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ARTICLE

# Influence of fruit color on the oil quality and seed germination of *Idesia polycarpa* Maxim.

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**ABSTRACT:** The aim of this study was to investigate the effects of *Idesia polycarpa* fruit blackening on fruit and seed morphological characteristics, oil content, fatty acid content, seed germination rate and physiological properties. Germination tests were conducted under dry and wet storage at 5 °C for 0 d, 20 d, 40 d, 60 d and 80 d. The fruit mass, the 100-grain weight, the moisture content, the oil content of seeds and oleic acid in unsaturated fatty acids of black fruit are significantly lower than red fruit (P < 0.05). The germination rate of black fruit seeds was higher than red under wet storage and the malondialdehyde content of black fruits decreased with increasing storage time. Our results demonstrated that black fruits of *I. polycarpa* should not be discarded indiscriminately and that the color of the fruits can be chosen according to the purpose of use. Black fruits are picked for species propagation, while red fruits are mainly harvested for oil extraction.

**Index terms:** black fruit, fatty acids, germination rate, morphological traits, oil content.

**RESUMO:** O objetivo do trabalho foi investigar os efeitos do escurecimento dos frutos de *Idesia polycarpa* nas características morfológicas dos frutos e sementes, teor de óleo, teor de ácidos graxos, taxa de germinação das sementes e propriedades fisiológicas. Os testes de germinação foram realizados sob armazenamento seco e úmido a 5 ° C por 0 d, 20 d, 40 d, 60 d e 80 d. A massa dos frutos, o peso de 100 sementes, o teor de umidade, o teor de óleo das sementes e ácido oleico nos ácidos graxos insaturados dos frutos pretos são significativamente inferiores aos dos frutos vermelhos (P < 0,05). A taxa de germinação das sementes de frutos pretos foi maior que dos frutos vermelhos sob armazenamento úmido e o teor de malonaldeído dos frutos pretos diminuiu com o aumento do tempo de armazenamento. Nossos resultados demonstraram que os frutos pretos de *I. polycarpa* não devem ser descartados indiscriminadamente e que a cor dos frutos pode ser escolhida de acordo com a finalidade de uso. Os frutos pretos são colhidos para propagação de espécies, enquanto os frutos vermelhos são colhidos principalmente para extração de óleo.

**Termos de indexação:** frutos pretos, ácidos graxos, taxa de germinação, características morfológicas, teor de óleo.

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## INTRODUCTION

*Idesia polycarpa* Maxim., a member of the *Idesia* genus in the Salicaceae family, is a deciduous tree species native to Asia and mainly distributed in Japan, Korea, and China (Kim et al., 2005; Zhang et al., 2018; Li et al., 2019). In China, it is distributed primarily in the Yangtze River basin, north China, and several provinces south of the northwest area. The oil content of fruit pulp and seed was 8.38 %～48.35 % and 12.6 %～28.17 %, respectively. The annual oil output per hectare is about 2.25~3.75 t (Yang et al., 2009), which has the reputation of “Tree Oil Depot.” Furthermore, the oil from its fruit contains a high quantity of unsaturated fatty acids, especially linoleic acid, with up to 81.74% (Dai, 2014), an essential fatty acid that can only be obtained from the diet or dietary supplements for humans (Warude et al., 2006; Fan et al., 2019). The oil of *I. polycarpa* has high nutritional value and can be used as high-quality edible oil (Guo et al., 2012). It is gaining increasing attention due to the extract of fruits can be used in medicinal applications, such as against obesity and preventing hyperlipidemia and atherosclerosis (Dai et al., 2011; Hwang et al., 2012; Lee et al., 2013), antioxidant and anti-skin-aging (Ye et al., 2014), and a feedstock for biodiesel in the industry (Yang et al., 2009).

As a valuable oilseed plant, the yield and quality of the fruit are very critical for the development of its industry. Moreover, large-scale seed breeding is an essential means of reproduction in *I. polycarpa*. However, during the ripening process, especially in October, one-third of the fruits may become “abnormal” black. If the black fruit is not studied in characteristics and discarded randomly, it will cause certain economic losses to the development of the *I. polycarpa* industry. Therefore, exploring whether the transition from red to black fruits is beneficial or unfavorable is significant.

Changes in fruit color are not necessarily negative. Variations in the color of *Elaeis guineensis* do not affect the germination capacity and the growth of seedlings (Norsazwan et al., 2022). Changes in *Allophylus edulis* seed color can be indicative of harvest time (Kaiser et al., 2016). Furthermore, several studies have shown that there are many differences in the chemical composition of essential oils (Messaoud et al., 2011), the compositions and contents of fatty acids (Messaoud and Boussaid, 2011), antioxidant activity (He et al., 2010; Liu et al., 2020), germination properties, and seed longevity (Bhatt et al., 2017a, 2017b; Bhatt and Santo, 2018) between different fruit colors.

The germination ability of seeds was affected by the colour of the fruit, seed moisture content temperature, and storage time of seeds (Sowmya et al., 2012; Pereira et al., 2013; Mira et al., 2015; Silva et al., 2017; Bhatt and Santo, 2018). The storage process of seeds is a natural aging process. A series of changes have taken place in the seeds, which were reflected in the changes of physiological and biochemical indexes, so the physiological and biochemical reactions of seeds can reflect the inner quality of seeds more accurately (Zhang et al., 2019). Oleaginous seeds are more susceptible to lipid peroxidation, producing higher levels of reactive free radicals that cause damage to membranes and proteins and lead to seed deterioration (Moncaleano-Escandon et al., 2013; Jose et al., 2018; Nyamayevu et al., 2018). MDA is one of the most critical products of membrane peroxidation. Its accumulation would affect the seed’s vigor and reduce the seed germination percentage (Nezamdoost et al., 2009; Li et al., 2016; Ebone et al., 2019).

The effects of different storage conditions and storage time on the germination of seeds of *I. polycarpa* have been studied previously (Wang et al., 2015). It was necessary to determine whether the fruit’s colors affect the seed germination characteristics under different conditions.

Therefore, this study aimed to assess the differences in oil content and fatty acid content between black and red fruits, as well as the biochemical and germination rates of seeds stored for a time under different environmental conditions, to provide a reference for determining the appropriate harvesting period, the color selection at harvest, and safe storage time and conditions for seeds.

## MATERIAL AND METHODS

The fruits of both colors (red and black) (Figure 1) were collected from robust 5-year-old trees of *I. polycarpa* on October 2018 at the experimental forestry station (113°38’E, 34°47’N), Zhengzhou, Henan province, China.

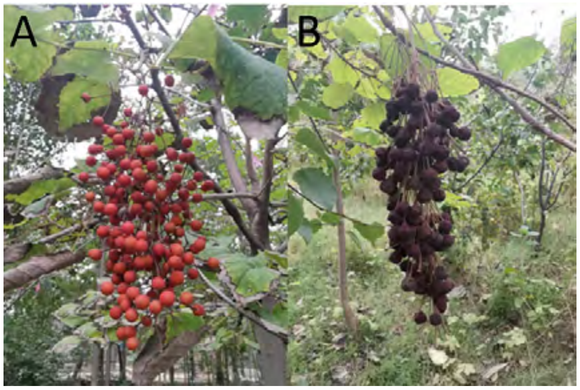


Figure 1. Variation in fruit colors of *I. polycarpa*: (A) Red, (B) black.

Immediately after collection, the measurement of fruit morphological characteristics includes fruit mass, 100-grain mass, number of seeds per fruit, fruit water content, and the length and width of fruit and seeds. All indicators were determined with three replications. Then, fruits were air-dried in laboratory shade and deposited at room temperature until the experiments started.

The Soxhlet extraction method was used for preparing oils from the seed and pulp of *I. polycarpa* (Kaushik and Bhardwaj, 2013). First, carefully separate the pulp and seeds after the fruits are dried in the shade, then dry them in the oven at 85 °C until the weight does not change. Subsequently, the samples were put into the pulverizer to crush and pass the 50-mesh sieve. For organic solvent extraction, 5g of powder was wrapped with filter paper, added in 250 ml of acetone, and placed in the Soxhlet apparatus at 70 °C for 10 h. The obtained essential oils were dried over anhydrous sodium carbonate and stored at 4 °C for further analysis. The residual powder was dried, weighed, and the percentage of mass change and dry weight were used to calculate the oil yield. All samples were carried out with three replications.

The gas chromatography-mass spectrometry (GC-MS) method was adopted to analyze the fatty acid components in the seed and pulp of *I. polycarpa* and was determined quantitatively by area normalization. The methyl esterification of fatty acids was based on the method used by Li et al. (2019) and has been modified. Put each oil sample (100 mg) into a test tube and add 2 mL of n-hexane with 2 ml of 0.4 mol.$L^{-1}$ KOH-methanol solution to dissolve, then place in a 40 °C constant temperature water bath for 30 minutes. The mixture was extracted with 5 ml saturated sodium chloride and kept at room temperature until the stratification was apparent, and then the supernatant was collected for the chromatographic analysis sample.

GC/MS analysis was performed on an Agilent 7890A-5975C GC/MS system equipped with an Agilent MSD detector and an HP-5fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a constant 1 mL.$min^{-1}$ flow rate. The mass detector has an ionization energy of 70 eV and a mass range of m/z 20-550. Samples of essential oils were diluted in hexane to a concentration of 40 µL.$mL^{-1}$, and 1 µL of the solution was injected in the split ratio (1:50). Keep the oven temperature at 60 °C for 1 minute. then gradually increased to 80 °C at 2 °C .$min^{-1}$, to 220 °C at 5 °C.$min^{-1}$, and then held at 220 °C for 5 min. The injector and transfer line temperatures were 230 °C and 250 °C, respectively.

The seeds were soaked in 2% detergent water for 4 hours to remove the wax from the seed coat and sterilized with 3% potassium permanganate solution for 30 min, then rinsed off with clear water. To assess the effect of water on the seed germination of red and black-colored fruits, dry storage and wet storage were set up. For dry storage, we were to put seeds in dry sterile glass bottles, affix labels, and seal them, while for wet storage, we Added the appropriate amount of sterile water to the bottle. Afterward, the treated seeds were stored in incubators for 0, 20, 40, 60, and 80 days during the dark at 5 °C in two storage methods before commencing the germination test.

The seeds were placed on germination beds prepared with 9 cm diameter Petri dishes and sterilized filter paper. The temperature regimes of the incubators were set (a day divided into two cycles) at 15 °C and 25 °C. In addition, the germination bed needs to be filled with the appropriate amount of sterile water as necessary. Three replications were set for the germination test, with 100 seeds per replication. The standard of seed germination was considered to be the emergence of the radicle. The germination percentage was observed and counted every 5 days until the 20th day, when there was no seed germination.

One gram of stored seeds was removed, ground into a powder with liquid nitrogen, and then stored at -80 $^{o}$C. Malondialdehyde (MDA) content was measured by the thiobarbituric acid reaction to indicate lipid peroxidation levels, according to Sudhakar et al. (2001). Soluble protein content was determined by the Komas Brilliant Blue G-250 method, as described by Li (2005). Soluble sugar content was detected by anthrone spectrophotometric methods (Li et al., 2005). All tests were repeated three times.

All data were sorted out by EXCELL (Version 2010; Microsoft Corp) and SPSS (Version 25.0; IBM Corp) software. An independent-samples T-test was conducted to assess the differences in fruit mass, the number of seeds per fruit, the length and width of fruit and seeds, moisture content, and the oil content between different fruit colors. The differences in germination percentage of seeds between different treatments were analyzed by one-way analysis of variance (ANOVA). Then, Duncan's new multiple range test (MRT) determined the significance of differences.

## RESULTS AND DISCUSSION

There was no significant difference in fruit length, fruit width, or number of seeds per fruit, but the weight of a single red fruit, the weight of 100 fruits, and the moisture content are significantly higher than those of black fruits ($P < 0.05$) (Table 1). This is consistent with the appearance of plump red fruits and wrinkled black fruits. Studies have shown that the color change of fruits is closely related to water content (Özkan et al., 2003), mainly because water content affects sugar metabolism (Chen et al., 2020), and sugar promotes the degradation of anthocyanin that determines the color of fruits (Cao et al., 2009; Liao et al., 2016). However, the 100-seed weight and the moisture content of seeds between the two fruit colors showed no significant difference, indicating that changes in fruit color did not affect the quality of the seeds.

GC-MS detected the fatty acid composition of the oil in the pulp and seed of *I.polycarpa*. The six chemical components were mainly analyzed as oleic acid (C18:1), linoleic acid (C18:2), stearic acid (C18:0), palmitoleic acid (C16:1), palmitic acid (C16:0), myristic acid (C14:0). The relative content of each component in the pulp and seed was calculated by peak area normalization method, as shown in Table 2. The result showed that the acids in the pulp and seed of *I. polycarpa* were mainly unsaturated fatty acids, accounting for 77.82% - 87.94% of the total composition. The content of saturated fatty acids was 10.76% - 20.28% of the total composition. The content of oleic acid in seeds and

Table 1. Characteristics of red and black color fruits in *I. polycarpa.*

| Trait | Red fruits | Black fruits | N | F | *p* |
|---|---|---|---|---|---|
| Fruit mass (g) | 0.24±0.04 | 0.22±0.03 | 90 | 0.74 | - |
| 100-grain weight of fruit(g) | 19.81±0.72 | 18.57±2.25 | 100 | 1.96 | 0.01 |
| 100-grain weight of seeds (g) | 0.31±0.07 | 0.30±0.06 | 100 | 0.31 | 0.49 |
| Number of seeds per fruit | 40.07±7.61 | 39.69±6.00 | 90 | 4.54 | 0.71 |
| Fruit length (mm) | 9.68±0.46 | 9.80±0.56 | 90 | 2.19 | 0.12 |
| Fruit width (mm) | 9.68±0.66 | 9.68±0.71 | 90 | 1.32 | 0.95 |
| The moisture content of fruit | 51.35±1.22 | 36.05±1.58 | 100 | 0.13 | 0.001 |
| The moisture content of seeds | 8.00±0.002 | 7.5±0.0001 | 20 | 0.4 | 0.16 |
| The oil content of pulp | 31.80±0.003 | 29.2±0.01 | 100 | 2.046 | 0.09 |
| The oil content of seed | 35.20±0.01 | 22.61±0.002 | 100 | 10.78 | - |

pulp was the highest, and the range of seeds was higher than that in pulp, with a significant difference, but there was no difference in fruit color. The fatty acid content of seeds showed a significant difference at C18:1, and the red fruit was 2.47% higher than the black fruit. The content of unsaturated fatty acids in red fruit seeds was also higher than that in black fruit, and there was a significant difference. As for the quality of seed oil, red fruit is better than black fruit. The data also exhibited no difference in the content of fatty acids in the pulp of *I. polycarpa* with different fruit colors.

The soluble sugar content in dry storage was significantly higher than that of wet storage, regardless of black or red fruit (Figure 2). This result showed a higher consumption of soluble sugar in seeds during the wet storage. As mentioned by Li and Min (2020), the respiration rate of the seeds of *Phoebe journey* could be improved in low stratification for some days, more energy needs to be consumed, and the soluble sugar content gradually decomposes, resulting in the decreased soluble sugar content. Under the dry storage condition, there was no significant difference ($P > 0.05$) in the soluble sugar content between red and black fruit except for 0 d. The soluble sugar content of seeds in black fruit did not change significantly with the increase in storage time, while red fruit showed a downward trend. Previous data demonstrated that storage reserves (soluble sugar, soluble protein) were used as the indicators of dormancy break in seeds and were involved in seed germination (Eichholtz et al., 1983; Bao and Zhang, 2011). The content of soluble sugar in seeds is not only related to the rate of starch degradation and fat conversion into sugars but also directly related to the rate of seed respiration consumption, reflecting the dynamic changes of material accumulation and consumption in seeds (Yang et al., 2006). In the present study, the accumulation and consumption of black fruit material was more stable.

During the dry storage, the soluble protein of seeds in the black fruits and red fruits showed a tendency of "up-down" and displayed significant differences in storage for 0, 60, and 80 days ($P < 0.05$) (Figure 3). The reduction in the soluble protein content may be due to the deterioration of seeds as storage time and the synthesis or activation of a large number of proteolytic enzymes during the deterioration process (Bewley et al., 2013).

During wet storage, the soluble protein in red fruit seeds showed a trend of first increasing and then decreasing. Research has shown that more than 50% of the soluble protein content in seeds is composed of enzyme proteins,

Table 2. The fatty acid compositions in the seed and pulp of *I. polycarpa* in different fruit colors.

| Fatty acid NO. | Chemical composition | Molecular formula | Percentage(%) | | | |
|---|---|---|---|---|---|---|
| | | | Seeds of red fruit | Seeds of black fruit | The flesh of red fruit | The flesh of black fruit |
| 1 | C16:1 | $C_{16}H_{30}O_2$ | 0.09±0.08b | 0.26±0.06b | 3.92±0.19a | 4.10±0.33a |
| 2 | C18:1 | $C_{18}H_{34}O_2$ | 8.71±0.54a | 6.24±1.09b | 5.18±0.34b | 6.33±0.69b |
| 3 | C18:2 | $C_{18}H_{32}O_2$ | 79.15±0.68a | 78.97±1.64a | 68.72±1.06b | 67.94±0.64b |
| | Unsaturated fatty acid | | 87.94±1.00a | 85.47±1.09b | 77.82±0.87c | 78.36±0.65c |
| 4 | C14:0 | $C_{14}H_{28}O_2$ | 0.17±0.06a | 0.19±0.07a | 0.23±0.06a | 0.23±0.04a |
| 5 | C16:0 | $C_{16}H_{32}O_2$ | 9.89±0.94b | 10.97±1.20b | 19.25±0.82a | 18.91±0.66a |
| 6 | C18:0 | $C_{18}H_{34}O_2$ | 0.70±0.04a | 0.80±0.12a | 0.79±0.12a | 0.73±0.02a |
| | Saturated fatty acid | | 10.76±0.91b | 11.96±1.21b | 20.28±0.79a | 19.87±0.70a |
| 7 | Others | ------ | 1.3±0.27c | 2.57±0.28a | 1.87±0.15b | 1.77±0.04b |

Note: Others including 9-Hydroxynonanoic acid (C9H18O2), 9,12-Hetpadecadienoic acid(C17H30O2), 12-Hydroxyoleic acid(C18H35O3), dodecanoic acid (C12H24O2), heptadecanoic acid (C17H32O2), arachidic acid (C20H40O2). All data represent the Mean (n = 3) ± SD. The different alphabet letters in the same line indicated that there are significant differences ($P < 0.05$).

which play a certain catalytic role in the process of seed germination and promote seed germination (Wang et al. 2013). During low-temperature storage, the increase of protease leads to the degradation of specific axis proteins, suggesting the mobilization of storage reserves before seed germination (Eichholtz et al., 1983). The soluble protein content of seeds in black fruit decreased at 80 d, but there was very little difference compared to the protein content stored for 0 d, 20 d, and 40 d.

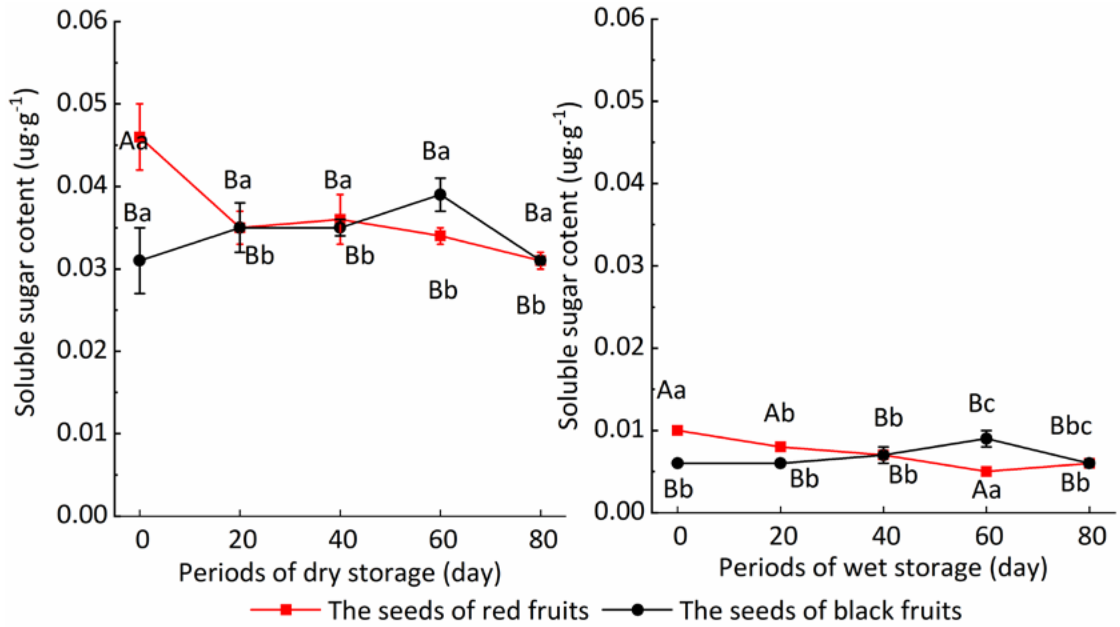


Figure 2. The changes in soluble sugar content of seeds in red and black fruit after different storage periods under dry and wet storage conditions. A-B represents the difference between black and red fruits under the same storage conditions and the same storage days (P < 0.05), and a-c represents the difference between different storage days under the same storage conditions (P < 0.05).

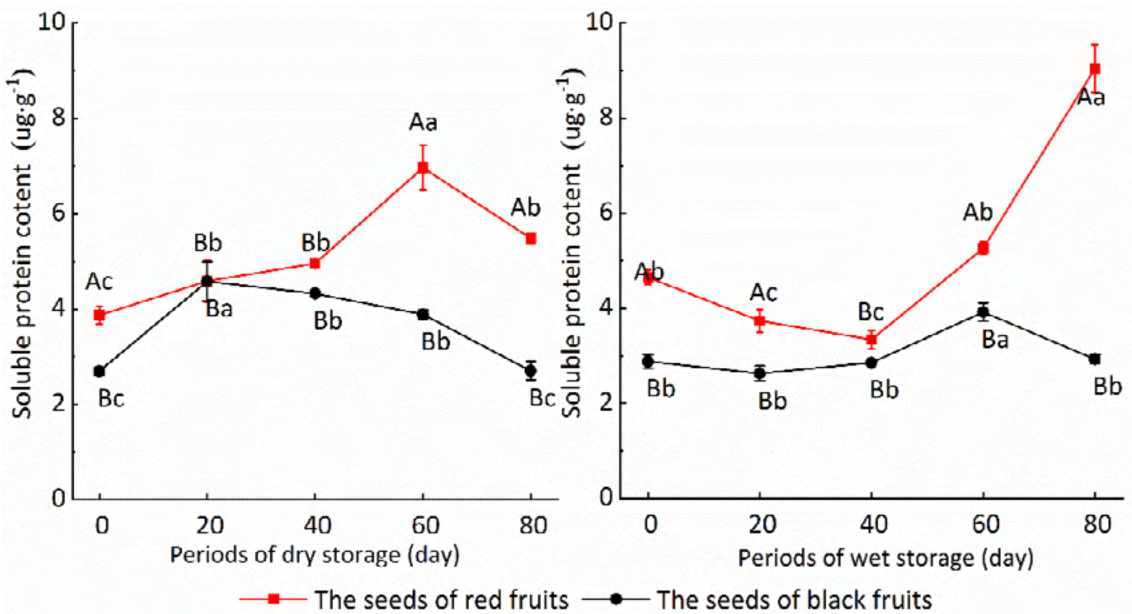


Figure 3. The changes in soluble protein content of seeds in red and black morph after different storage periods under dry and wet storage conditions.

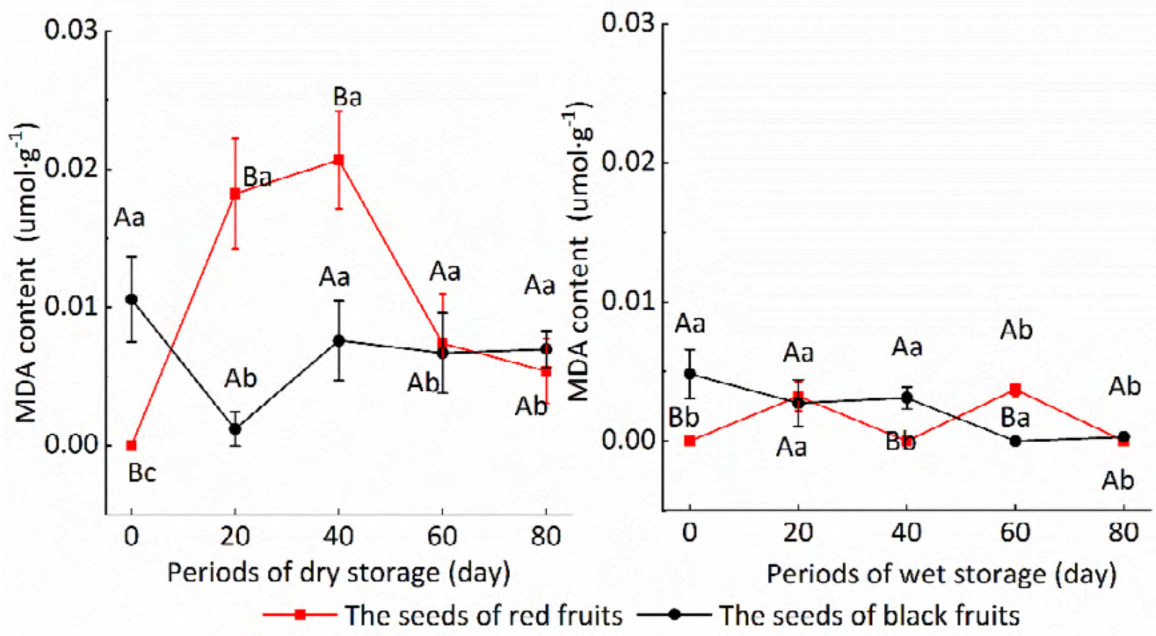


Figure 4. The changes in MDA content of seeds in red and black fruit after different storage periods under dry and wet storage conditions.

The variation range of MDA content in dry storage was significantly greater than that in wet storage, consistent with the research results of *Aesculus hippocastanum* seeds (Obroucheva et al., 2016), indicating that wet storage had good protection for the plasma membrane permeability of *I. polycarpa* seeds. Under the condition of wet storage, the content of MDA did not accumulate with the extension of storage time but decreased (Figure 4). Therefore, it infers that black fruits may prevent the occurrence of lipid peroxidation in seeds by operating cell repair mechanisms. Einali and Valizadeh (2017) verified that seeds of *Pistacia vera* stored in moist and cold (5 °C) conditions could prevent lipid peroxidation by increasing the activity of antioxidant enzymes, such as CAT and APX, thus enhancing the potential of seed germination.

The germination rate of seeds in wet storage is significantly higher than in dry storage, regardless of red or black fruit (Figure 5). This is consistent with the research results of Wang et al. (2015), which demonstrated that *I. polycarpa* seeds have dormancy characteristics and require wet-cold storage to release dormancy and promote germination. The germination rate of seeds in red fruit was significantly higher than that of black fruit in 0, 20, and 40 days of dry storage ($P < 0.05$). With the increase in storage time, the germination rate of seeds decreased significantly ($P < 0.05$). This study indicated that the longer the dry treatment during storage, the greater the impact on seed viability. Research has shown that drying reduces seed antioxidant enzyme activity and increases membrane lipid peroxidation, thereby reducing seed germination (Feng et al., 2017). For example, *Coffea arabica* and *Carex* seeds have lower germination rates under dry-cold conditions (Budelsky and Galatowitsch, 1999; Rosa et al., 2011). The germination rate of black fruit seeds wet stored at 5 °C for 40 and 60 days was higher than that of red fruit, and the difference was significant. Different results were reported by Nyamayevu and Mashingaidze (2013), who showed the highest germination at the yellow fruit color stage, and the seeds begin to degenerate when they turn brown and black.

This is extremely important to determine the appropriate harvest time based on different usage goals, avoiding the waste of seeds or fruits (Ozdemir and Topuz, 2004; Gonçalves et al., 2018). For example, Camu Camu (*Myrciaria dubia*) fruit is physiologically mature from 88 to 116 days after anthesis (DAA) and showing overripe behavior at 116 DDA. When the fruit is at 88 DAA, its bioactive compound accumulation (AOX) reaches its highest, making it appropriate for the nutritional market, while 88-102DAA fruit is more suitable for the juice industry and the fresh market due to the

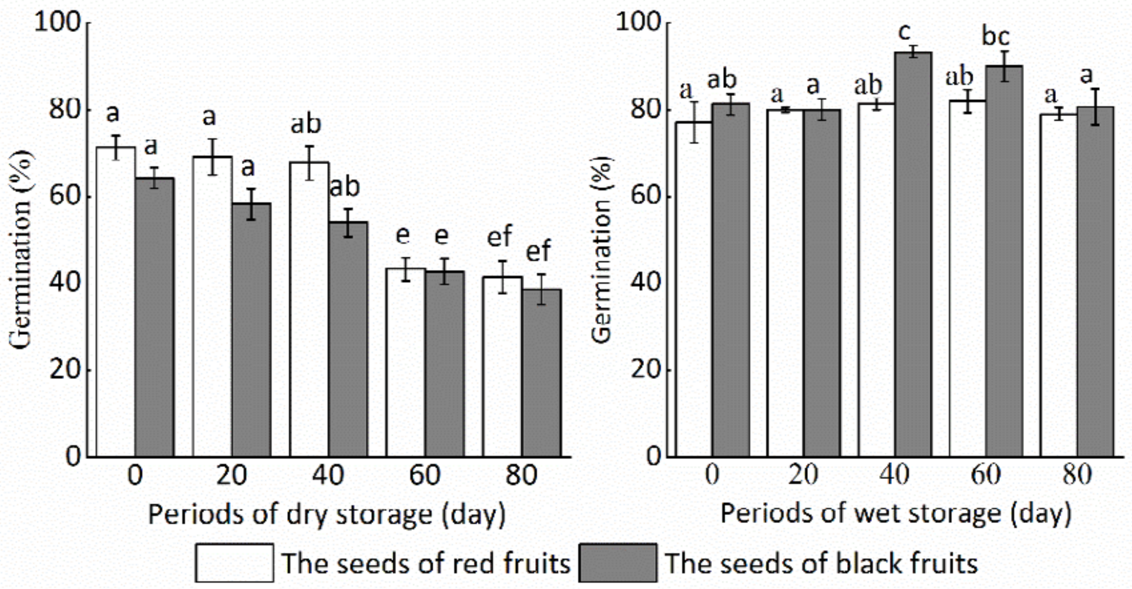


Figure 5. Effect of different storage conditions and storage duration on germination percentage of seeds collected from red and black fruit of *I. polycarpa.* Each bar represents means ± SD. The different letters showed significant differences (P < 0.05).

fruit contains higher soluble solids, sugar, and lower starch (Neves et al., 2017). For the potential use of *Jatropha curcas* as a raw material for biodiesel, the yellow capsule stage of fruit was the optimal harvesting stage, during which seeds not only exhibit maximum germination efficiency but also have higher oil content (Ahmad and Sultan, 2015). In this study, black fruits have low seed oil content but a high germination rate, while red fruits have higher oil content, which means we can choose different fruit colors based on the purpose of use.

## CONCLUSIONS

There are differences in the morphology of fruits of different colors, with red fruits having significantly higher moisture and oil content than black fruits.

Under wet storage conditions, the germination rate of black fruit seeds is higher than that of red fruit black fruits, and the content of MDA and soluble protein does not change with increasing storage time, indicating that black fruit seeds are more stable and less sensitive to membrane lipid peroxidation.

Fruits of different colors can be collected according to different commercial purpose. Black fruits are picked for species propagation, while red fruits are mainly harvested for oil extraction.

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