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Research article

# Safflower seeds development: physical changes and the role of gibberellic acid, light, and temperature

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**ABSTRACT**: Safflower is a crop with seeds containing high amounts of oleic and linoleic acids, which have applications in cosmetics, food, feed, and pharmaceutical industries. Safflower seeds are generally reported to undergo after-ripening and to have physiological dormancy. This study comprehensively analyzed the physical changes and the induction of dormancy and germinability in developing seeds to determine whether the safflower population is adequately developed during seed dispersal. From the 80$^{th}$ day after flowering (DAF), seeds were collected separately from plants on a weekly basis until 131 DAF, resulting in seven sampling dates. The changes in length, diameter, thickness, water content, and fresh and dry weight during seed development were measured. Simultaneously, the effects of dark and light, temperature, and gibberellic acid ($GA_3$) (0, 50, and 100 µM) on seed germination, dormancy and germination characteristics of safflower seeds were investigated. The seeds ceased to increase in length, width and thickness, reaching a mature appearance at 131 DAF, with a dry mass per seed of 0.0443 g. The water content decreased between 117 and 124 DAF, indicating an obvious process of desiccation during the final stage of seed maturation. Germination capacity is acquired at 117 DAF, as evidenced by an increase in germination and a reduction in dormant seeds, particularly at low temperatures (10°C) and with $GA_3$ supplementation. At 131 DAF, seeds exhibited 7.9 % water content, and there was an increase in seed germination and a decrease in seed dormancy status, regardless of $GA_3$ supplementation and temperature.

**Keywords**: *Carthamus tinctorius*, after-ripening, cold stratification, germination, physiological dormancy

## Introduction

Safflower (*Carthamus tinctorius* L., family Asteraceae) is cultivated primarily for its seed, called achenes, which contains high amounts of oleic and linoleic acids (Khalid et al., 2017). These acids are a valuable source of phenols with antioxidant and anti-aging activity (Zemour et al., 2019). Additionally, the seed contains macro and micro-nutrients that can be used as livestock feed (Kereilwe et al., 2020).

Previous studies have suggested that safflower seeds are dormant (Kotecha and Zimmerman, 1978; Dajue and Mündel, 1996). Despite the evidences reported over time, the precise events involved in safflower seed dormancy remain unclear. Mature safflower seeds gradually overcome dormancy during storage for 240 days in an uncontrolled environment, with no temperature and relative humidity control. Storage associated with cold stratification led to an increase in germination (Oba et al., 2017). Later, these authors confirmed the presence of physiological dormancy (PD) in safflower seeds, which was predominant in the first 120 days of storage (Oba et al., 2019).

Despite the significance of the PD, none of the observations reported from any direct embryo or measurements to assess their full development when still on the plant were conducted. It is known that there is also a coexistence of developing seeds during the formation of safflower buds. However, there are no detailed studies to confirm whether embryos undergo physiological post-maturation. Understanding the development dynamics can help detect the appropriate time to collect seeds capable of establishing seedlings and increase crop sustainability. The initial step in understanding seed germination is to ascertain which environmental stimuli can overcome dormancy (Zhang et al., 2020). In addition to temperature, water, and oxygen, a dormant seed can also be responsive to other factors, including hormones and light (Willis et al., 2014).

For other species of the Asteraceae family, such as *Helianthus annuus* L. and *Helianthus petiolaris* Nutt., exogenous gibberellic acid ($GA_3$, 1 mM) enhances germination of immature achenes 20 DAF (Seiler, 1998). Subsequently, it was demonstrated that sunflower seed dormancy has two origins: the embryo itself and the seed envelopes. At 10 °C, the inability of sunflower seeds to germinate results mainly from the embryo, as pericarp removal had almost no effect on germination. However, it was concluded that higher temperatures and less rainfall were associated with lower seed dormancy at harvest (Lachabrouilli et al., 2021).

The acquisition, maintenance, cycling, and loss of dormancy are integrated into the central paradigm, which is dependent on the plant hormone abscisic acid (ABA) and gibberellic acid ($GA_3$). The latter stimulates

seed germination (Dekkers and Bentsink, 2015). The primary objective of this study was to determine whether safflower seeds are dormant. The study will observe the entire process from seed development to maturation and shedding. It will examine the relationship between seed development time and germination according to cold stratification, light, and $GA_3$.

## Materials and Methods

### Plant material and seed production

Safflower plants were cultivated in the Agrarian Sciences Farm (22°14′ S, 54°59′ W, altitude 434 m) at Dourados, Mato Grosso do Sul state, Brazil. The soil in the experimental sites is classified as a typical Dystroferric Red Latosol (FAO, 2006) and the biome is the Brazilian savannah.

Seeds were sown on 10 May 2019 at a mean rate of 15 $m^{-1}$. The experimental plots comprised 13 rows wide (0.50 m spacing) and 20 m long. The useful area was 95 $m^2$, excluding 50 cm of borders at each edge. Seeds were sown on corn crop residues using a seeder (Jumil®, model 2680 TD). The rainfall volume and mean temperatures were monitored daily throughout the seed production period (Figure 1A).

Soil chemical characteristics (0-20 cm) prior to sowing the safflower seeds were: pH = 5.8, H + Al = 5.6 $cmol_c\ dm^{-3}$; $K^+$ = 1.53 $cmol_c\ dm^{-3}$; $Ca^{2+}$ = 5.34 $cmol_c\ dm^{-3}$; $Mg^{2+}$ = 1.87 $cmol_c\ dm^{-3}$; and V% = 58. There was no chemical correction of the soil or application of fertilizers at the time of sowing and management practices were applied to develop the safflower crop fully. The weed control was done through manual weeding; disease control was unnecessary.

Flowering was monitored daily by observing newly opened flowers during the reproductive period. The floral buds were previously marked with ribbons to differentiate the order of occurrence of the flower openings. Upon the opening, the flowers were characterized and harvested within the useful area at a 7-day interval from 80 to 131 DAF. Immediately after each harvest period, the inflorescences were placed inside plastic bags for immediate transport to the Laboratório de Tecnologia de Sementes of Universidade Federal da Grande Dourados (UFGD). The seeds were extracted manually.

The physical characteristics of the seeds and the germination process were evaluated during the maturation progress, including immediately following the harvest of the seeds and after they were subjected to a cold stratification process, treated with $GA_3$, and subjected to light.

### Physical characteristics of the seed

The physical characteristics of the seeds were represented by the length, diameter, and thickness in four samples comprising 25 seeds at harvest. These characteristics were evaluated with a digital caliper (0.01 mm). The length corresponded to the measurement range between the apex and base of the seed, and the diameter was determined in the basal region of the seeds. Thickness was determined at the intermediary portion of the seeds. The seeds were weighed using an electronic balance (0.0001 g), and the individual fresh mass was determined and expressed in grams per seed.

### Seed water content and seed dry weight

The water content was determined gravimetrically via the oven method at 105 ± 3 °C for 24 h, in accordance with the Brazilian Seed Analysis Rules (MAPA, 2009). This was conducted on four samples of ten seeds for each harvest time. The results were expressed as a percentage on a fresh weight basis. The dry matter of the seeds was quantified at 105 ± 3 °C for 24 h in a drying oven. The seeds of each replica were weighed to determine the average dry matter content (g per seed).

### Effect of $GA_3$, light and cold stratification on seed germination

Following each harvest, the germination test was performed in a germination chamber (Biochemical Oxygen Demand). The seeds were imbibed in germination boxes on blotting paper moistened with distilled water (0 µM $GA_3$) or $GA_3$ at 50 and 100 µM for 24 h. Solutions of $GA_3$ were prepared by dissolving the compound in ethanol (EtOH) prior to dilution in water. Subsequently, the seeds were sown in germination boxes on paper moistened with distilled water. In both cases, the volume used to wet the substrate was equal to 2.5 times the mass of the paper.

To investigate the influence of light during the imbibition phase, germination tests were conducted for each sample in the absence of light or under continuous white light (200 µmole $m^{-2}$ $s^{-1}$). During observations of imbibition occurring in the dark, the seeds were illuminated with a green safety light.

The effect of temperature on dormancy release was determined in each sample by incubating the germination boxes at a constant temperature of 10 °C or alternating between 20 and 30 °C for 16 h at the lowest temperature and 8 h at the highest temperature. For experiments using hormones, seeds were imbibed in the dark or under continuous white light as previously described in the two temperatures (MAPA, 2009).

Four samples with 25 seeds were used for each $GA_3$ concentration, dark or light exposure, and temperature at each harvest time. Seeds were considered germinated when they exhibited an increase in root length greater than 2 mm for up to 14 days. In order to confirm the presence of dormant seeds, non-germinated seeds were incubated in a solution of 1 % 2,3,5- triphenyltetrazolium chloride for 2 h. Viable seeds stained red, and data were not analyzed statistically. For each treatment, the controls corresponded to seeds imbibed in water under continuous

white light at 20-30 °C. The percentage of germination and dormant seeds were calculated (MAPA, 2009).

### Statistical analyses

The data were analyzed with Excel software (Microsoft Corporation) for statistical analyses of physical increment during seed development. Values are expressed as the mean ± standard deviation of four replicates in each independent experiments; *p*-values < 0.05 were considered statistically significant.

The Shapiro-Wilk test was conducted to analyze the adjusted residuals to normal distribution for statistical analyses of dormant seeds and germination during development. A completely randomized split-plot design was employed to analyze the influence of light (plot), temperature (sub-plot), and $GA_3$ (sub-subplot) during seed development. For each of the seven harvest times, the seeds were subjected to analyses under light and in the dark, at temperatures of 10 °C and 20-30 °C, and in three $GA_3$ solutions (0, 50, and 100 µM $GA_3$). The means were subjected to the Tukey test with a critical *p*-value of 5 % ($p < 0.05$).

## Results

### Water content and seed morphometrics

Safflower plants exhibit long and uniform vegetative growth (Figure 1B). Each capitulum of safflower (Figure 1C and D) contains achenes (hereafter seeds). The color of the safflower seeds was milky white and became gray as the maturation process progressed (Figure 1D).

The water content of the seeds immediately following harvest was 76.2 % at 80 DAF. Thereafter, a gradual decrease occurred throughout the maturation process until the seeds reached hygroscopic equilibrium with the relative humidity (7.9 %), at 131 DAF (Table 1). Concomitantly, the seed fresh mass reached its maximum value (0.0639 g) at 80 DAF and subsequently decreased to a minimum value (0.0482 g) at 131 DAF. This reduction in seed fresh mass was parallel to the reduction in the water content of the seeds, which occurred mainly from 124 DAF (Table 1).

Concurrently, the seed dry weight reached a minimum value (0.0152 g) at 80 DAF and subsequently increased, reaching the maximum value at 131 DAF (Table 1).

The water content exhibited a gradual decline between 117 and 124 DAF. However, a sharp reduction was observed from 124 to 131 DAF (Table 1), which is indicative of the anticipated desiccation process during the final stage of maturation in safflower seeds.

The morphometric characteristics of safflower seed underwent changes during development. The safflower seed reached a mean length of 0.65 mm from 80 to 124 DAF. In subsequent harvests, the mean values of seed length tended to decrease (Table 2). In contrast, the increase in width did not follow a similar pattern, as the mean value was identified at 110 DAF, followed by decreases in subsequent harvest times (Table 2). The measurement of seed thickness revealed that it reached

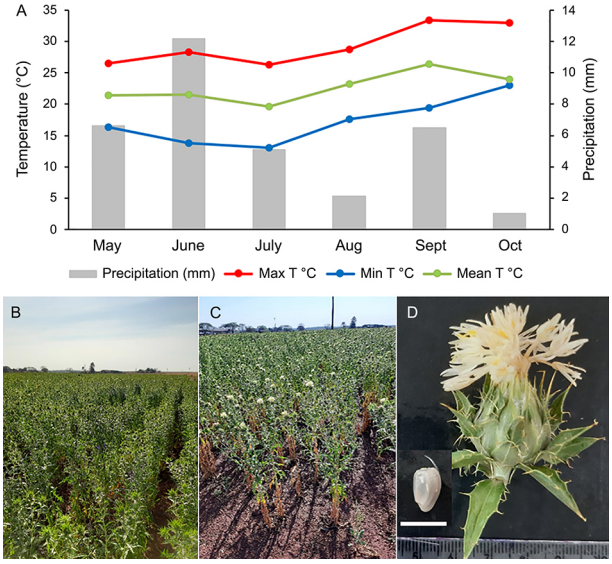


**Figure 1** – A) Climatic conditions during the development of safflower seeds in the field in 2019; B) Field growth of *Carthamus tinctorius* during the winter crop in 2019; C) Flowering of *C. tinctorius* plants; D) Close up view of capitulum and *C. tinctorius* seed. Bars indicate 1 cm.

**Table 1** – Water content and mass characterization of safflower seeds during seed development in the 2019 winter crop.

| | Days after flowering | | | | | | |
|---|---|---|---|---|---|---|---|
| | 80 | 87 | 94 | 110 | 117 | 124 | 131 |
| Water content (%) | $76.2^{a} \pm 0.9$ | $64.1^{b} \pm 9.2$ | $50.0^{bc} \pm 3.5$ | $47.6^{c} \pm 3.4$ | $29.4^{d} \pm 3.3$ | $26.4^{d} \pm 0.6$ | $7.9^{e} \pm 1.3$ |
| CV (%) | 1.18 | 14.35 | 7.00 | 7.14 | 11.22 | 2.27 | 16.45 |
| Fresh mass per seed | $0.0639^{ab} \pm 0.0025$ | $0.0712^{a} \pm 0.0064$ | $0.0611^{bc} \pm 0.0035$ | $0.0617^{bc} \pm 0.0015$ | $0.0623^{bc} \pm 0.0018$ | $0.0554^{c} \pm 0.0027$ | $0.0482^{d} \pm 0.0018$ |
| CV (%) | 3.91 | 8.98 | 5.72 | 2.40 | 2.88 | 4.87 | 3.73 |
| Dry mass per seed | $0.0152^{c} \pm 0.0006$ | $0.0255^{b} \pm 0.0023$ | $0.0305^{b} \pm 0.0017$ | $0.0323^{ab} \pm 0.00081$ | $0.0411^{a} \pm 0.0013$ | $0.0439^{a} \pm 0.0020$ | $0.0443^{a} \pm 0.0016$ |
| CV (%) | 3.94 | 9.01 | 5.57 | 2.50 | 3.16 | 4.55 | 3.61 |

Data represents mean values, standard deviation (±) and coefficient of variation (CV). Mean values followed by the same superscript in a row do not differ among themselves (Tukey test, $p \leq 0.05$).

**Table 2** – Morphometric characterization of safflower during seed development in the 2019 winter crop.

| | Days after flowering | | | | | | |
|---|---|---|---|---|---|---|---|
| | 80 | 87 | 94 | 110 | 117 | 124 | 131 |
| Length (mm) | $0.66^{abc} \pm 0.1$ | $0.68^{a} \pm 0.1$ | $0.67^{ab} \pm 0.07$ | $0.64^{bcd} \pm 0.09$ | $0.63^{bc} \pm 0.08$ | $0.66^{abc} \pm 0.06$ | $0.59^{c} \pm 0.07$ |
| CV (%) | 15.15 | 14.70 | 10.44 | 14.06 | 12.69 | 8.67 | 11.86 |
| Width (mm) | $0.47^{ab} \pm 0.06$ | $0.48^{a} \pm 0.07$ | $0.47^{ab} \pm 0.05$ | $0.51^{a} \pm 0.07$ | $0.46^{c} \pm 0.08$ | $0.48^{a} \pm 0.03$ | $0.46^{c} \pm 0.05$ |
| CV (%) | 12.76 | 14.58 | 10.63 | 13.72 | 17.39 | 6.25 | 10.86 |
| Thickness (mm) | $0.36^{b} \pm 0.06$ | $0.37^{ab} \pm 0.06$ | $0.36^{b} \pm 0.05$ | $0.38^{a} \pm 0.05$ | $0.37^{ab} \pm 0.02$ | $0.34^{c} \pm 0.04$ | $0.34^{c} \pm 0.04$ |
| CV (%) | 16.66 | 16.21 | 13.88 | 13.15 | 5.40 | 11.76 | 11.76 |

Data represents mean values, standard deviation (±) and coefficient of variation (CV). Mean values followed by the same superscript in a row do not differ among themselves (Tukey test, $p \leq 0.05$).

0.37 mm, on average, from 80 to 117 DAF, after which it remained relatively constant (0.34 mm) from 124 to 131 DAF (Table 2).

### Effects of temperature, light/dark, and $GA_3$

Germination varied significantly during seed development, with the effects of $GA_3$ solutions, temperatures, and light or dark conditions and their interaction being statistically significant ($p < 0.05$). The interaction of these factors was employed to illustrate the acquisition of germinability during seed development (Tables 3, 4, and 5). Additionally, the average numerical results were utilized to demonstrate the correlation between germination and dormancy alleviation during safflower seed development (Figure 2A-I).

In general, seeds were collected from 80 to 94 DAF but did not germinate at any of the temperatures tested (Figure 2A and B). To assess the status of the seeds at the end of the germination test, those that did not germinate were removed from the paper substrate and exposed to the tetrazolium solution to detect their viability. This process revealed that all the seeds were viable and, therefore, considered dormant (Figure 3).

The seeds collected at 110 DAF exhibited 4 and 5 % germination at 10 °C, in the dark with 50 and 100 µM of $GA_3$, respectively. These results were not statistically significant ($p > 0.05$) (Figure 2C). At temperatures between 20 and 30 °C, germination did not occur under light conditions (Figure 2D).

From 117 DAF onward, it was observed that the treatments of temperature, light/dark, and gibberellin (GA) had a significant effect ($p \leq 0.05$) on seed germination, indicating the overcoming of seed dormancy (Figure 2E and F). The concentration of 50 µM $GA_3$ resulted in a higher germination rate than the control (without GA supplementation) only at a temperature of 10 °C, both in dark/light conditions (Table 3). However, the positive effect of 100 µM of $GA_3$ supplementation was evident in seed germination in the two temperatures, with and without light (Table 3). The germination results were higher at 10 °C than at 20-30 °C in all GA applications (Table 3).

As seed development proceeded at 124 DAF (Figure 2G and H), the beneficial effect of GA supplementation on seed germination became apparent, exhibiting a positive correlation with temperature and light conditions, except seed germination with 50 µM $GA_3$ supplementation at 20-30 °C/dark, which did not differ from the control (Table 4). At 124 DAF, the results of seed germination were higher at the temperature of 10 °C, in

**Table 3** – Germination (%) of safflower seeds collected at 117 days after flowering under variations in gibberellic acid ($GA_3$) solutions, temperatures, and light or dark conditions.

| | Light | | Dark | |
|---|---|---|---|---|
| | 10 °C | 20-30 °C | 10 °C | 20-30 °C |
| 0 µM | 72 Ba*A* | 16 Bb*B* | 48 Ba*B* | 9 Bb*A* |
| 50 µM | 93 Aa*A* | 14 Bb*B* | 59 Ba*B* | 19 ABb*A* |
| 100 µM | 92 Aa*A* | 32 Ab*A* | 84 Aa*B* | 33 Ab*A* |

Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row; italic letters refer to mean comparisons in the same $GA_3$ solution and temperature under light and dark conditions (Tukey test, $p \leq 0.05$).

**Table 4** – Germination (%) of safflower seeds collected at 124 days after flowering under variations in gibberellic acid ($GA_3$) solutions, temperatures, and light or dark conditions.

| | Light | | Dark | |
|---|---|---|---|---|
| | 10 °C | 20-30 °C | 10 °C | 20-30 °C |
| 0 µM | 7 Ba*B* | 7 Ba*A* | 18 Ba*A* | 5 Bb*B* |
| 50 µM | 31 Aa*B* | 20 Ab*A* | 71 Aa*A* | 26 Bb*A* |
| 100 µM | 38 Aa*B* | 28 Ab*B* | 61 Ab*A* | 91 Aa*A* |

Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row; italic letters refer to mean comparisons in the same $GA_3$ solution and temperature under light and dark conditions (Tukey test, $p \leq 0.05$).

**Table 5** – Germination (%) of safflower seeds collected at 131 days after flowering under variations in gibberellic acid ($GA_3$) solutions, temperatures, and light or dark conditions.

| | Light | | Dark | |
|---|---|---|---|---|
| | 10 °C | 20-30 °C | 10 °C | 20-30 °C |
| 0 µM | 82 Aa*A* | 92 Aa*A* | 91 Aa*A* | 88 Aa*A* |
| 50 µM | 89 Aa*A* | 77 Ba*B* | 97 Aa*A* | 91 Aa*A* |
| 100 µM | 88 Aa*A* | 95 Aa*A* | 98 Aa*A* | 96 Aa*A* |

Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row; italic letters refer to mean comparisons in the same $GA_3$ solution and temperature under light and dark conditions (Tukey test, $p \leq 0.05$).

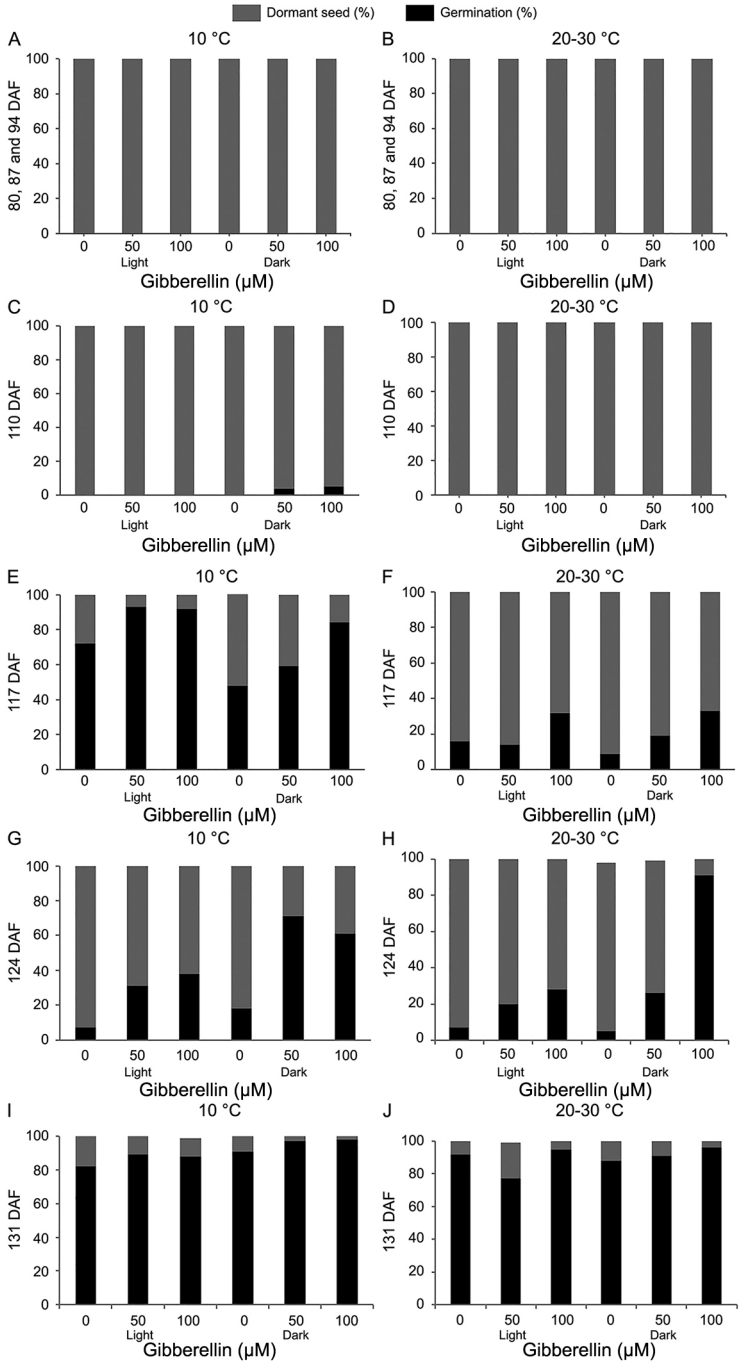


**Figure 2** – Dormant seeds and germination of *Carthamus tinctorius* seeds of different ages and exposed to gibberellin supplementation and light/dark. A) Seeds collected at 80, 87, and 94 days after flowering (DAF) and imbibed at 10 °C and B) 20-30 °C; C) Seeds collected at 110 DAF and imbibed at 10 °C and D) 20-30 °C; E) Seeds collected at 117 DAF and imbibed at 10 °C and F) 20-30 °C; G) Seeds collected at 124 DAF and imbibed at 10 °C and H) 20-30 °C; I) Seeds collected at 131 DAF and imbibed at 10 °C and J) 20-30 °C.

dark/light conditions and $GA_3$ supplementation, except the control (0 µM) in the presence of light and with supplementation of $GA_3$ 100 µM/dark, which exhibited higher germination at the temperature of 20-30 °C (Table 4).

The results obtained at 117 DAF indicate that the seeds could germinate, as evidenced by an increase in germination and a reduction in dormant seeds (Figure 2E and F). However, when observing the results of seeds collected at 124 DAF and exposed to a temperature

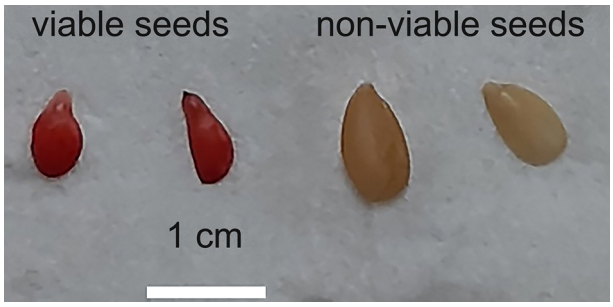


**Figure 3** – Tetrazolium test performed on non-germinated seeds of *Carthamus tinctorius* following exposure to gibberellic acid ($GA_3$) solutions, light/dark, cold stratification, and 20-30 °C after 14 days. Viable seeds stained red and were considered dormant seeds. Non-viable seeds were unstained even after 14 days of imbibition.

of 10 °C, which was the temperature that promoted the highest germination results, it was observed that there was a peak in dormancy status in the seeds (Figure 2G). The results observed at 124 DAF indicate that the seeds overcame dormancy with the highest $GA_3$ supplementation. Overall, seed germination at a temperature of 20-30 °C was practically unchanged, except in the presence of $GA_3$ 100 µM/dark (Figure 2H), which exhibited superior performance compared to the light condition and the temperature of 10 °C (Table 4).

At 131 DAF, regardless of $GA_3$ supplementation and temperature, the seeds exhibited an increase in germination and a reduction in dormancy status (Figure 2I and H). Only the seeds exposed to darkness at temperatures between 20 and 30 °C and treated with $GA_3$ at a concentration of 50 µM exhibited higher germination rates than when seeds were exposed to light ($p \leq 0.05$) at 131 DAF. The lowest germination result (82 %) was observed in seeds that were not exposed to $GA_3$ (control/10 °C/light) (Table 5). Although not significantly different, supplementation with 100 µM $GA_3$ provided higher numerical results for germination at both temperatures and light/dark conditions (Table 5). These findings suggest that at 131 DAF, the safflower seeds produced under these conditions were mature, dispersed, and able to germinate, with a low number of dormant seeds.

## Discussion

Studying how plants disperse and acquire the characteristics necessary for successful dispersal has been a research subject. The diversity of morphological and biomechanical shapes and structures inherent to plant seeds results from the search for different strategies for successful dispersal and adequate germination time (Baskin and Baskin, 2014; Arshad et al., 2019). The evaluation of safflower seed development, including the overall shape of the seed and the physiological status at the time of dispersal, is of significant importance to both breeders and growers.

The measurement of plant morphometry and anatomy provides insights into the growth patterns of plants in different environments. Biometric identifications are a classification system based on recognition that distinguishes individuals by defining their authenticity using a specific physiological or behavioral characteristic (Campbell et al., 2021).

There was a minimal increase in the dry mass of safflower seeds from 117 to 131 DAF (Table 1). This was likely due to the primary metabolic process occurring in the seed at this stage, which encompassed the conversion of nutrition and cell histological differentiation and development. Biomass accumulation was less critical during this period, as it was more pronounced between 80 and 117 DAF. This phenomenon is further supported by the findings of Ellis (2019), which indicate that the seed-filling phase starts with a consistent or nearly consistent gradient and culminates in a plateau (maximum dry weight). Subsequently, the water content decreases rapidly during the initial stage of maturation, followed by a gradual reduction to a minimum content prior to seed shedding (Table 1). This is an expected phenomenon, as the decline is a consequence of the disproportionate accumulation of assimilates relative to water throughout the plant development and maturation (Ellis, 2019).

Despite the lengthy period of development, seeds exhibited no further increase in length, width, and thickness (Table 2). The resulted in a complete mature appearance at 131 DAF. An indirect indicator, such as the appearance of grey seeds with an average length of 0.59 mm, width of 0.46 mm, and thickness of 0.34 mm, was applied to represent this maturation visually. Despite the complete maturation and full development, safflower seeds were not naturally shed at 131 DAF. This phenomenon may cause confusion among researchers, as safflower seeds frequently exhibit low germination and are generally considered dormant immediately after harvest.

Previous studies have examined the seeds shortly after harvest. Safflower seeds harvested at a period close to the point of physiological maturity [25.8 % wet basis (wb)] exhibited low germination rates, and increases in germination occurred only after five days of cold stratification of the seeds (Oba et al., 2019). These findings suggest that the germination results of seeds harvested at 124 DAF were also low, with a water content of 26.4 % wb and without the application of exogenous GA (Table 4). This means that the relationship in which fresh safflower seeds are less desirable is described because the seeds require post-maturation periods to overcome seed dormancy. Freshly harvested safflower seeds gradually break dormancy only during a storage period of 240 days in an uncontrolled environment (Oba et al., 2017). However, seeds stored for 180 and 240 days exhibited less considerable increases in root protrusion when subjected to a longer period of cold stratification. However, this explanation needs to consider the crucial physiological factors involved in the acquisition of

germinability and the determination of dormancy status during seed development.

Our findings contradicted our initial expectations, indicating that seeds require a prolonged after-ripening period. At 131 DAF, a minimum of 82 % germination was observed in the seeds exposed to low temperature/light without needing GA supplementation (Table 5). Furthermore, our results demonstrated that safflower seeds overcome dormancy in a synergistic action of gibberellin/abscisic acid (GA/ABA) imbalance, according to seed development.

At 117 DAF, the safflower seeds were responsive to the temperature of 10 °C, exposure to light, and supplementation with exogenous GA. This indicates that the seeds had already developed all the anatomical structures necessary for germination. The application of these factors in synergism appears to have altered the ABA balance. Subsequently (124 DAF), the seeds exhibited reduced sensitivity to the application of multiple factors, potentially due to increased internal ABA. In general, safflower seeds are typically harvested at this stage of development. According to the official recommendations for seed germination testing, which specify a temperature range of 20-30 °C (MAPA, 2009), the germination results are often low, as has been observed by Oba et al. (2017) and Bidgoly et al. (2018).

Notably, seeds harvested at a later stage (131 DAF) exhibited greater sensitivity to multiple associations that enhanced seed germination. The application of alternating temperatures (20-30 °C) or the sole use of low temperatures was sufficient to achieve the maximum germination potential for the seeds (Table 5). These findings suggest that a GA/ABA equilibrium is naturally established during safflower seed permanence or delayed harvest, which initiates seed germination. Prior to this maturation stage, internal GA levels may be insufficient to stimulate germination, thereby explaining the necessity of the after-ripening process observed in freshly harvested safflower seeds (Oba et al., 2017). Consequently, mechanisms that impede germination metabolism similarly impact embryo growth, resulting in a shift in the balance in favor of dormancy (Lando et al., 2020).

In the case of freshly dispersed seeds, it is necessary to consider the germination process and embryonic development. It is important to ensure that there are no physical blockages to the entry of water from the environment to the dispersion units. For example, safflower seeds are botanically achenes, and in other achenes like sunflowers, the remaining structures, such as the pericarp, prevent germination (Lachabrouilli et al., 2021). In addition, non-deep physiological dormancy (PD) is the type of dormancy found in seeds of various families, including Asteraceae (Baskin and Baskin, 2020), to which safflower belongs. Seeds with PD have fully developed embryos with a physiological mechanism inhibiting germination (Baskin and Baskin, 2020).

It has been demonstrated that non-deep PD can be released by dry storage, also known as after-ripening (Finch-Savage and Bassel, 2016; Baskin and Baskin, 2020). The term "after-ripening" is defined as the loss of dormancy achieved by exposing a seed to a specific set of environmental conditions after maturation and separation from the mother plant. These conditions may include soil (Hidayati et al., 2019) or dry storage (Abrantes et al., 2021) and additionally reported in seeds remaining on dead mother plants in the field (Baskin and Baskin, 2020). Once the safflower seeds have reached the point of physiological maturity and remained attached to the mother plant until 131 DAF, it is possible to interpret this question within the context of the after-ripening process.

Seeds can overcome dormancy during the post-maturation process. Temperature-dependent changes in seed dormancy level are expressed as changes in environmental conditions permissive for seed germination (Batlla and Benech-Arnold, 2015). After-ripening occurs in non-soaked viable seeds under very low water potentials (i.e., 5 ~ 15 % moisture content, wb). Some species respond to warm conditions, while others respond to cool, moist conditions (i.e., stratification or chilling). These species also respond to both sets of conditions (Iglesias-Fernández et al., 2011). The decline in ABA content, the decrease in ABA sensitivity, and the increase in GAs sensitivity are involved in the mediated transition after maturation from the dormant to the non-dormant state of many species, such as sunflower (Baskin and Baskin, 2020; Xia et al., 2019).

It has been demonstrated that certain factors, such as temperature, play a significant role in the modulation of after-ripening processes. It is widely understood that seed dormancy is also strongly regulated by the environmental conditions that prevail during seed development in the mother plant, including the maternal environment (Kerdaffrec and Nordborg, 2017). This is particularly evident in sunflower seeds, where the most extended duration of the developmental cycle from sowing to harvest is associated with the lowest dormancy at harvest (Lachabrouilli et al., 2021). The precise molecular mechanisms involved remain unclear. It has been observed that there are limited changes in gene expression between dormant and non-dormant dry seeds. This is likely due to the lack of free water for enzymatic reactions in the dry state. In contrast, passive and non-enzymatic reaction events, such as oxidation and Amadori-Maillard reactions, which occur during after-ripening, have been postulated to be associated with the release of dormancy (Sano et al., 2020; Sano and Marion-Poll, 2021).

It is not surprising that dormancy in safflower seeds has been widely reported to be overcome only after dry storage (Oba et al., 2017, 2019), given that the average seed size undergoes little or no change during seed development, as demonstrated by a very accurate morphometric analysis of the seeds (Table 2).

The results of this study indicate that safflower seeds remain immature up to 110 DAF, and they begin to germinate at 117 DAF. However, the GA/ABA equilibrium

remains a significant factor in the seed behavior. This allows us to conclude that dormancy break in safflower seeds is driven by a synergistic association between seed maturation and GA/ABA balance. As the seeds of safflower developed and became sensitive to treatment with $GA_3$, which promoted germination, the results indicated that the optimal harvest time for the seeds is essential for obtaining safflower seeds that are fully capable of germinating. However, it is necessary to emphasize that safflower seeds apparently do not present a mechanical barrier to dormancy and germination. In this sense, dormancy is solely a physiological phenomenon, not a combined mechanical-physiological type.

Another significant contribution of the present study is the development of a technology for germination in safflower seeds. Dormancy represents a significant challenge in the production of technology and genetic improvement of this species (Berville et al., 2005; McPherson et al., 2009), as the precise manner in which dormancy status changes and drives germination characteristics remains to be determined. To overcome this barrier, the current literature offers a practical solution in the form of cold stratification (Oba et al., 2017, 2019; Bidgoly et al., 2018). Seeds can be stored for up to 240 days (Oba et al., 2017, 2019) and subjected to laser treatment with 300 mJ (Perveen et al., 2021). The results indicate the necessity of considering a harvest delay to achieve high seed germination and field establishment.

In a plant population, the decision about the point of seed harvest is influenced by a range of factors, including the phenological state of the majority of the plant population and climatic conditions that can threaten the physiological quality of the seeds. From this perspective, harvesting in advance can be a strategy to avoid damage to the crop. However, when climatic conditions do not threaten the viability of the seeds on the mother plant, safflower seeds should be harvested after 131 DAF to obtain seeds with high germination. Nevertheless, a delay in harvest can result in the induction of secondary dormancy or seed aging (loss of vigor) and death. Consequently, further studies are necessary to elucidate the impacts of after-ripening on the mother plant the physiological mechanisms of safflower plants in subsequent growth stages, and the final seed yield and quality.

In order to consider late harvesting a useful tool rather than a problem for overcoming dormancy, storage of safflower seeds to dormancy alleviation is recommended, in agreement with Oba et al. (2017) and Oba et al. (2019). Adding $GA_3$ can increase seed numbers for optimal field conditions when fresh seed is required.

In conclusion, safflower seeds exhibit a mature appearance at 131 DAF, with an average length, width, and thickness of 0.59, 0.46, and 0.34 mm, respectively. Safflower seeds remain immature up to 110 DAF, and at 117 DAF, they begin to germinate and become sensitive to $GA_3$ supplementations, light exposure, and low temperatures. During the later stages of seed maturation, the sensitivity of seeds to the environment regulates seed germination and dormancy status. Seeds undergo an after-ripening process after 124 DAF, becoming insensitive to $GA_3$ supplementation, low temperature, light/dark conditions, and exhibiting high germination at 131 DAF.

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