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RESEARCH NOTE

Dormancy of safflower seeds: effect of storage and cold stratification[1]

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ABSTRACT - Safflower seeds exhibit dormancy soon after dispersion from the mother plant, making it impossible to sow newly harvested seeds. Thus, the aim of the present study was to evaluate breaking the dormancy of safflower seeds during storage associated with the use of different periods of cold stratification. Seeds with a moisture content of 7.2% were stored for 0, 60, 120, 180, and 240 days, and for each storage period, they were stratified for 0 (control), 1, 2, 3, 4, 5, 6, and 7 days at 10 °C in the dark. After stratification, seeds were subjected to the germination test and evaluated for percentage of root protrusion, germination speed index, and percentages of first count and final count of normal seedlings. Safflower seeds gradually overcome dormancy during storage for 240 days in a non-controlled environment. Storage associated with periods of cold stratification for 5 to 7 days lead to an increase in germination and are effective in breaking the physiological dormancy of safflower seeds.

Index terms: *Carthamus tinctorius* L., physiological dormancy, germination.

Dormência das sementes de cártamo: efeito do armazenamento e estratificação a frio

RESUMO - As sementes de cártamo apresentam dormência logo após a dispersão da planta-mãe, tornando inviável a semeadura de sementes recém-colhidas. Desse modo, objetivou-se com o presente estudo avaliar a superação da dormência das sementes de cártamo durante o armazenamento associado com a utilização de diferentes períodos de estratificação a frio. As sementes, com teor de água de 7,2%, foram armazenadas por 0, 60, 120, 180 e 240 dias e, a cada período de armazenamento, foram submetidas à estratificação durante 0 (controle), 1, 2, 3, 4, 5, 6 e 7 dias, a 10 °C e em ausência de luz. Após a estratificação, as sementes foram submetidas ao teste de germinação e avaliadas quanto à porcentagem de protrusão radicular, índice de velocidade de germinação e porcentagens de primeira contagem e contagem final de plântulas normais. As sementes de cártamo superam gradualmente a dormência durante o armazenamento por 240 dias em ambiente não controlado. O armazenamento associado aos períodos de estratificação a frio durante 5 a 7 dias promovem o aumento da germinação, sendo eficientes para a superação da dormência fisiológica das sementes de cártamo.

Termos para indexação: *Carthamus tinctorius* L., dormência fisiológica, germinação.

Introduction

Safflower (*Carthamus tinctorius* L.), a species belonging to the Asteraceae family, is a herbaceous annual plant that has high economic value due to its utility as a medicinal plant, ornamental plant, and source of carthamin, a type of dye used in fabric, cosmetics, and food industries. In addition, it is a promising source of raw material for extraction of food-grade vegetable oil and a feed supplement for poultry due to the protein (15-20%) and lipid (20-50%) contents contained in its seeds. The oleic (16-20%) and linoleic (71-75%) acids in the seeds are also important for human consumption (Ekin, 2005; Emongor, 2010; Laursen and Mouri, 2013).

In spite of the interest in cultivation, there are still difficulties

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related to adequate crop management practices, mainly due to the physiological dormancy that newly harvested safflower seeds have (Dolatabadian and Sanavy, 2008; Mayerhofer et al., 2011), a characteristic common to seeds in species belonging to the Asteraceae family (Baskin and Baskin, 2004), such as *Helianthus annuus* L. (Pallavi et al., 2010).

Dormancy is an innate property exhibited by seeds in most higher plants that causes delay in germination, allowing them to germinate only under quite restricted physical environmental conditions (water, temperature, and oxygen), so as to ensure successful plant development and perpetuation of the species (Baskin and Baskin, 2004). Due to the large diversity of climates and habitats, dormancy has evolved in a differentiated manner among species, resulting in a vast gamut of classes and levels (Finch-Savage and Leubner-Metzger, 2006; Graeber et al., 2012).

Induction of physiological dormancy occurs throughout seed maturation, due to an expressive increase in biosynthesis and abscisic acid (ABA) signaling in this phase (Weitbrecht et al., 2011). In after-ripening, the hormonal balance among germination-inhibiting substances (e.g., ABA) and germination-promoting substances (e.g., gibberellic acid - GA) in the internal and external tissues of the seeds will determine maintenance of this state of dormancy (Kumar et al., 2015).

When not intense, physiological dormancy can be broken with weeks or months of storage under dry conditions (Bazin et al., 2011; Basbouss-Serhal et al., 2016) or immediately through seed treatment with GA, among other substances (Liu et al., 2010), or by cold stratification at temperatures from 4 °C to 10 °C for 2 to 4 days (Bolingue et al., 2010; Tuttle et al., 2015; Xu et al., 2016). Some studies have shown that breaking of dormancy of safflower seeds can be attained by soaking them in a 3% hydrogen peroxide solution (Dolatabadian and Sanavy, 2008) or in GA_3 at 0.3%. Storage from four to six months is also recommended for breaking their dormancy (Mayerhofer et al., 2011). However, there are no reports of tests with cold stratification for these seeds.

Cold stratification may also be able to break the dormancy of newly harvested safflower seeds, and with gradual breaking of dormancy during storage, seeds require a shorter period of stratification to express their real germination potential. Thus, the aim of this study was to evaluate breaking of dormancy in safflower seeds during storage associated with the use of different periods of cold stratification, and also to determine ideal periods of stratification for different periods of seed storage.

Materials and Methods

The safflower seeds used were produced on the Agrarian Sciences Experimental Farm of the Universidade Federal da Grande Dourados (UFGD), Dourados, MS, Brazil, in the period from April to September of the 2015 crop season. The experimental farm is at 22º14'S and 54º59'W at a mean altitude of 434 m.

Safflower capitula were harvested near the point of seed physiological maturity, with a moisture content of 25.8% (Franchini et al., 2014), which were later transported to the Agricultural Product Pre-Processing and Storage Laboratory and Oilseed Grain Quality Laboratory at UFGD to conduct the procedures and analyses described below.

After manual shelling of the capitula and selection of healthy seeds, the seeds were dried in an experimental fixed-bed dryer at a temperature of 40 °C and airflow of 0.2 $m^3.s^{-1}.m^{-2}$ until reaching 7.2% moisture content, considered adequate for safe storage of the seeds of this species (Desai, 2004). The initial and final moisture contents were determined by the laboratory oven method at 105±1 °C for 24 hours and in triplicate, according to the Rules for Seed Testing - RST (Brasil, 2009).

After drying, the seeds were placed in a non-hermetic rigid plastic container and stored for 0, 60, 120, 180, and 240 days under non-controlled temperature and relative humidity (RH) conditions and shaded from direct light. The variation in temperature and RH of the storage environment was registered daily with the aid of two thermo hygrometers. In each period, the moisture content of the seeds was determined according to the RST (Brasil, 2009).

For each storage period, the seeds were pre-moistened in mini-chambers (Gerbox) at the temperature of 25 °C and RH of 100% for 24 hours for the purpose of preventing damage during their imbibition in water, which could affect seed germination and vigor. Then, the seeds were disinfected using a 2.5% sodium hypochlorite solution for five minutes, followed by rinsing in running water.

After that, cold stratification of the seeds was performed, arranging them uniformly between the substrate of sheets of paper, consisting of three sheets of paper for germination previously moistened with distilled water at a volume of 2.5 times the weight of the paper. The substrates were kept in a Biochemical Oxygen Demand (B.O.D.) incubation chamber at a temperature of 10 °C in the dark for periods of 0 (control), 1, 2, 3, 4, 5, 6, and 7 days.

The experiment was conducted in a completely randomized design in a double factorial arrangement with five storage periods (0, 60, 120, 180, and 240 days) and eight periods of cold stratification (0, 1, 2, 3, 4, 5, 6, and 7 days) in four replications of 50 seeds.

Immediately after removal of the substrates from the B.O.D. chamber (10 °C) after each period of stratification, the seeds were checked for root protrusion (minimum of 1.0

mm) during the procedure, and the results were presented in percentage. This evaluation was made to determine the maximum period that the seeds can be stratified at 10 °C without exhibiting root protrusion during the treatment for breaking dormancy.

After cold stratification for breaking dormancy, the substrates containing the seeds (already germinated or not) were transferred to a Mangelsdorf germination chamber with a temperature of 25 °C under constant white light, beginning the standard germination test (Brasil, 2009). On the fourteenth day of the germination test, the final percentage of root protrusion (minimum of 1.0 mm) of the seeds was determined. The percentage of normal seedlings in the first count (fourth day) and final count (fourteenth day) were also computed, according to the criteria established by the RST (Brasil, 2009).

At the same time, the germination speed index was determined (Maguire, 1962) through a daily count of the number of normal seedlings generated from the first to the fourteenth day of the germination test.

The data were subjected to analysis of variance using the SISVAR computational program (Ferreira, 2011). The effects of the stratification periods, in each storage period, on root protrusion, germination speed index, and first count and final count of normal seedlings in the germination test were evaluated by means of regression analysis, and the model was selected based on the magnitude of the coefficient of determination, level of significance of the equation, and knowledge of the biological phenomenon under study. For root protrusion during stratification of newly harvested and stored seeds, only the mean results were presented.

Results and Discussion

Over the 240 days of seed storage, there was considerable variation in temperature and relative humidity (Figure 1) since this procedure was conducted in a non-controlled environment. The mean temperature registered was 24.7 °C, the minimum was 18.5 °C, and the maximum was 30.2 °C. For relative humidity, the mean value was 59.3%, the minimum was 37.0%, and the maximum was 82.0%.

Changes in the moisture contents of the seeds occurred over the periods of storage. This response may be attributed to the oscillations in temperature and relative humidity during storage (Figure 1), which led to alterations due to the hygroscopic equilibrium of the seeds (Carvalho and Nakagawa, 2012). At the end of 240 days of storage, the seeds had 8.8% moisture content, a value higher than the initial moisture content (7.2%).

There was interaction between the storage periods and stratification periods on all the dependent variables analyzed ($p<0.01$); the breakdown of the period of stratification within each level of the storage period is shown.

Figures 2, 3A, 3B, and 3C show the results of root protrusion, germination speed index, and first count and final count of normal seedlings, respectively, during the germination test of seeds after different periods of storage and cold stratification.

The storage periods were important for gradual breaking of seed dormancy, shown by the increase in the percentages of root protrusion of the unstratified seeds (0 days of stratification) from 0 (10%) to 180 days of storage (75%); 50% of root protrusion was attained approximately at 134 days (Figure 2). In *Helianthus annuus* L. seeds, physiological dormancy was fully broken after 60 days of storage (Pallavi

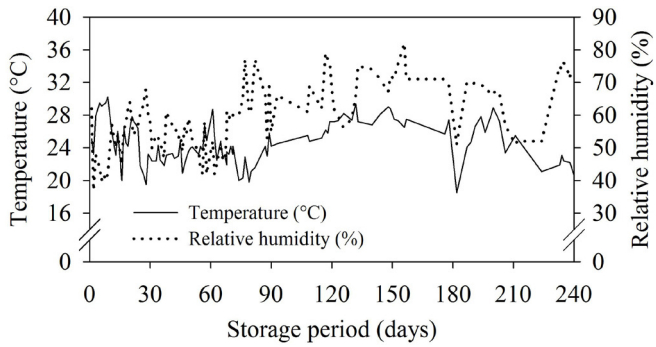
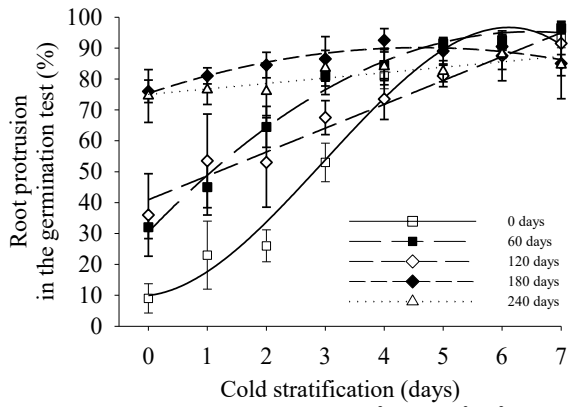


Figure 1. Temperature and relative humidity data of the storage environment of safflower seeds.



0 days = 9.9545 + 2.1647x + 6.2581x^2 -0.702x^3, R^2= 0,98**
60 days = 30.4375 + 19.9792x -1.5387x^2, R^2= 0,99**
120 days = 40.9167 + 7.7202x, R^2= 0,97**
180 days = 75.3333 + 6.4226x -0.6964x^2, R^2= 0,92**
240 days = 75.0417 + 1.756x, R^2= 0,78**

Figure 2. Root protrusion in the germination test of safflower seeds after different periods of storage and cold stratification. The bars represent mean standard deviation.

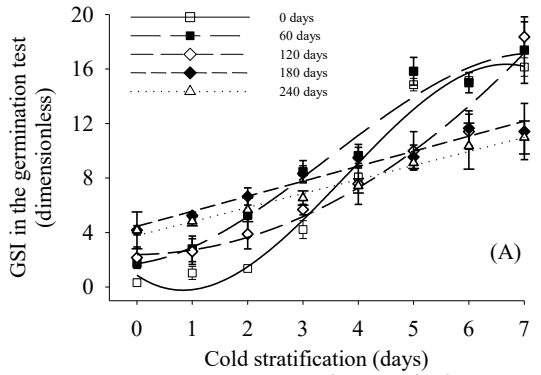
et al., 2010), suggesting that seed dormancy in this species is less intense than in safflower seeds, although they belong to the same family (Asteraceae).

The process by which seeds with physiological dormancy gradually acquire the ability to germinate during dry storage is called after-ripening (Baskin and Baskin, 2004). In many cases, this is related to the degradation of abscisic acid (ABA) and/or increase in sensitivity of the seeds to endogenous gibberellins (GA) (Kumar et al., 2015), as already observed in seeds of *Triticum aestivum* L. (Tuttle et al., 2015). It is possible that breaking seed dormancy by storage in the present study (Figure 2) is also involved with the ABA:GA hormonal balance dynamic in the internal and external tissues of the seeds, revealing the need for deeper studies regarding dormancy in safflower seeds.

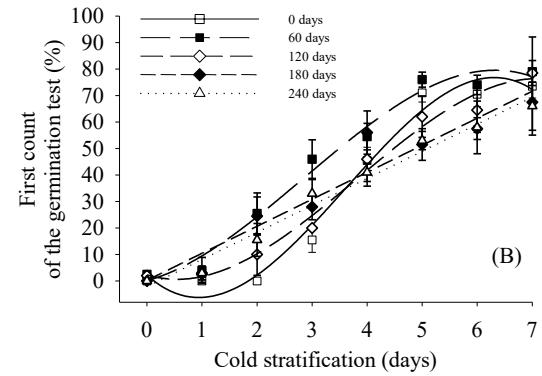
Along with breaking of seed dormancy through storage, the increase in the period of cold stratification also resulted in significant increases in the percentages of root protrusion, above all for the seeds stored from 0 to 120 days (Figure 2). Percentages higher than 90% were obtained from seed stratification for 5 (at 0 and 60 days of storage) and 7 days (at 120 days), indicating that the dormancy of these seeds was satisfactorily broken (Figure 2). Positive results in relation to cold stratification were also reported for *Triticum aestivum* L. seeds; the percentage and speed of seed root protrusion increased with an increase in the period of stratification at 4 °C. These events were associated with an increase in the levels of GA and with expressive reduction in ABA in the seed tissues (Xu et al., 2016).

In relation to the seeds that remained stored for 180 and 240 days, the increase in the period of stratification resulted in less considerable increases in root protrusion. This occurred because most of the seeds of the lot had already broken dormancy by the after-ripening process in these periods, above all at 240 days of storage (Figure 2). This is confirmed through observing that to achieve percentages of root protrusions similar to those obtained by the unstratified seeds at 180 and 240 days of storage (75% on average), the seeds stored for 0, 60, and 120 days needed a stratification period of 3 to 4 days (Figure 2). Similar results were found for *Medicago truncatula* Gaertn. seeds which, after breaking dormancy after six months of storage, did not exhibit a significant response in relation to stratification in temperatures from 10-17 °C for 4 days (Bolingue et al., 2010).

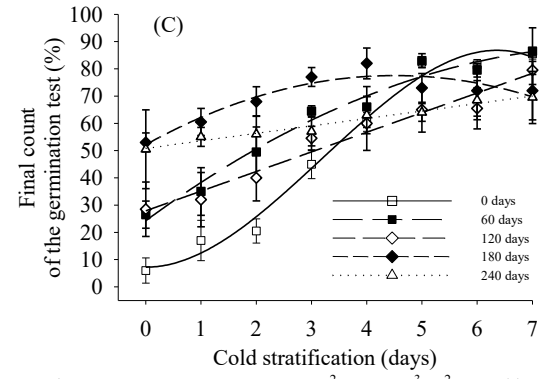
Regardless of the storage period, the increase in the period of stratification resulted in significant increases in the values of the germination speed index (GSI) of the seeds (Figure 3A), and the highest indices were obtained beginning at 5 days of stratification. Corroborating Weitbrecht et al. (2011), the results of the present study showed that,



0 days = 0.8742 -2.7701x + 1.8711x^2 -0.1661x^3, R^2= 0,98**
60 days = 1.7237 + 0.5157x + 0.749x^2 -0.0726x^3, R^2= 0,97**
120 days = 2.3815 + 0.0419x + 0.2959x^2, R^2= 0,97**
180 days = 4.4572 + 1.1018x, R^2= 0,96**
240 days = 3.7776 + 1.0297x, R^2= 0,98**



0 days = 2.3106 -19.0913x + 11.6677x^2 -1.0732x^3, R^2= 0,98**
60 days = 0.1515 + 5.6131x + 4.1872x^2 -0.4886x^3, R^2= 0,98**
120 days = 2.2576 -6.2004x + 6.1802x^2 -0.5404x^3, R^2= 0,99**
180 days = 0.25 + 10.1964x, R^2= 0,93**
240 days = -2.0417 + 10.2083x, R^2= 0,98**



0 days = 7.2652 -0.1925x + 5.9556x^2 -0.6225x^3, R^2= 0,99**
60 days = 24.2708 + 14.8839x -0.8601x^2, R^2= 0,98**
120 days = 27.9167 + 7.2024x, R^2= 0,96**
180 days = 52.1458 + 11.2768x -1.253x^2, R^2= 0,89**
240 days = 50.8333 + 2.744x, R^2= 0,97**

Figure 3. Germination speed index (A) and first count (B) and final count (C) of normal seedlings in the germination test of the safflower seeds after different periods of storage and cold stratification. The bars represent mean standard deviation.

in addition to promoting breaking of dormancy and root protrusion (Figure 2), cold stratification of the safflower seeds made the formation of normal seedlings faster and more uniform in the germination test (Figure 3A).

Just as for root protrusion (Figure 2), the increase in the GSI, through an increase in the period of stratification, was more accentuated for the seeds that were stored from 0 to 120 days in relation to those stored for 180 and 240 days (Figure 3A). Up to 1 day of stratification, the seeds stored for 180 and 240 days exhibited a GSI higher than that observed for seeds stored in the previous periods, indicating lower intensity of dormancy for these seeds. Nevertheless, beginning at 5 days of stratification, the GSI values of the seeds stored for 180 and 240 days were considerably lower than those obtained by the seeds with up to 60 days of storage (Figure 3A). These results may be related to loss of seed vigor due to natural deterioration caused by aging, which is an inevitable and irreversible process (Marcos-Filho, 2015).

For all storage periods, the increase in the stratification period resulted in significant increases in the percentages of first count of normal seedlings (performed on the fourth day of the germination test), and higher values were observed beginning at 5 days of stratification (Figure 3B). These results are related to the increase in the GSI of the seeds with the increase in the stratification period (Figure 3A). It is not yet clearly known how cold stratification acts on breaking seed dormancy, nor what mechanism detects the signal of low temperature during soaking. However, it is known that some inhibitor genes of GA biosynthesis lose their activity after stratification (Graeber et al., 2012). In addition to facilitating resumption of production of bioactive GA and repressing ABA biosynthesis by seeds, cold stratification is apparently able to degrade pre-existing ABA and increase seed sensitivity to endogenous GA, favoring breaking of dormancy and germination (Finch-Savage and Leubner-Metzger, 2006). For safflower seeds, the positive effects of cold stratification can be seen by the results of root protrusion, GSI, and first count of normal seedlings (Figures 2, 3A, and 3B).

Even with 2 days of stratification, the newly harvested seeds (0 days of storage) exhibited a percentage of first count of only 2% and were considerably lower than the values observed for seeds stored in the other periods (18% on average). These results are linked to greater intensity of dormancy for these seeds (Figure 3B). Nevertheless, from 5 to 7 days of stratification, higher percentages of first count were observed for the seeds stored from 0 to 60 days (73% on average), followed by values achieved at 120 days of storage (69% on average), and closing with a mean percentage of 60% for the seeds stored for 180 and 240 days (Figure 3B). The association of these results of first count (Figure 3B) with those of the germination speed index (Figure 3A) confirms that safflower seeds exhibited a gradual loss of vigor during storage, and this was more accentuated after 120 days.

With the increase in the period of seed stratification, increases were also found in the percentages of normal seedlings in the final count of the germination test. This effect was more pronounced for the seeds that remained in storage from 0 to 60 days, which exhibited percentages from 83% to 86% beginning at stratification for 6 days. At 120, 180, and 240 days of storage, maximum percentages were detected after stratification of seeds for 7 (78%), 4 (77%), and 7 (70%) days, respectively (Figure 3B). These results reinforce the applicability of cold stratification for breaking dormancy and stimulation of germination of newly harvested or stored safflower seeds.

Moreover, as shown in Figure 3C, the passage of the storage periods led to considerable increases in the percentages of normal seedlings in the final count of the unstratified seeds (0 days of stratification), with a mean increase of 7% every 30 days of storage up to 180 days, stabilizing from that period on. To achieve percentages similar to those obtained by seeds stored for 60 to 120 days and not stratified (26% on average), the newly harvested seeds needed a minimum period of 2 days of stratification, while 4 days of stratification were needed to go beyond the percentage found for the unstratified seeds that remained in storage for 180 and 240 days (52% on average) (Figure 3C).

According to Tuttle et al. (2015), the responses of the seeds to stratification vary according to the intensity of dormancy, such that seeds with a high degree of dormancy require longer periods of stratification and vice-versa. The results of final count of normal seedlings (Figure 3A), associated with those of root protrusion (Figure 2), confirm the hypothesis initially presented that as the time of storage passes, safflower seeds require less time of cold stratification to break dormancy and germinate.

The kinetics of release from dormancy by the periods of after-ripening is considerably affected by environmental air temperature and especially by the moisture content of the seeds during storage (Bazin et al., 2011; Basbouss-Serhal et al., 2016). Since safflower seeds were not stored under controlled conditions (Figure 1), it is not possible to determine the real effect of temperature on breaking dormancy. However, the seed moisture content oscillated from 7.2% to 8.8% over the period evaluated, which is within the range of moisture content (5.7% to 15.0%) considered optimal for the occurrence of after-ripening (Weitbrecht et al., 2011; Graeber et al., 2012).

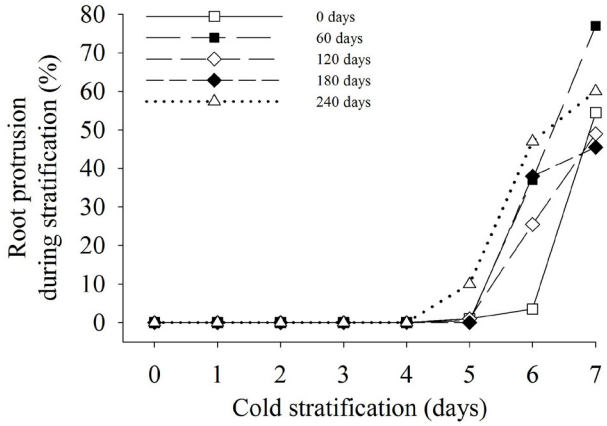


Figure 4. Occurrence of root protrusion in safflower seeds (newly harvested and stored up to 240 days) for different periods of stratification at 10 °C.

In Figure 4 are the mean results of root protrusion when cold stratification (10 °C) was being carried out. Conducting the method of breaking dormancy for 6 and 7 days led to high percentages of root protrusion of the newly harvested or stored seeds (44% on average). These results indicate that stratification periods longer than 5 days promote breaking of dormancy and trigger the germination process of safflower seeds even at low temperature (10 °C). Thus, the use of stratification in safflower seeds during 6 and 7 days may be inadequate in cases of substrate transfer, due to the possibility of mechanical damage to tissues of the primary root.

Conclusions

Safflower seeds gradually break dormancy over a storage period of 240 days in an environment that is not controlled.

Storage along with periods of cold stratification for 5 to 7 days leads to an increase in germination and is effective in breaking physiological dormancy of safflower seeds.

Acknowledgments

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