C under alternating light/dark	**ODS-15-038**	22.25B	44.33D	0.37C	4.47C
	ODS-15-053	19.25B	42.92E	0.32C	6.12A
	TOT7196	20.75B	44.42D	0.35C	5.49B

Means within a column followed by the same letter are not significantly different by LSD (P > 0.05).

5. DISCUSSION

The findings showed that seeds subjected to 30°C in constant darkness broke dormancy and enhanced seed germination of spider plants compared to an alternating temperature of 30°C (12 h) in the light and 20°C (12 h) in the dark (Figure 3), as observed in previous studies by (Ochuodho & Modi, 2005;2007);(Saifullah et al., 2023). Subjecting seed at 30°C in constant darkness might have given a favorable environmental circumstance since temperature ranges of 20 to 30°C and darkness are optimal for spider plant germination (K'Opondo, Muasya, & Kiplagat, 2005). This may be attributable to the negative photoblastic nature of the seeds, which is supported by the findings of (Bewley & Black, 1994);(Baskin et al., 1998);(Ochuodho & Modi, 2005). Seeds germinated under light for 12 h at 20°C did not favor germination. This is probably due to the natural phenomenon that occurs when the seeds are buried in the soil, which entirely experiences the presence of darkness and there is photo-inhibition of seed germination (Thanos et al., 1991); (Bewley & Black, 1994).This explains why the seeds germinated better at 30°C under constant darkness.

The seed germination percentage was lower under pre-heating of 40ºC for 24 h (1 d) and soaking in 1M sulfuric acid for 10 min. Similar results were reported by (Ochuodho & Modi, 2005). However, in contrast to De Villiers et al. (2002) observed improved seed germination of *Brassica tounefortii* through pricking and acid scarification, this species exhibited a more robust seed coat as it demonstrated better germination rates following exposure to sulfuric acid for 32 min. Conversely, Ekpong (2009) and Ochuodho (2005) reported that preheating at 40ºC for 1 to 5 d or 15 days was the most effective treatment for breaking seed dormancy and increasing seed germination. However, for accessions ODS-15-038 and ODS-15-053, application of GA3 500 ppm increased seed germination at 30°C in constant darkness. This could be because of its hormonal effects in breaking dormancy by inhibiting the activity of abscisic acid (ABA), prompting the action of alpha-amylase, which induces seed germination, as observed by (Muasya et al. 2009) and (Bewley & Black, 1994). Plant growth regulators like gibberellic acid have been found to effectively break dormancy in seeds by disrupting metabolic processes that hinder seed germination, as per various sources (Bewley & Black, 1994). The accessions showed variation in germination characteristics under temperature and light conditions (Tables 1).

These differences in seed germination between *C. gynandra* accessions could be due to the influence of biotype-specific factors that originated from different environment reflecting

habitat-specific selection, which require different environmental conditions for breaking seed dormancy (Zharare, 2012). Variation among accessions may be due to seed dormancy, which is influenced by many genes, some of which are regulated by environmental factors, as reported by Caboche et al. (1998).

The differences between seed viability and germination were significant across the accessions (Figure 5), this big difference indicating low-quality seeds of *C. gynandra*. This finding shows a high seed viability of spider plants, which, however, does not signify subsequent germination. The final seed viability was calculated by including both the seeds that germinated and those that were stained with TZ, showing that the stained seeds were dormant yet viable but failed to germinate.

6. CONCLUSION

This study showed that spider plant seeds are generally negatively photoblastic. Seed dormancy and germination of *Cleome gynandra* should be performed at 30°C in constant darkness to obtain optimum results. This implies that, in practice, the seeds are to be properly covered to eliminate light and not sown on the soil surface, as it renders them photo-dormant. Hence, exposure of seeds to light for 12 h at 30°C and 20°C 12 h in the dark significantly reduced seed germination. Indeed, the seeds have physiological dormancy, which overcomes the imbibition of GA3 500 ppm and effectively improves germination of *C. gynandra* seeds. The fact that the spider plant has a high seed viability, as shown by this study, does not necessarily mean that the seeds will germinate immediately. This study provides valuable insights into the seed viability of spider plants, which can be utilized for crop improvement. Further studies are needed to investigate the relationship between genetic diversity and seed dormancy and which technologies can bypass seed dormancy and enhance germination.

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10. LIST OF ABBREVATIONS

GA_3	Gibberellic acid
GA	Gibberellic acid
ABA	Abscisic acid
GBioS	Laboratory of Genetics, Biotechnology and Seed Sciences
ppm	Parts per million
RH	Relative humidity
C4	Carbon fixation
TTC	Triphenyl tetrazolium Chloride
TZ	Tetrazolium Test
UNAM	University of Namibia
FGP	Final germination percentage
FSV	Final seed viability
MGT	Mean germination time
MGR	Mean germination rate

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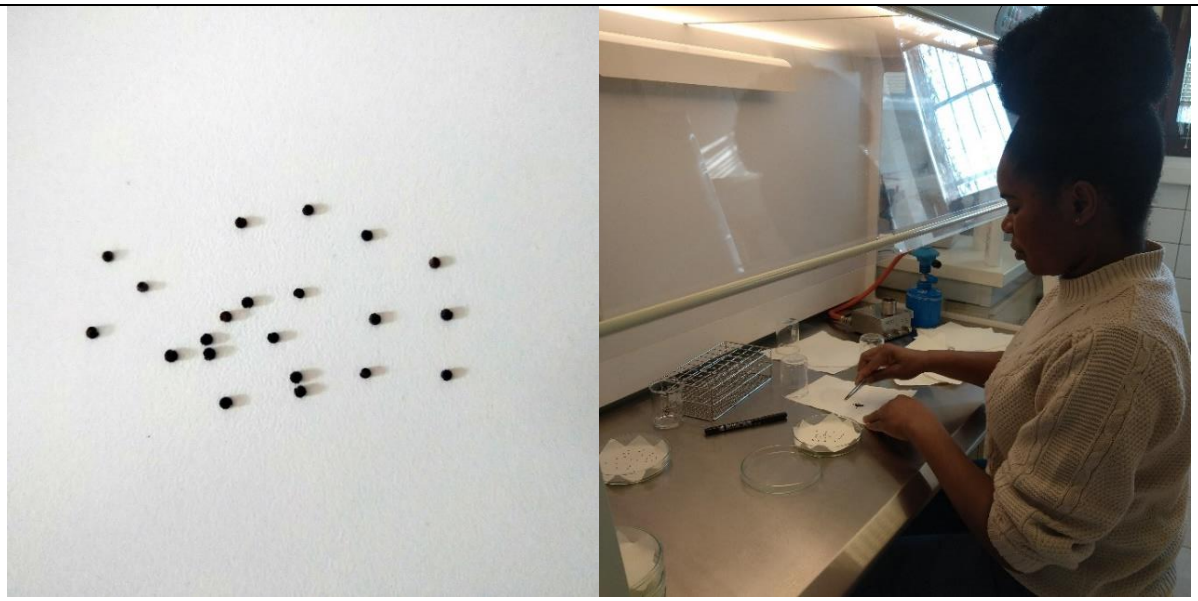
Appendix 1 Experimental evidence



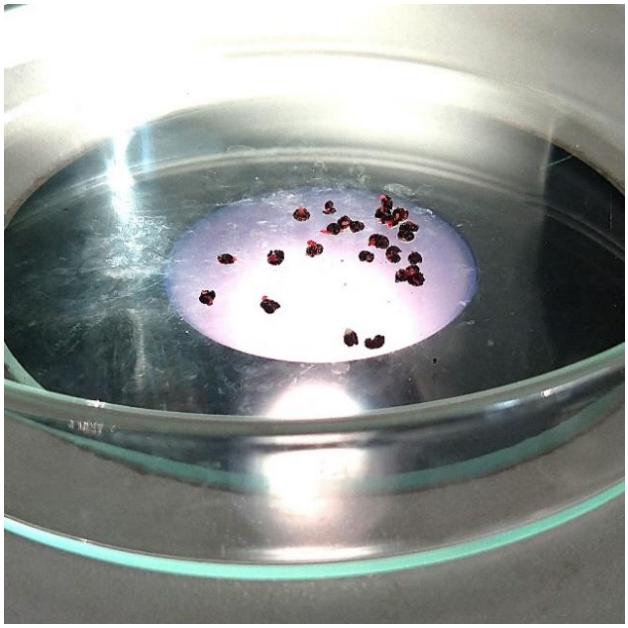
A **B**

Figure 1. **A.** Images of spider plants seeds used during the experiments: **B.** Seed counting before the experiment.

Figure 2. **A.** Incubation of seed in the growth chamber during the experiments **B.** image of germinated seeds in petri dishes.

A

B



C

Figure 3.**A** Poking of seeds with a needle to weaken the seed coat and allow tetrazolium solution to access the embryo. **B.** Seeds in 0.5% (w/v) tetrazolium chloride solution before incubation. **C.** TZ stained seeds under

Appendix 2 Experimental layout

Replication 1						
Growth chamber (Main plot) (constant darkness & Temp.)				Growth chamber (Main plot) (Alternating Light & Temp.)		
C1	**C2**	**C3**		**C1**	**C2**	**C3**
C1C	C2S	C3H		C1C4	C2H4	C3S4
C1G	C2C	C3G		C1S4	C2G1	C3C2
C1S	C2H	C3S		C1G1	C2S4	C3H4
C1H	C2G	C3C		C1H2	C2C3	C3G1

Replication 2						
Growth chamber (Main plot) (Constant darkness & Temp.)				Growth chamber (Main plot) (Alternating Light & Temp.)		
C1	**C2**	**C3**		**C1**	**C2**	**C3**
C1S	C2H	CG4		C1C3	C2S3	C3H3
C1H	C2C	C3S		C1G2	C2C4	C3G2
C1C	C2G	C3H		C1S3	C2H1	C3S3
C1G	C2S	C3C		C1H3	C2G2	C3C1

Main plot: Light and temperature conditions in Growth chamber
Sub-plot: Seed accessions
Sub-sub-plot: Germination treatments
Replicate3 & 4 were but randomly arranged.
Note: One plot represents one petri-dish

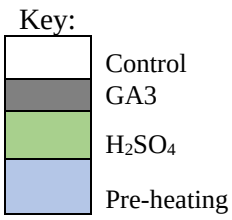


Figure 2. Laboratory layout of the experiment.

Appendix 3 List of plant species used in the study

TABLE 1 | List of spider plant accessions used in the study.

No	Accession name	Institution	Country of Origin	Region
1	TOT1048	World Vegetable Center	Thailand	Asia
2	TOT7198	World Vegetable Center	Malaysia	Asia
3	TOT7197	World vegetable center	Malaysia	Asia
4	TOT7486	World Vegetable Center	Lao People's Democratic Republic	Asia
5	TOT3536	World Vegetable Center	Lao People's Democratic Republic	Asia
6	TOT3527	World Vegetable Center	Lao People's Democratic Republic	Asia
7	TOT7196	World Vegetable Center	Malaysia	Asia
8	TOT4976	World Vegetable Center	Thailand	Asia
9	TOT5799	World Vegetable Center	Thailand	Asia
10	ELG 19/07A	KENRIK	Kenya	East Africa
11	BAR 1807B	KENRIK	Kenya	East Africa
12	TOT8926	World Vegetable Center	Kenya	East Africa
13	TOT6420	World Vegetable Center	Tanzania	East Africa
14	KF-07	KENRIK	Kenya	East Africa
15	KSI 2407A	KENRIK	Kenya	East Africa
16	NAM2232	Vergenoeg	Namibia	Southern Africa
17	LA 1	Ogongo campus	Namibia	Southern Africa
18	BC-01B	LUANAR	Malawi	Southern Africa
19	BC-03A	LUANAR	Malawi	Southern Africa
20	BC-03B	LUANAR	Malawi	Southern Africa
21	BC-01A	LUANAR	Malawi	Southern Africa
22	CZ-01	Chitedze Research Station	Malawi	Southern Africa
23	NAM-6	Ogongo	Namibia	Southern Africa
24	TOT6439	World Vegetable Center	Zambia	Southern Africa
25	BC-02A	LUANAR	Malawi	Southern Africa
26	ODS-15-038	GBioS	Benin	West Africa
27	ODS-15-053	GBioS	Togo	West Africa
28	ODS-15-121	GBioS	Ghana	West Africa
29	ODS-15-045	GBioS	Togo	west Africa
30	ODS-15-013	GBioS	Benin	west Africa
31	ODS-15-020	GBioS	Benin	west Africa
32	ODS-15-037	GBioS	Benin	west Africa
33	ODS-15-044	GBioS	Benin	west Africa

KENRIK, Kenya Resource Centre for Indigenous Knowledge; LUANAR, Lilongwe University of Agriculture and Natural Resources; GBioS, Laboratory of Genetics, Biotechnology and Seed Science.

DECLARATION

on authenticity and public assess of final mater's thesis

Student's name:	Veronica Popyeni Ndakolute
Student's Neptun ID:	BWD60J
Title of the document:	Effect of temperature, light and pre-germination treatments on seed germination and viability of spider plant (*Cleome gynandra*)
Year of publication:	2024
Department:	Vegetable and Mushroom Growing

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