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Research Article
Effect of Temperature, Light and Sowing Depth on Seed Germination of *Celosia argentea* L.

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Abstract

Background and Objective: *Celosia argentea* is an annual leafy vegetable that has gained prominence for its role in combating nutritional challenges and as a herb of therapeutic importance in many parts of the world. Despite the reported nutritional and pharmacological applications of *C. argentea* in most countries of the world, it is little known or not recognized in many other countries like South Africa, where there is a dearth of information on its utilization in any form whatsoever. In an effort to improve and encourage the domestication of this neglected nutraceutical plant, this study was designed to evaluate the effect of temperature, light and sowing depth on seed germination of *Celosia argentea* in order to put forth the best environmental condition necessary for its germination and domestication in South Africa. **Methodology:** Seed viability was tested using triphenyltetrazolium chloride, seed germination under different light, temperatures and sowing depths were evaluated. One-way analysis of variance (ANOVA) and MINITAB was used for statistical analysis. **Results:** The results revealed that the average seed weight of 100 seeds was 78.4 mg and the viability of the seeds was 88.0±2.6%. The optimum condition for germination were 25°C, alternating light and dark and a sowing depth of 1 cm. Although, maximum cumulative percentage germination was recorded at the temperature range of 20-30°C, seeds of *Celosia argentea* showed remarkable tolerance at the low temperature of 15°C. **Conclusion:** It is evident from this study that the seed germination of the species is solely dependent on appropriate temperature (25°C), alternating light and dark and a sowing depth of 1 cm. Therefore, this species has the potential of thriving under varying environmental conditions.

Key words: *Celosia argentea*, seed germination, domestication, viability, sowing depth

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Celosia argentea L. is an annual plant of tropical, subtropical and temperate zones of Africa, South America and South East Asia[1]. The seeds of *C. argentea* are extremely small, with black shiny seed coat and diameter of 1-1.5 mm. The flowers of the species are hermaphroditic in nature and are commonly referred to as wool-flowers[2,3]. This species has gained prominence for its role in combating nutritional challenges and has also found great application as a herb of therapeutic importance[4,5]. It is an important source of nutrients and minerals needed to maintain health and could be used to promote food security for millions of consumers in many parts of the world[6,7]. The leaves, flowers and tender stems are edible and are consumed as a potherb in sauces or soups[8].

Traditionally, *Celosia argentea* has been used in the treatment of diarrhea, piles, bleeding nose, inflammation, mouth sores, blood diseases, hemorrhoids, leucorrhea, uterine bleeding[2,9], haematological disorders and for the treatment of diabetes mellitus[6,10]. The anti-inflammatory, antioxidant and anti-bacterial properties of this plant have been attributed to the presence of a number of polyphenols in the seeds, roots and leaves[11-13].

Propagation of this plant is primarily through the seeds[14]. The physiological sequence of events that takes place during seed germination is usually influenced by some intrinsic and extrinsic factors like dormancy, physiological immaturity, genotype, light, temperature, water availability and sowing depth[15]. Each factor has a specific influence on the germination process, either acting alone or in combination with other factors, as a result, the sensitivity of each species must be considered[15].

Early reports Badra[16] and Schippers[17] suggested that both high light intensity and high temperature are required for the good growth of *Celosia argentea* since it is believed to have originated from tropical regions. Seed germination of *Celosia argentea* has been reported to fail under the slightest drought conditions, during the dry season and depending on the severity of heat and evapotranspiration. Reports from various authors, including Chauhan and Johnson[18] and Schmidt[19] suggested that when conditions that favor germination of a seed are not met, a viable seed may not necessarily germinate and may, therefore, become dormant [20,21]. Seed dormancy has also been linked to seasonal temperature variations, where cold stratification weakens seed dormancy in summer species leading to germination[22].

Deficiencies in nutrients such as vitamin A, calcium, iron and zinc are prevalent in South African children[23]. There have been reports of vitamin A deficiency in one out of every three preschool children especially in the rural areas of South Africa[24]. Green leafy vegetables such as *C. argentea* are good, cheap and readily available sources of these nutrients[25]. The Eastern Cape Province is one of the poorest and rural areas of South Africa which are prone to nutrient and dietary deficiencies as a result of poverty. The inclusion of *C. argentea* in the diet could help in combating these vitamins, mineral and other dietary deficiencies. The species could also replace rather expensive commercially consumed vegetables common in the South African market as well as enhance the socio-economic status of the rural communities.

This study therefore aimed at evaluating the effect of sowing depth, light and temperature on the germination and growth of *Celosia argentea*, in order to determine the best environmental conditions suitable for its germination towards encouraging its domestication in the Eastern Cape of South Africa.

MATERIALS AND METHODS

Seed collection: The experiment was carried out in the Medicinal Plants and Economic Development (MPED) Research Centre Laboratory, University of Fort Hare, South Africa from May-July, 2017. Mature seeds of *Celosia argentea* were purchased from an Agroshop in Nigeria and the seeds were stored in an airtight container at room temperature (15-25°C) for 4 months.

General experimental procedures: For all the treatments, 3 replicates of 25 seeds were used, except for the seed weight and viability test where 100 and 50 seeds were used, respectively.

Unless otherwise stated, seeds were placed on 2 layers of Whatman No1 filter paper in 9 cm petri dishes. Initially, the filter papers were moistened with 3 mL of distilled water, then subsequently when necessary, with 1 mL of distilled water to maintain adequate moisture. Radicle emergence was the criterion for germination. Germinated seeds were counted and removed daily for a period of 14 days for each experiment. Counts for the dark treatments were performed under safe green light. All the experiments were performed in triplicates.

Viability test: The viability and quality of seeds used for the experiment were tested using the triphenyltetrazolium reagent as described by Peters[26]. Three replicates of 50 seeds were used for each seed lot. The seeds were soaked in water for 24 h, cut along the margin without damaging the embryo then soaked in colourless 0.1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) solution at 25°C in the dark. The seeds were removed from the TTC solution after 16 h, rinsed in distilled water, then soaked in 10 mL of 95% ethanol to permit direct observation of the embryo. The embryos of viable seeds appeared reddish in colour.

Seed weight determination: Seed weight was determined by weighing 100 seeds using an analytical balance and the mean weight of 1 seed was calculated.

Germination trial: Germination trial was conducted by sowing 50 seeds in 9 cm diameter petri dish layered with a double sheet of Whatman No 1 liter paper, moistened with distilled water. Seeds were monitored daily for 14 days and germinated seeds were recorded and removed.

Effect of temperature: The effect of temperature on germination was investigated by placing petri dishes of seeds in incubators set at 5, 10, 15, 20, 25, 30, 35 and 40°C for 14 days. These temperatures were approximate mean of daily minimum and maximum temperatures in Alice during the period of the experiment (May-July, 2017).

Effect of light: The effect of continuous light, alternating light and dark (12 h light/12 h dark photoperiod) and continuous darkness were accomplished under controlled environments. Continuous light treatments were achieved with continuous illumination produced by four white fluorescent tubes. For alternating light and darkness treatment, another set of 3 petri dishes of seeds were placed in a room with 12 h light/12 h dark photoperiod, while for the dark treatment, Petri dishes with seeds were covered with three layers of aluminum foil and kept in a dark room. The petri dishes were saturated with distilled water and observed daily for germination under green light for 14 days.

Effect of sowing depth: This was carried out by sowing 10 seeds at different sowing depths of 0 (soil surface), 1, 2, 3, 4 and 5 cm in rectangular seedling trays measuring 65×100 cm^2 with 200 bottom holes. The trays were filled with top loamy soil and irrigated daily. Seedling emergence was monitored daily and germination count was taken over 14 days.

Statistical analysis: All data were expressed as mean$\pm$standard deviation (SD) of 3 replicates. One-way analysis of variance (ANOVA) was used to determine significant differences in parameters and the effect of various factors on germination. Mean values were considered significant at $p<0{\cdot}05$. All analyses were performed using MINITAB student version 17 for Windows software. Fischer's LSD test was used for comparison of multiple means and to determine the general pattern of germination.

RESULTS

Seed weight and viability: The average weight of 100 seeds was 78.4 mg, therefore a single seed weighed 0.784 mg. The viability test, using 0.1% triphenyltetrazolium chloride on 50 seeds of C. *argentea* triplicates revealed that 88.0$\pm$2.6% of the seeds were viable.

Effect of temperature on germination: Data regarding daily percentage seed germination and average germination time of *Celosia argentea* seeds at different temperatures are shown in Table 1. There was no germination at 5°C all through the

Table 1: Effect of different temperature conditions on daily (%) germination

Days	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
0	NG	NG	NG	NG	NG	NG	NG	NG
1	NG	NG	NG	NG	2.67±1.15	2.67±0.58	NG	5.33±0.58
2	NG	NG	NG	2.67±1.15	21.33±2.31	10.67±0.58	4.00±1.00	12.00±2.00
3	NG	2.67±1.15	NG	2.67±1.15	24.00±3.61	10.67±1.52	12.00±1.75	5.33±0.58
4	NG	NG	NG	6.67±1.15	8.00±1.00	9.33±1.52	NG	10.67±2.52
5	NG	NG	1.33±0.58	4.00±1.73	12.00±2.65	1.33±0.58	NG	2.67±0.58
6	NG	2.67±1.15	NG	2.67±0.58	1.33±0.58	8.00±1.00	NG	NG
7	NG	NG	1.33±0.58	4.00±1.00	17.33±3.21	NG	1.33±0.58	2.67±0.58
8	NG	NG	NG	5.33±0.58	1.33±0.58	5.33±0.58	1.33±0.58	2.67±1.15
9	NG	NG	NG	2.67±1.15	NG	4.00±0.00	1.33±0.58	1.33±0.58
10	NG	NG	1.33±0.58	2.67±1.15	NG	NG	NG	NG
11	NG	NG	NG	6.67±0.58	NG	5.33±0.58	NG	NG
12	NG	NG	1.33±0.58	2.67±0.58	NG	2.67±0.58	NG	NG
13	NG	NG	1.33±0.58	2.67±0.58	NG	NG	1.33±0.58	NG
14	NG	NG	NG	2.67±0.58	1.33±0.58	NG	NG	1.33±0.58

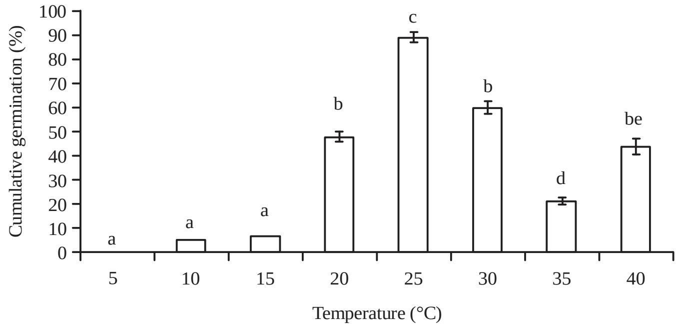

Fig. 1: Effect of different temperature conditions on mean cumulative percentage germination

Values are Mean±Standard deviation (n = 3). Error bars with different letters indicate significant difference ($p<0.05$) in germination across different temperatures

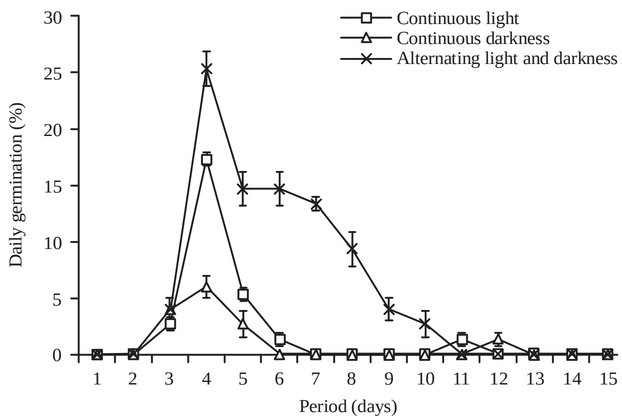

Fig. 2: Effects of different light conditions on the percentage of mean germination on the seed germination

Values are Mean±Standard deviation of 3 replications (n = 3)

period of observation. Interestingly, 2.67±1.15% germination was observed on 3 and 6 days at 10°C, also, 1.33±0.58% germination was observed on 5, 7, 10, 12 and 13 days at 15°C. Germination at 20°C started on the 2nd day and continued until the last day of observation (14th day). Most of the germination was recorded between 2-7 days with the highest daily germination of 24±2.31, 21.33±3.61 and 17.33±3.21% recorded on 3, 2 and 7 days, respectively at 25°C.

The mean cumulative germination presented in Fig. 1 shows a significantly higher ($p<0.05$) cumulative germination (89.33±2.08%) at 25°C compared to other temperatures investigated. The mean percentage germination at 5°C (0±0%), 10°C (5.33±1.15%) and 15°C (6.67±0.58) were significantly lower ($p<0.05$) than at 20, 25, 30, 35 and 40°C. Although the mean percentage germination at 30°C was numerically higher than at 20 and 40°C, there was no statistical significant difference between their values.

Effect of light on germination: The effect of continuous light (CL), continuous darkness (CD) and alternating light and dark, (ALD) on the mean daily and cumulative percentage seed germination are shown in Fig. 2 and 3, respectively. Germination in all the light conditions started on the 2nd day with CL having a percentage germination of 2.67±0.58, CD 4.00±1.00 and ALD 4.00±1.00%. Peak germination was observed on the 3rd day in all the conditions evaluated with a mean percentage germination of 17.33±0.58% values for CL, CD (4.00±1.00%) and ALD (25.33±1.53%). Germination continued steadily until day 9 with lag phases of no growth from day 6-9 in CL and 5-10 days in CD (Fig. 2).

The mean cumulative percentage germination (Fig. 3) revealed a significantly higher ($p<0.05$) germination success of 88±4% in the ALD treatment. The germination for CL and CD were 28±0 and 12±1.73%, respectively.

Effect of sowing depth on germination: The effect of different sowing depths on the mean percentage of seed germination is shown in Fig. 4 and 5. The mean daily percentage of germination did not follow a specific pattern but germination was first observed at 0 cm on 5th day

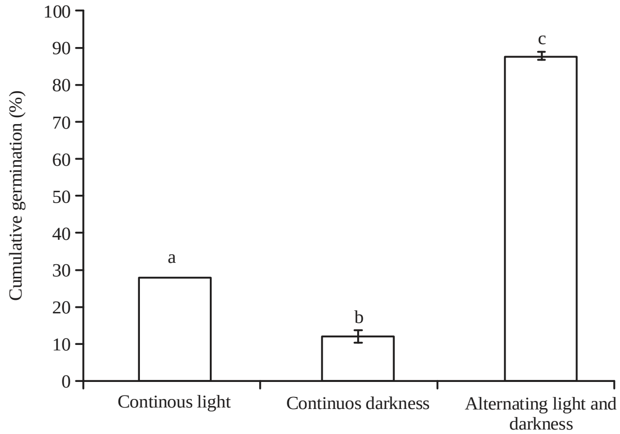

Fig. 3: Cumulative percentage of germination of *C. argentea* seeds as affected by different light conditions

Values are Mean±Standard deviation (n = 3). Bars with different letters indicate significant differences ($p<0.05$)

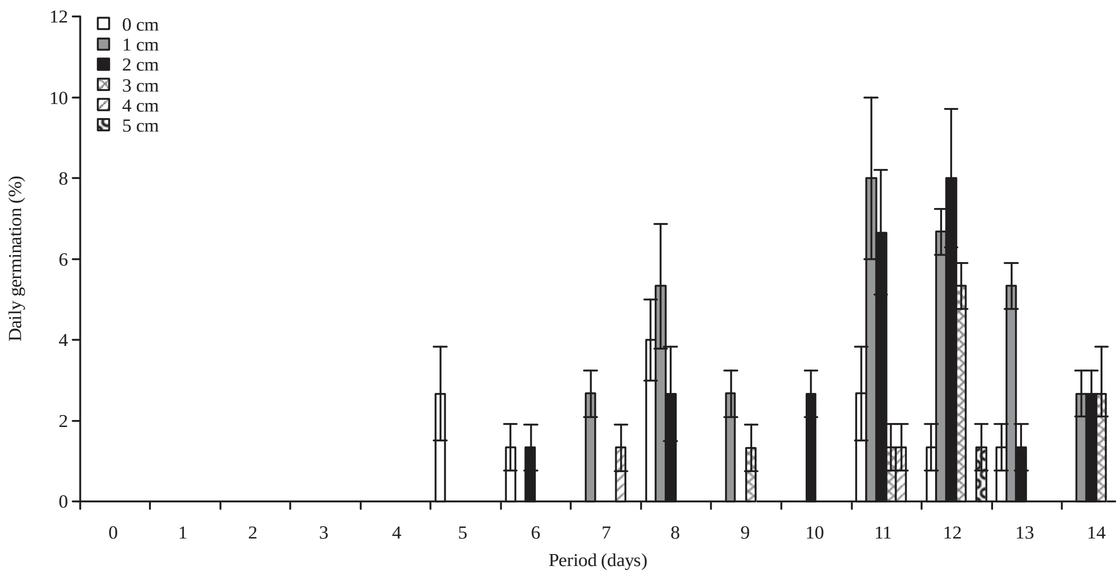

Fig. 4: Percentage of daily germination of *C. argentea* seeds at different sowing depths over 14 days

Values are Mean±Standard deviation, n = 3

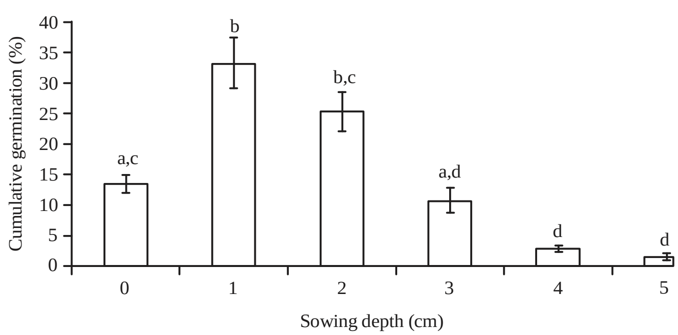

Fig. 5: Effect of sowing depth on the germination of *Celosia argentea* seeds

Values are Mean±Standard deviation (n = 3). Bars with different letters indicate significant differences ($p<0.05$) among the different sowing depths whereas, germination started on 7th day at sowing depths of 1 and 4 cm and on 6 days at the sowing depth of 2 cm. Seeds sowed at 5 cm depth only showed sparing growth on the 12th day. Cumulatively, a pattern of germination was observed with progressive decrease in germination as the sowing depth increased from 1-5 cm, while the maximum cumulative percentage germination of 33.33±4.16% was recorded at a depth of 1 cm. The lowest percentage germination of 1.33±0.58% was recorded at the depth of 5 cm. Seeds sowed at the soil surface (0 cm) had a mean cumulative percentage germination of 13.33±1.53%.

DISCUSSION

In the life cycle of a plant, seeds have the highest resistance to extreme environmental stress[27]. For a species to colonize a new habitat successfully, seeds must be able to germinate well under prevailing environmental conditions. Matured seeds of *Celosia argentea* are non-dormant and the viability test showed that 88% of the seeds were viable.

Temperature is one of the main factors affecting seed germination because it acts directly on seed water absorption and the biochemical reactions that regulate the metabolism involved in the germination process[28]. Additionally, the period of germination may be altered completely in response to temperature[29]. According to Ballare[30], the quality, intensity and duration of light radiation also influence seed germination.

The result of this study showed that the maximum temperature for germination of *Celosia argentea* seeds ranged from 20-30°C while the mean optimum temperature was 25°C. Significantly low percentage germination was recorded at 10 and 15°C. These observations are similar to the findings of Kashmir *et al.*[31], who reported that the best conditions for germination of *Silybum marianum* seeds are high temperatures (25 -30°C), which is similar to those of *Corchorus olitorius*, *Eupatorium odoratum* and some other tropical species. This is in contrast to the findings of Okusanya[32], who reported that no germination occurred at 15°C for *Celosia cristata*. Cumulatively, there was no germination at 5°C, this could be attributed to the fact that low temperatures can have lethal effects on viable seeds[33] although, Merritt *et al.*[34] reported that cold stratification could break the dormancy of viable seeds and enhance germination in many species.

Worthy of note in this investigation is the successful germination observed at 10 and 15°C. Although, significantly low percentage germination was recorded at these temperatures, it is an indication that *C. argentea* seeds have remarkable tolerance for low temperatures, an attribute similar to *Luffa aegyptiaca* and some temperate species which successfully germinated at 15-31°C. However, the delay of 3-5 days before germination observed at these temperatures (10 and 15°C, respectively) could be attributed to the seeds acclimatizing to lower temperatures[35] since the species is originally a tropical plant.

In accordance with the findings of El-Keblawy[36], alternating the temperature from cold to warm and vice versa could result in higher germination success in some plants as observed in this study. While extreme cold temperatures (5°C) could be deleterious for germination, extreme heat (30-40°C) did not also produce optimum germination.

Light is one of the most important environmental factors that interact with temperature to regulate seed germination in many plant species. Optimum seed germination was observed in the alternating light and dark regime. A similar observation was recorded by Kambizi *et al.*[37] for *Withania somnifera.* The implication of this is that *C. argentea* requires a specific amount of light and dark phases to break the seed dormancy and trigger the series of biochemical reactions needed for germination. Hence, neither continuous light nor continuous darkness produced significant germination. Okusanya[38] reported similar results for *Celosia cristata*. The sensitivity of seeds to the quality of light spectrum is mediated by phytochrome which is a frequent natural process with species that grows in open areas[39]. Therefore, the occurrence of germination under different light conditions may be due to the active-form phytochrome present in the seed in a quantity sufficient to induce the germination process[40]. In general, small seeded species are more sensitive to light for germination than species with larger seeds[41]. This could account for the pattern observed with *C. argentea* which has fairly small seeds (mean seed mass = 0.784 mg). Similar to the results observed in this study, seeds of several species such as *Clitoria fairchildiana* and *Dalbergia cearensis* showed considerable germination under alternating light and dark treatment[42]. Thus, the enhanced germination under light or alternating light and dark are an appropriate response to simulated natural conditions. It is not evident how much light intensity could be reduced that will result in significant reduction in seed germination, however *Celosia argentea* seeds have a high compensation point and therefore, will be able to tolerate limited shading, an attribute worthy of note for field establishments.

Seeds of *C. argentea* sown at depth of 1 cm gave the highest seed germination percentage, although, sowing depth of 2 cm could also support germination. The result of seed germination at 0 cm (soil surface) was low when compared to 1, 2 and 3 cm. This could be ascribed to limited soil-to-seed contact, reduced water availability and other environmental conditions that may limit germination of seeds on the soil surface[43,44]. Another reason for low germination at soil surface can be attributed to the higher water potential and longer imbibition time potential of the seeds for germination[44].

Generally, it was observed that seed germination decreased progressively from 1 cm as the depth increased. The reduced germination of C*. argentea* seeds at deeper depths was explained by Odeleye and Olufolaji[25], who reported that the radicle has to develop a pressure high enough to pierce through the soil, so as the distance the hypocotyl has to traverse in the soil to the surface increases, the pressure needed to pierce the soil also increases, while the inherent seed energy decreases. This is similar to the findings of Chauhan and Johnson[45], who observed high germination trend (73-76%) at sowing depth of 1 cm with this same species but progressive decline in germination as sowing depth increased.

The light requirement for germination of *C. argentea* ensures that the seeds will germinate successfully near the soil surface when other conditions are suitable for seedling emergence.

CONCLUSION

This study clearly shows that the optimal temperature for germination of *C. argentea* seeds is 25°C, although it may do well between 20-30°C. The species germinates better under alternating light and dark conditions and at the sowing depth of 1 cm. In the field, many of these species' seeds, if left on the soil surface are not expected to germinate but remain viable. It is evident that for field establishment, seed germination of this plant will be solely dependent on light, temperature and sowing depth conditions. It is therefore, imperative that these conditions should be taken into consideration for economic cultivation of this species.

SIGNIFICANCE STATEMENT

The present study gives insights into the effect of temperature, light and sowing depth on seed germination of *Celosia argentea*, a leafy vegetable and medicinal plant used in some countries of the world. This study revealed that the optimal temperature for germination of *C. argentea* seeds is 25°C. The species germinates better under alternating light and dark conditions and at the sowing depth of 1 cm.

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