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Original Article

Regulation of dormancy break and germination of safflower seeds: the role of GA_3, light and cold temperatures

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Abstract

The safflower crop is considered a great alternative for crop rotation since drought tolerance and low production cost are attractive for its choice. However, safflower seeds show dormancy soon after dispersal from the mother plant, making it difficult to successfully establish plants using newly harvested seeds. The influence of temperature, gibberellin and light/dark on dormancy break of safflower seeds during storage were investigated. In a completely randomized design, freshly harvested seeds or stored for 100 and 200 days (paper bag, 20 °C/ 60% UR) were treated with GA_3 (0 and 100 µM), at 4, 10 and 25 °C, in the presence and absence of light, during the germination test. Seeds were evaluated for germination percentage, germination speed and seedling survival after 21 days. The temperature of 10 °C, in combination with GA_3 (0/100 µM), or light/dark, provided the highest seed germination results, for freshly-harvested seeds and stored seeds. Collectively, these observations indicate that dormancy was not affected by gibberellic acid (100 µM GA_3) and the germination results at 21 days were significantly higher, in relation to the use of GA_3, under light or dark. Recently harvested seeds could efficiently germinate at 10 °C in the dark, while seeds dry-stored at 20 °C had decreased germination percentages.

Keywords: storage, *Carthamus tinctorius*, gibberellic acid-3, root protrusion.

Resumo

A cultura do cártamo é considerada uma ótima alternativa para a rotação de culturas na segunda safra, uma vez que a tolerância à seca e o baixo custo de produção são atrativos para sua escolha. Entretanto, as sementes de cártamo apresentam dormência logo após a dispersão da planta-mãe, dificultando o êxito do estabelecimento de plantas com a utilização de sementes recém-colhidas. A influência da temperatura, giberelina e luz/escuro na superação de dormência de sementes de cártamo durante o armazenamento foram investigados. Em um delineamento inteiramente casualizado, as sementes recém-colhidas ou armazenadas durante 100 e 200 dias (em saco de papel, 15 °C/ 60% UR) foram submetidas às doses de GA_3 (0 e 100 µM), às temperaturas de 4, 10 e 25 °C, na presença e ausência de luz, durante o teste de germinação. As sementes foram avaliadas quanto à germinação, velocidade de germinação e sobrevivência das plântulas após 21 dias. A temperatura de 10 °C, em combinação com o uso de GA_3 (0/100 µM), ou luz/escuro, proporcionou os maiores resultados de germinação de sementes em relação às demais temperaturas, para as sementes recém-colhidas e armazenadas. Coletivamente, essas observações indicam que a dormência não foi afetada pelo ácido giberélico (100 µM GA_3) e os resultados de germinação aos 21 dias foram significativamente maiores, em relação ao uso de GA_3, no claro ou no escuro. As sementes recém-colhidas germinam eficientemente na temperatura de 10 °C no escuro, enquanto as sementes armazenadas a 20 °C apresentaram redução da germinação.

Palavras-chave: armazenamento, *Carthamus tinctorius*, 3-ácido giberélico, protrusão da raiz primária.

1. Introduction

Safflower (*Carthamus tinctorius* L.) is cultivated due to the properties of its seeds, called achenes, which contain a high oil content consisting of high amounts of oleic and linoleic acids compared to other oilseeds (Khalid et al., 2017). In addition, there is interest in safflower seeds as a source of phenols with antioxidant and anti-aging activity (Zemour et al., 2019; Özçinar, 2021), source of macronutrients (P, K, Ca, Mg, Na) and micronutrients (Zn, Fe, Mn and Cu) for animal feed (Kereilwe et al., 2020).

Even though this species has been cultivated for hundreds of years and in different countries around the world, safflower seeds show dormancy, which is a characteristic of many species belonging to the Asteraceae family (Dolatabadian and Modarres, 2008; Lachabrouilli et al., 2021). In safflower seeds, dormancy is more pronounced soon after removal from the parent plant, making sowing unfeasible after harvest (Oba et al., 2017).

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Seed dormancy is considered as a characteristic that prevents germination, even when environmental factors such as water, temperature, oxygen, light/dark are apparently favorable for viable seeds to germinate in period of time (Baskin and Baskin, 2021). Even while the seed coat still remains impermeable to water, mature seeds of certain species may need an additional period, either in the field or during storage, to overcome dormancy. This phenomenon in seeds is considered as non-deep dormancy (Baskin and Baskin, 2004) and, during post-maturation of seeds, preparation for germination requires strong stimulation of gibberellic acid synthesis (GA) (Iglesias-Fernández et al., 2011), which acts in the resumption of embryonic growth after water imbibition by the seeds.

Dormancy release that occurs in the post-maturation of seeds can be induced by several agents, especially in species belonging to the Asteraceae family. Temperature is one of the factors that influences the overcoming of sunflower seed dormancy during storage; after 60 days at 25 °C there is an increase in gibberellin synthesis and sensitivity to exogenous GA, overcoming dormancy faster than seeds stored at 5 °C (Rodríguez et al., 2018). On the other hand, in safflower seeds, exposure the seeds to 10 °C/dark during the germination test was more effective in overcoming dormancy than storing the seeds for 60 days at 25 °C (Oba et al., 2019). After storage at different temperatures, persistence of dormancy in sunflower seeds may involve changes in metabolism and/or sensitivity to ABA that manifest after soaking, although other hormonal pathways may also play a role in seed dormancy (El-Maarouf-Bouteau et al., 2015; Cuena-Lombraña et al., 2020).

It must also be considered that light through the conversion of phytochrome triggers the synthesis of gibberellins (GA) which finally results in the promotion of germination through the activation of any of the mechanisms controlled by GA that promote the seed germination, such as weakening of the cell wall and growth of the embryo (Batlla and Benech-Arnold, 2015).

Freshly harvested safflower seeds may require up to 8 months of storage (an environment with an average temperature of 25 °C and 60% relative humidity) to overcome dormancy (Oba et al., 2017). This characteristic hinders the propagation and management of the crop and raises doubts about the true physiological potential of the seed lots right after harvest. However, it is also important to consider the seedlings survival from seeds stored after long periods of time.

It is still unclear about the potential effects of storage, temperature and germination inducers for overcoming safflower seed dormancy. Furthermore, it has not been elucidated whether the effect of storage is mediated by changes in seed dormancy and changes in metabolism and/or hormone sensitivity. The hypotheses of this work are that: (1) different temperatures, light/dark conditions and exogenous GA act to overcome dormancy and promote safflower seed germination; (2) the dormancy that seeds present right after harvesting can be overcome due to the hormonal balance that occurs in seeds during storage and; (3) seed storage increases sensitivity to exogenous GA, light and temperature during seed imbibition.

Considering that seed germination is an important aspect that affects safflower production (Soleymani, 2019), this study aimed to evaluate the effect of exogenous GA, temperatures and light/dark on the germination of freshly harvested seeds submitted to storage.

2. Material and Methods

2.1. Seeds production

Safflower seeds (*Carthamus tinctorius* L.) were produced at Experimental Farm of Agricultural Sciences da Universidade Federal da Grande Dourados (UFGD), in Dourados, Mato Grosso do Sul state, Brazil, from May to October of the winter season of 2019. The production area is at an average altitude of 434 m and 22º14'S e 54º59'W. The soil is classified as a typical Distroferric Red Latosol (Santos et al., 2018).

Sowing occurred on May 10, 2019, at an average rate of 15 seeds m^{-1}. The experimental plots consisted of rows 13 m wide (0.50 m spacing) and 20 m long. The useful area was 95 m^2, excluding 50 cm of borders at each end. There was no chemical correction of the soil or application of fertilizers at the time of sowing and management practices were applied for the full development of the safflower crop. Weed control was carried out with manual weeding and it was not necessary to carry out disease control. Figure 1 shows the climatic data during the seed production season.

The seeds were manually harvested on October 27, 2019. After harvesting and processing the seeds, the seed lot was taken to the Seed Technology Laboratory of the Faculty of Agricultural Sciences (UFGD). The time interval between seed processing and the installation of treatments in the laboratory was 2 days, so the seed samples were considered to have the same physiological status, and therefore without starting after-ripening process.

The water content of the seeds was determined by the gravimetric method after drying in an oven at 105 ± 3 °C, 24 h according to the Rules for Seed Analysis (Brasil, 2009) and the seeds presented moisture content of 12.1%.

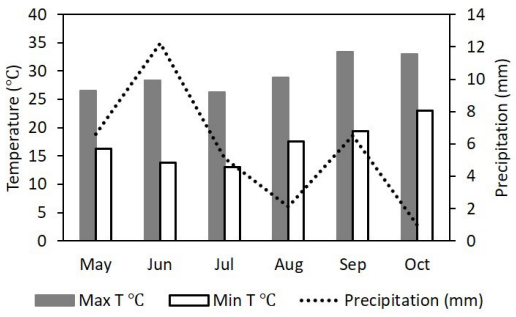

Figure 1. Maximum, minimum temperature and precipitation during safflower seed production in the winter season in Dourados, Mato Grosso do Sul, Brazil. 2019. Source: Guia Clima (EMBRAPA, 2019).

2.2. Seed storage and germination

The seed lot was divided into three samples; and one a sample was evaluated immediately. The other two samples were placed inside paper bags (31cm × 19cm × 33cm, 120 g of grammage) and were stored for 100 and 200 days in an acclimatized chamber (dark, 20 °C and 60% UR). After each period, the seeds were removed from the packages and the water content of the seeds was determined by the gravimetric method and oven drying (Brasil, 2009). For the germination test, the seeds were disinfected with sodium hypochlorite solutions 2,5% for five minutes, followed by rinsing in running water. The test was conducted by placing the seeds between paper towels moistened with distilled water (0 µM) equivalent to 2.5 times the mass of dry paper (Brasil, 2009), in eight repetitions with 25 seeds.

2.3. Effect of temperature, GA_3 and light/dark

In order to evaluate the effect of cold temperatures on safflower seed germination, temperatures of 4 and 10 °C were applied. The temperature of 25 °C was used because it is recommended by the Brazilian Rules for Seed Analysis (Brasil, 2009). Gibberellic acid solutions GA_3 (100 µM) were applied to moisten the substrates in place of water. The GA_3 solution was diluted with KOH 1N and pH adjusted to 7.0, with HCl 1N. The amount of GA_3 solution applied to wet the substrates was equivalent to 2.5 times the mass of dry paper.

Simultaneously, the samples were kept in germination chambers (B.O.D.) (*Biochemical Oxygen Demand*) at 4 °C and 10 °C with white light or dark. The evaluations of treatments conducted in the dark were performed under green light. The combinations with the temperature of 25 °C, presence of light and substrate moistened with water (0 µM) constituted the control.

2.4. Germination characteristics

To evaluate the effect of treatments, the germination criterion used was primary root protrusion, with a minimum length of 2 mm. Germination monitoring was performed daily and at the same time. The germination results evaluated at 4 and 21 days after the initiation of the tests (Brasil, 2009), were expressed as a percentage. Non-germinated seeds were incubated in the 2,3,5- tetrazolium triphenyl-chloride (1%) for two hours to confirm the seeds dormancy status. Seeds stained red were considered dormant and these data were not submitted to statistical analysis.

The germination speed (GS), expressed as an index, was calculated using the formula: $GT = \Sigma(Gt/Tt)$; Gt is the number of seeds that germinated in the "t th" day, and Tt is the number of days required for the seed to germinate (Ranal and Santana, 2006).

2.5. Seedling development potential

The ability to continue the development of seeds that germinated in "apt" (normal development) and "inapt" (abnormal development) seedlings under ideal field conditions (Brasil, 2009) was evaluated. The germinated seeds were kept in the substrate for 21 days after germination characteristics assessments. The results were expressed as percentage of seedling survival.

The experiment was conducted in a completely randomized design. For freshly harvested seeds and seeds stored for 100 and 200 days, a factorial scheme was used 3 × 2 × 2 [GA_3 (0 or 100 µM) × temperature × white light or dark], in eight repetitions of 25 seeds for each treatment. Data were subjected to analysis of variance and, in case of significance, temperatures were compared using Tukey's test. The doses of gibberellin and the presence or absence of light were compared using the test "t", both at a 5% probability level, with the statistical software SISVAR® (Ferreira, 2019).

3. Results

3.1. Freshly harvested seeds

The interaction effect between GA_3 x light/dark, GA_3 x temperature and light/dark x temperature was significant ($p \leq 0,05$) for all evaluated characteristics. The triple interaction between the factors was not significant ($p > 0,05$), therefore, seed germination at four days was not influenced by treatments (Figures 2A and 2B). The average seed germination result was less than 10%.

However, after 21 days, significant differences were observed between treatments in seed germination (Figures 2C and 2D). The temperature of 10 °C, in combination with the use of gibberellin (0/100 µM), or light/dark, provided the highest seed germination results in relation to the other temperatures, which was 73% (Figure 2C). Seed germination at a temperature of 25 °C, in combination with the factors, showed intermediate results (42%), and a temperature of 4 °C provided the lowest germination results (25%) (Figure 2C).

With GA_3 (100 µM), safflower seeds subjected to temperatures of 4 °C and 25 °C showed sensitivity (2% and 23% germination, respectively); and in the absence of the hormone, the germination results of seeds subjected to these temperatures were higher (25% and 42%, respectively) (Figure 2C). The seeds submitted to the temperature of 10 °C did not show significant differences in germination (on average, 70%) at both GA concentrations (Figure 2C).

The results obtained with the temperature of 10 °C were superior to the other temperatures during germination, in both GA concentrations and light/dark conditions (Figure 2C). Seeds subjected to those at light/dark conditions at temperatures of 10 °C and 25 °C showed no significant difference in germination; however, the temperature of 4 °C associated with the dark was more favorable to seed germination in relation to the presence of light (Figure 2C). Regarding the interaction between GA x light/dark, without GA_3, the exposure of the seeds in the dark enhanced the seeds germination (59%) in relation to the presence of light (33%) (Figure 2D). However, with the application of GA_3, there was no significant difference between light and dark and an average of 30% germination was observed (Figure 2D). Similar results were observed in germination speed (GS); the temperature of 10 °C in both GA applications accelerated seed germination, followed by the temperature of 25 °C and the temperature of 4 °C, which was lower than the other temperatures (Figure 2E).

Without GA, seeds presented the highest GS results, regardless the temperature, when compared to the use of GA (Figure 2E). Regarding the interaction between temperatures and light/dark, GS was higher at 10 °C in the light and at 25 °C in the dark (Figure 2E).

Without GA, darkness also enhanced GS (Figure 2F). However, with GA application, there was no significant difference between light and dark in GS (Figure 2F).

Similar results to seed germination were observed in seedling survival. With the temperature of 10 °C, the highest results of seedling survival were verified in relation to the other temperatures associated or not with the application of GA (64%) and light/dark (63%) (Figure 2G). Temperatures of 4 °C and 25 °C did not differ from each other, except when in the dark, since seedling survival was lower at 25 °C (22%) (Figure 2G). Seedlings originated from treatments without GA showed greater survival at all temperatures tested, except for the temperature of 10 °C where there was no significant difference with or without GA (Figure 2G).

Only at 4 °C associated with the dark there was a superior result (39%) in relation to the presence of light (10%) whereas with the other temperatures, there was no significant difference between light and dark for seedling survival (Figure 2G). In the presence of light, there was no significant difference between GA treatments; whereas in the dark, there was greater survival of seedlings (60%) without GA supplementation (Figure 2H).

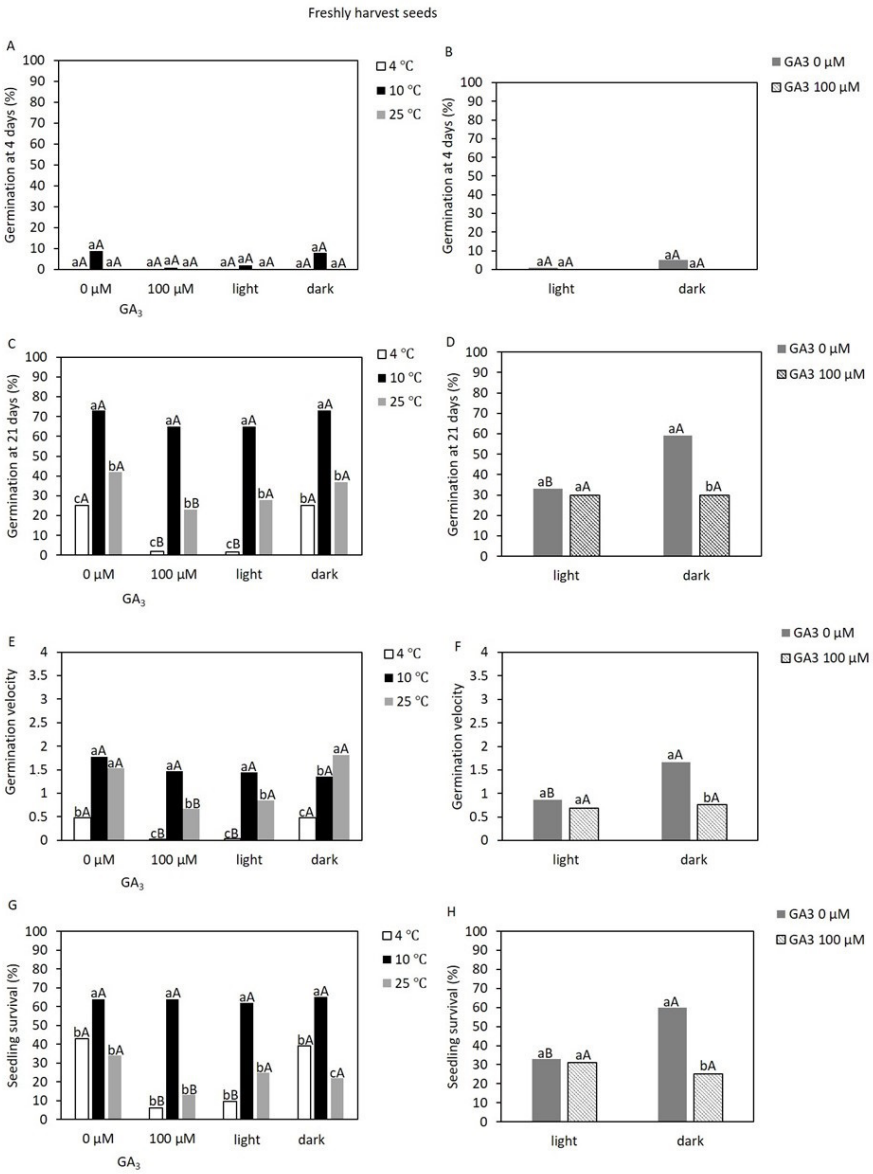

Figure 2. Lowercase letters compare each temperature in the same GA treatments (0 or 100 µM) or light/dark. Capital letters compare the same temperature in each GA treatment (0 ou 100 µM) or light/dark.

3.2. Stored seeds

Previous studies indicated the significant effect of the storage period in order to achieve high germination of safflower seeds, hence the effect of temperature, GA_3 and light/dark treatments were evaluated after seeds storage. After 100-days storage, no significant difference was observed between treatments in seed germination at 4 days (Figures 3A and 3B), nor after 200 days of storage (Figures 4A and 4B). It is important to note that the seeds that did not germinate even before or after storage were dormant. However, as observed for freshly harvested seeds, after seeds storage, there was a significant effect of treatments on seed germination results at 21 days. 100-days stored seeds imbibed at 10 °C with or without GA supplementation in the light/dark presented the highest germination results in relation to the other temperatures, which did not differ from each other (Figure 3C). However, at the same temperature, with GA_3 100 µM, the germination result was significantly lower (28%) compared to the absence of GA (62%) (Figure 3C). Without GA, the germination results were higher (24% and 25%, light and dark, respectively) in relation to the one obtained with 100 µM GA (14% and 9%, light and dark, respectively) (Figure 3D).

After 200-days seeds storage without GA_3 supplementation, the germination results after 21 days imbibed seeds at 10 °C were higher (59%), followed by imbibition at 25 °C (24%) and at 4 °C, seeds did not germinate (Figure 4C).

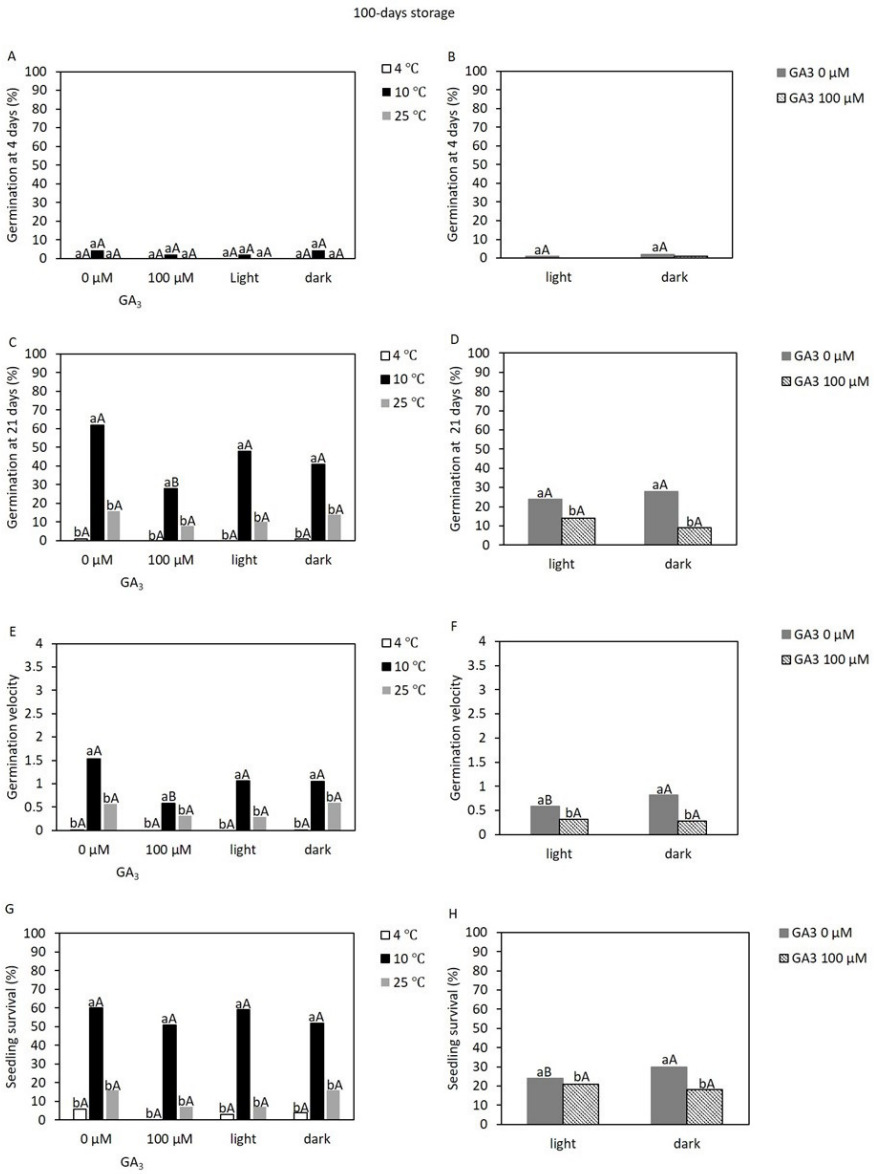

Figure 3. Lowercase letters compare each temperature in the same GA treatments (0 or 100 µM) or light/dark. Capital letters compare the same temperature in each GA treatment (0 ou 100 µM) or light/dark.

However, with 100 µM GA_3, there was no significant difference between the temperatures at seed germination (on average, 2% of germination; Figure 4C), indicating that after prolonged storage (200 days), the exogenous application of GA may negatively affect the hormonal balance that trigger seed germination. Without GA supplementation, the germination results at 21 days were significantly higher in relation to the one obtained with GA, under light or dark (Figure 4D).

Similar results to those obtained for seed germination were observed for germination speed. After 100-days storage seeds showed a higher germination speed at 10 °C compared to the other temperatures, in both conditions of GA 0/100 µM and light/dark; whereas no significant difference between the other temperatures (Figure 3E). However, only imbibed seeds at 10 °C showed a significant increase in germination speed in the absence of GA (1.53) compared to the seeds imbibed with 100 µM (0.58) whereas the other temperatures did not differ significantly regarding the use or not of GA and light/dark (Figure 3E). However, without GA, the germination speed was higher in both light (0.52) and dark (0.82) conditions, compared to GA supplementation. There was no significant difference between light/dark for each GA treatment in seedling survival (Figure 3F).

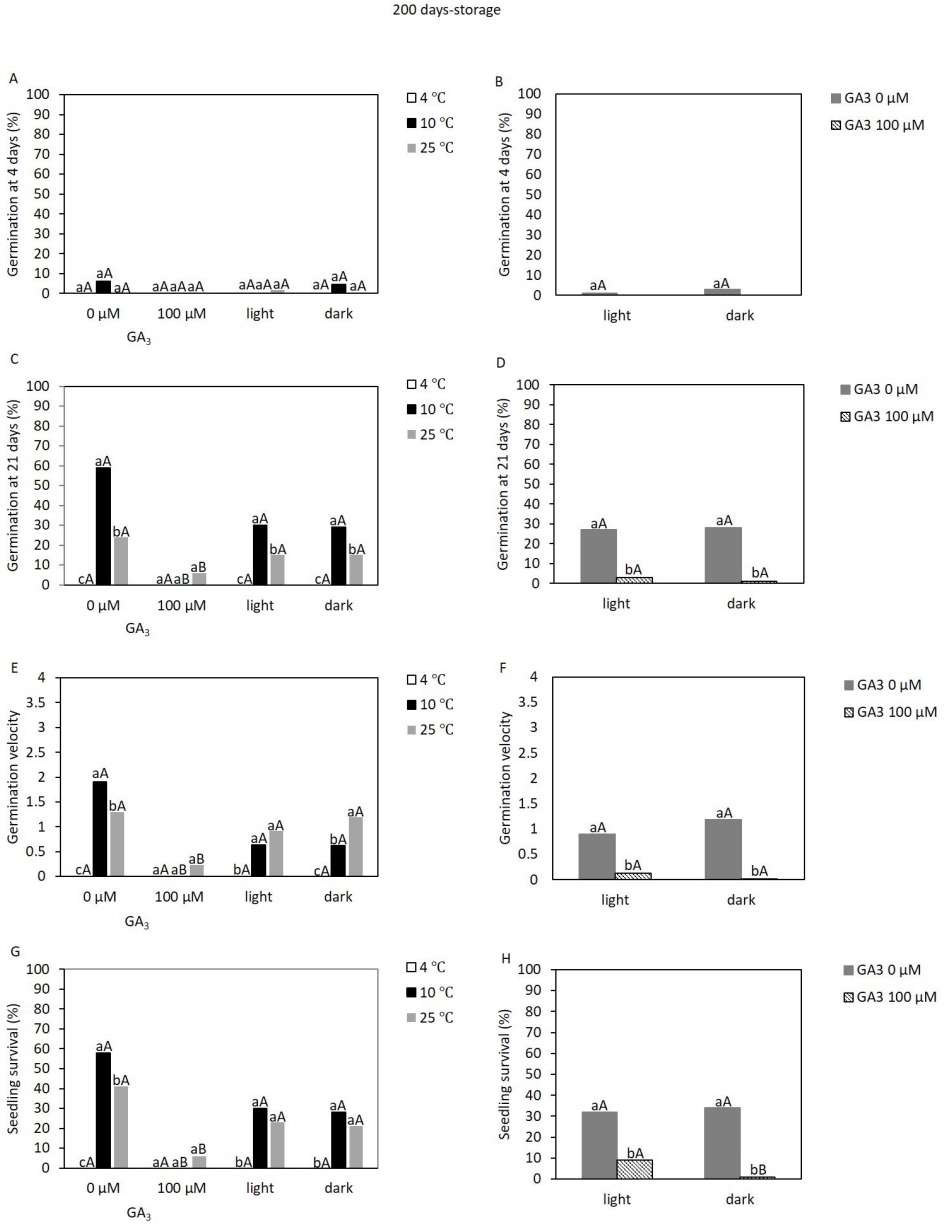

Figure 4. Lowercase letters compare each temperature in the same GA treatments (0 or 100 µM) or light/dark. Capital letters compare the same temperature in each GA treatment (0 ou 100 µM) or light/dark.

For 200-days stored seeds, regarding the interaction between GA x temperatures, similar results were observed in the speed germination, indicating the significant effect of the temperature of 10 °C, especially without the application of GA (Figure 4E). However, in the presence of light there was no significant difference between the temperatures of 10 °C and 25 °C; while in the dark, the germination speed was higher at 25 °C, followed by the other temperatures (Figure 4E). The germination speed was higher without GA, but seeds treated or not-treated with GA_3 showed no significant difference between light/dark in the germination speed (Figure 4F).

After 100-days seeds storage, in both conditions with or without GA and light/dark, the seedlings survival was higher at 10 °C in detriment of the other temperatures, which did not differ from each other (Figure 3G). For each temperature, there was no significant difference between GA and light/dark treatments (Figure 3G). Without GA, seedling survival was higher in both light/dark, when compared to seeds-treated with 100 µM GA (Figure 3H). However, seedling survival in the dark was higher without GA application (30%) compared to the one treated with 100 µM GA (18%) whereas under light no significant difference with GA treatments (on average, 23%) (Figure 3H).

Interactions between GA x temperatures were significant for seedlings survival from seeds 200-days stored (Figure 4G). In the absence of GA_3, there was a positive effect of the temperature of 10 °C on the continuity of seedling development (58%) in relation to the temperature of 25 °C (41%) and the temperature of 4 °C, which did not provide seedling survival (Figure 4G).

In both light/dark conditions, no significant differences were observed for seedling survival between seeds imbibed at 10 °C and 25 °C, which were superior to the one imbibed at 4 °C (Figure 4H). Seeds not-treated with GA showed higher seedling survival than seeds GA-treated, both in light or dark. However, there was no significant difference between light/dark for seedlings survival from treated seeds (on average, 5%) or without GA_3 (on average, 32%) (Figure 4H).

4. Discussion

The seed industry requires high quality seeds available for processing soon after harvest (i.e. non dormant). Apparently, this possibility is limited for safflower seeds. According to the results, right after harvesting, and even after storing seeds for more than 100 days, which is a practice used intuitively by producers to increase plant establishment in the field, temperature appears as a preponderant factor to overcome seed dormancy and trigger seed germination.

However, this important factor is not considered officially in the analysis of safflower seeds. (Brasil, 2009). According to seed germination at 21 days, the temperature of 10 °C was more favorable for freshly harvested seeds (Figure 2C) or stored seeds (Figures 3C and 4C) in relation to the temperature of 25 °C, that is the recommended for safflower seed germination test (Brasil, 2009).

Some factors are related to slow temporal changes. These factors (e.g., temperature) are integrated over time to change the depth of dormancy and sensitivity to other factors (e.g., light) (Finch-Savage and Footitt, 2012). We found that temperature seems to be the most determining factor for safflower seeds germination. Previous studies indicated that cold stratification (10 °C) for 7 days followed by the temperature of 25 °C would be promising for safflower seeds, but after a minimum period of 60-days seed storage, the effect of the stratification period could be reduced (Oba et al., 2017). Later, the authors reported the cold stratification at 10 °C as a premise to overcome the dormancy in safflower seeds, instead 120-days seeds storage (Oba et al., 2019).

The presented results indicate the low temperature (10 °C) during the germination test is more effective than the high temperature (25 °C), which is currently recommended for the species. The lowest temperature (4 °C) was not efficient for the germination process, indicating there is a thermal limit to overcome dormancy and trigger the safflower seeds germination. In *Arabidopsis thaliana* seeds, cold temperature increased dormancy and is correlated with increased ABA (Huang et al., 2018). On the other hand, in *Halenia elliptica* seeds, cold temperatures were more effective in overcoming dormancy than relatively higher temperatures whereas seed germination decreased when the temperature of 2 °C was substituted at 8 °C (Chen et al., 2020). Our results indicate that constant temperature of 10°C is prominent for safflower seed germination, germination speed and seedlings survival originated from freshly harvested seeds without the need for seeds storage or to change the temperature to 25 °C during the germination test.

The type of dormancy that most diaspores of Asteraceae species have is non-deep physiological dormancy (Baskin e Baskin, 2014), that can be overcome through removing seed cover structures (Nur et al., 2014) and/or by exposing the seeds to predetermined temperatures (Schütz et al., 2002). For many species exposure to temperature variations acts as an exogenous signal to modulate seed dormancy (Penfield and MacGregor, 2017) and the ideal temperature to overcome seed dormancy differs from that ideal for seed germination (Baskin and Baskin, 2014). However, seed soaking under sub-optimal temperature conditions induces genes expression that positively regulate ABA signaling in the embryo and in endosperm tissues, and transcriptional activation of genes that negatively regulate GA signaling in the embryo (Tuan et al., 2018).

One of our research hypotheses was based on the potential effect of exogenous gibberellin to overcome dormancy and promote safflower seed germination. However, all variables analyzed - as in freshly harvested or stored seeds - indicated the exogenous GA did not alter the seed dormancy status. Conversely, other factors may immediately indicate the conditions are more suitable for seed germination (for example, temperature) have altered the dormancy status, but without altering the seeds sensitivity to exogenous GA. It is unclear whether this was a problem of penetration of GA through the surrounding seed layers or a genuine insensitivity.

After seed imbibition, dormancy release occurs through ABA degradation, which precedes activation of germination by a second plant hormone, gibberellin. The balance between these two hormones integrates signals from light, temperature or nitrate, acting antagonistically on embryo growth and endosperm weakening (Sano and Marion-Poll, 2021).

Environmental factors, such as low temperatures, can induce GA biosynthesis during the early stages of germination (Debeaujon and Koornneef, 2000). For safflower seeds, the application of 100 µM of GA_3 did not replace the temperature requirement to overcome dormancy and promote seed germination, since the seeds are exposed to the determining factor to dormancy release the wide range of environmental conditions permissive for seed germination (Batlla and Benech-Arnold, 2015).

Darkness is an important cue to be considered in the physiology of safflower seed germination according to seed germination at 21 days, germination speed and survival of seedlings from freshly harvested seeds (Figure 2) mainly without GA_3. For many species of Asteraceae, white light inhibits seed germination. Freshly harvested barley seeds exposed to continuous white light induced dormancy, with increase of ABA in the embryo, and seed imbibition in the darkness promotes seed germination (Ma et al., 2017). Seeds of *Epilasia acrolasia* overcame dormancy and presented higher germination when imbibed at 20/10 °C in the dark than when imbibed in other conditions (Nur te al., 2014). Seeds of *Brachypodium distachyon* exposed to white light during imbibition induced dormancy with no change in embryo ABA levels (Barrero et al., 2012).

It is noteworthy that up to 100 days of storage, safflower seeds remained sensitive to white light during imbibition, but such sensitivity was not significant after 200 days of dry storage (Figure 4). Afterripening would then lead to decrease in this sensitivity, however, insufficient to become responsive to exogenous GA or able to germinate at different temperatures than 10 °C. Safflower seeds germinated at the constant temperature of 10 °C. The results elucidated there is a thermal limit for and germination; temperatures of 4 °C and 25 °C are not required to trigger germination of safflower seeds. In contrast when seed imbibition occurs at 10 °C it is not necessary seeds dry-storage or exogenous GA_3 to dormancy release. Apparently, safflower seeds do not undergo after-ripening during dry-storage at 20 °C to dormancy release. We also demonstrated that white light enhances rather than reduces the expression of dormancy in safflower seeds, especially in those recently harvested or 100-days stored (20 °C, 60% UR).

It is widely known that under tropical conditions, the daily temperature range is high, even during the winter season (see Figure 1). In this sense, it remains to be investigated the effect of constant low temperature (10 °C) during dry-storage considering the interval between seed harvest and the next crop to provide seeds suitable for field establishment where climate conditions are not controlled. It would be efficient to elucidate whether the temperature of 10 °C could be an immediate factor to overcome dormancy even when it is not associated with moist conditions.

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References

BARRERO, J.M., JACOBSEN, J.V., TALBOT, M.J., WHITE, R.G., SWAIN, S.M., GARVIN, D.F. and GUBLER, F., 2012. Grain dormancy and light quality effects on germination in the model grass Brachypodium distachyon. *The New Phytologist*, vol. 193, no. 2, pp. 376-386. http://dx.doi.org/10.1111/j.1469-8137.2011.03938.x. PMid:22039925.

BASKIN, J.M. and BASKIN, C.C., 2004. A classification system for seed dormancy. *Seed Science Research*, vol. 14, no. 1, pp. 1-16. http://dx.doi.org/10.1079/SSR2003150.

BASKIN, C.C. and BASKIN, J.M., 2014. *Seeds: ecology, biogeography and evolution of dormancy and germination*. 2nd ed. San Diego: Academic Press/Elsevier.

BASKIN, J.M. and BASKIN, C.C., 2021. The great diversity in kinds of seed dormancy: a revision of the Nikolaeva-Baskin classification system for primary seed dormancy. *Seed Science Research*, vol. 31, no. 4, pp. 249-277. http://dx.doi.org/10.1017/S096025852100026X.

BATLLA, D. and BENECH-ARNOLD, R.L., 2015. A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Science Research*, vol. 25, no. 2, pp. 147-158. http://dx.doi.org/10.1017/S0960258514000452.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento – MAPA, 2009. *Regras para análise de sementes*. Brasília: MAPA/ACS. 399 p.

CHEN, D.L., LUO, X.P., YUAN, Z., BAI, M.J. and HU, X.W., 2020. Seed dormancy release of Halenia elliptica in response to stratification temperature, duration and soil moisture content. *BMC Plant Biology*, vol. 20, no. 1, pp. 352. http://dx.doi.org/10.1186/s12870-020-02560-8. PMid:32723291.

CUENA-LOMBRAÑA, A., SANNA, M., PORCEDDU, M. and BACCHETTA, G., 2020. Does storage under gene bank conditions affect seed germination and seedling growth? the case of senecio morisii (asteraceae), a vascular plant exclusive to sardinian water meadows. *Plants*, vol. 9, no. 5, pp. 581. http://dx.doi.org/10.3390/plants9050581. PMid:32370198.

DEBEAUJON, I. and KOORNNEEF, M., 2000. Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology*, vol. 122, no. 2, pp. 415-424. http://dx.doi.org/10.1104/pp.122.2.415. PMid:10677434.

DOLATABADIAN, A. and MODARRES, S.S.A.M., 2008. Effect of the ascorbic acid, pyridoxine and hydrogen peroxide treatments on germination, catalase activity, protein and malondialdehyde content of three oil seeds. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 36, no. 2, pp. 61-66. http://dx.doi.org/10.15835/nbha36270.

EL-MAAROUF-BOUTEAU, H., SAJJAD, Y., BAZIN, J., LANGLADE, N., CRISTESCU, S.M., BALZERGUE, S., BAUDOUIN, E. and BAILLY, C., 2015. Reactive oxygen species, abscisic acid and ethylene interact to regulate sunflower seed germination. *Plant, Cell & Environment*, vol. 38, no. 2, pp. 364-374. http://dx.doi.org/10.1111/pce.12371. PMid:24811898.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA, 2019 [viewed 1 March 2019]. *Guia clima: sistema de monitoramento agrometeorológico de Mato Grosso do Sul* [online]. Available from: https:// https://clima.cpao.embrapa.br/

FERREIRA, D.F., 2019. SISVAR: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria*, vol. 37, no. 4, pp. 529-535. http://dx.doi.org/10.28951/rbb.v37i4.450.

FINCH-SAVAGE, W.E. and FOOTITT, S., 2012. To germinate or not to germinate: a question of dormancy relief not germination stimulation. *Seed Science Research*, vol. 22, no. 4, pp. 243-248. http://dx.doi.org/10.1017/S0960258512000165.

HUANG, Z., FOOTITT, S., TANG, A. and FINCH-SAVAGE, W.E., 2018. Predicted global warming scenarios impact on the mother plant to alter seed dormancy and germination behaviour in *Arabidopsis*. *Plant, Cell & Environment*, vol. 41, no. 1, pp. 187-197. http://dx.doi.org/10.1111/pce.13082. PMid:29044545.

IGLESIAS-FERNÁNDEZ, R., DEL CARMEN RODRÍGUEZ-GACIO, M. and MATILLA, A.J., 2011. Progress in research on dry afterripening. *Seed Science Research*, vol. 21, no. 2, pp. 69-80. http://dx.doi.org/10.1017/S096025851000036X.

KEREILWE, D., EMONGOR, V.E., OAGILE, O. and PHOLE, O., 2020. Nutritional value of safflower whole seed as animal feed in semi-arid southern African conditions. *African Crop Science Journal*, vol. 28, no. s1, pp. 103-115. http://dx.doi.org/10.4314/acsj.v28i1.8S.

KHALID, N., KHAN, R.S., HUSSAIN, M.I., FAROOQ, M., AHMAD, A. and AHMED, I., 2017. A comprehensive characterization of safflower oil for its potential applications as a bioactive food ingredient: a review. *Trends in Food Science & Technology*, vol. 66, pp. 176-186. http://dx.doi.org/10.1016/j.tifs.2017.06.009.

LACHABROUILLI, A.S., RIGAL, K., CORBINEAU, F. and BAILLY, C., 2021. Effects of agroclimatic conditions on sunflower seed dormancy at harvest. *European Journal of Agronomy*, vol. 124, pp. 126209. http://dx.doi.org/10.1016/j.eja.2020.126209.

MA, Z., BYKOVA, N.V. and IGAMBERDIEV, A.U., 2017. Cell signaling mechanisms and metabolic regulation of germination and dormancy in barley seeds. *The Crop Journal*, vol. 5, no. 6, pp. 459-477. http://dx.doi.org/10.1016/j.cj.2017.08.007.

NUR, M., BASKIN, C.C., LU, J.J., TAN, D.Y. and BASKIN, J.M., 2014. A new type of non-deep physiological dormancy: evidence from three annual Asteraceae species in the cold deserts of Central Asia. *Seed Science Research*, vol. 24, no. 4, pp. 301-314. http://dx.doi.org/10.1017/S0960258514000300.

OBA, G.C., GONELI, A.L.D., MASETTO, T.E., HARTMANN FILHO, C.P., MICHELS, K.L.L.S. and ÁVILA, J.P.C., 2019. Artificial drying of safflower seeds at different air temperatures: effect on the physiological potential of freshly harvested and stored seeds. *Journal of Seed Science*, vol. 41, no. 4, pp. 397-406. http://dx.doi.org/10.1590/2317-1545v41n4197808.

OBA, G.C., GONELI, A.L.D., MASETTO, T.E., HARTMANN FILHO, C.P., PATRICIO, V.S. and SARATH, K.L.L., 2017. Dormancy of safflower seeds: effect of storage and cold stratification. *Journal of Seed Science*, vol. 39, no. 4, pp. 433-439. http://dx.doi.org/10.1590/2317-1545v39n4181244.

ÖZÇINAR, A.B., 2021. Safflower (*Carthamus tinctorius* L.): utilisation, genetics and agronomy. *MAS Journal of Applied Sciences*, vol. 6, no. 5, pp. 1130-1136. http://dx.doi.org/10.52520/masjaps.179.

PENFIELD, S. and MACGREGOR, D.R., 2017. Effects of environmental variation during seed production on seed dormancy and germination. *Journal of Experimental Botany*, vol. 68, no. 4, pp. 819-825. http://dx.doi.org/10.1093/jxb/erw436. PMid:27940467.

RANAL, M.A. and SANTANA, D.G.D., 2006. How and why to measure the germination process? *Revista Brasileira de Botânica*, vol. 29, no. 1, pp. 1-11. http://dx.doi.org/10.1590/S0100-84042006000100002.

RODRÍGUEZ, M.V., BODRONE, M.P., CASTELLARI, M.P. and BATLLA, D., 2018. Effect of storage temperature on dormancy release of sunflower (Helianthus annuus) achenes. *Seed Science Research*, vol. 28, no. 2, pp. 101-111. http://dx.doi.org/10.1017/S0960258518000065.

SANO, N. and MARION-POLL, A., 2021. ABA metabolism and homeostasis in seed dormancy and germination. *International Journal of Molecular Sciences*, vol. 22, no. 10, pp. 5069. http://dx.doi.org/10.3390/ijms22105069. PMid:34064729.

SANTOS, H.G., JACOMINE, P.K.T., ANJOS, L.H.C., OLIVEIRA, V.A., LUMBRERAS, J.F., COELHO, M.R., ALMEIDA, J.A., ARAUJO FILHO, J.C., OLIVEIRA, J.B. and CUNHA, T.J.F., 2018. *Sistema brasileiro de classificação de solos*. 5ª ed. Brasília: Embrapa.

SCHÜTZ, W., MILBERG, P. and LAMONT, B.B., 2002. Seed dormancy, after-ripening and light requirements of four annual Asteraceae in south-western Australia. *Annals of Botany*, vol. 90, no. 6, pp. 707-714. http://dx.doi.org/10.1093/aob/mcf250. PMid:12451026.

SOLEYMANI, A., 2019. Safflower (*Carthamus tinctorius* L.) seed vigor tests for the prediction of field emergence. *Industrial Crops and Products*, vol. 131, pp. 378-386. http://dx.doi.org/10.1016/j.indcrop.2017.03.022.

TUAN, P.A., KUMAR, R., REHAL, P.K., TOORA, P.K. and AYELE, B.T., 2018. Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. *Frontiers in Plant Science*, vol. 9, pp. 668. http://dx.doi.org/10.3389/fpls.2018.00668. PMid:29875780.

ZEMOUR, K., LABDELLI, A., ADDA, A., DELLAL, A., TALOU, T. and MERAH, O., 2019. Phenol content and antioxidant and antiaging activity of safflower seed oil (*Carthamus tinctorius* L.). *Cosmetics*, vol. 6, no. 3, pp. 55. http://dx.doi.org/10.3390/cosmetics6030055.