A test on pollen viability and fruits forming ability of some melon varieties by using storage treatment

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Abstract. Climate change decreases biodiversity through hybridization. The warming climate affects the spread of hybridization. The production of melon fruit in Indonesia fluctuated from time to time from the seeds' lousy quality. An effort to improve melon seeds' quality was needed using hybridization. However, the low availability of high viability pollen became one of the problems in the hybridization process. This research aimed to study the pollen's viability and produce two varieties of melons using some storage treatments. The research used Randomized Complete Block Design with split-plot design by using two treatment factors, the main plot and storage treatment were used as the subplot. The combination of varieties and pollen storage was significant for the viability of the pollen. Golden Melon Variety A and B had different pollen viability. Using a two-day storage treatment at low temperatures, Golden Melon Variety B showed the highest pollen viability. Storage treatment took effect on the percentage of the number and the weight of the fruits produced. The 0-day storage treatment at low temperature showed the optimum yield. Pollen viability was positively correlated with fruit weight.

1. Introduction
Melon (Cucumis melo L.) is included in the Cucurbitaceae family, including important economic vegetables, such as cucumber, watermelon, and Siamese pumpkin, and yellow pumpkin [1]. Indonesian production of melon in 2017 was only 92,434 tons and it was lower than its number in 2016, which was 117,344 tons [2]. One of the causes of the reduction in low-quality seeds [3]. This is because climate change decreases biodiversity through hybridization. The warming climate affects the spread of hybridization. Hybrid seeds play a very important role in improving the quality of plant production, but some problems obstruct the production of hybrid seeds. That is why other means of enhancing the production of high-quality melons are needed; one of them is synthetic pollination through mating or smearing the pollen of male pants into the bud head of female plants [4]. It has to be done at the right time to get optimum fruit setting and good yield. However, the asynchrony in the flowering process from male melon plants to females often occurs.

The availability of pollen has become one of the solutions to solve the asynchrony in the flowering process. The availability of the pollen guarantees the collection of germplasm, improves the efficiency of breeder land usage because we do not need to plant male elder plants, and pertains the high viability pollen into a specific period. One way to maintain the availability of the pollen is by storing the pollen. Pollen viability is very interesting for breeders and researchers. The breeding project aims to keep the pollen for a long time. High-quality genetic material could be saved for future usage or biotechnology
and the application of gene editing can create a fast and effective method in determining pollen viability [5]. However, the viability and the germination of the pollen could change during the storage period. It can cause a loss in production [6]. Pollen water content and storage temperature play an important role in maintaining pollen viability during storage [7]. The viability and the germination of the pollen are also affected by environmental conditions, such as temperature [8]. Flowering plants in their reproduction period are responsive toward temperature [9]. Maximum pollen germination is at 15 °C and the minimum is at 25 °C [10]. Reduction of the temperature from 32°C to 10°C could improve the pollen viability [11]. High temperature and water pressure cause sterility in five genotypes of rice pollen [12]. Other than that, there is no research about pollen quality improvement by storing melon pollen at low temperatures yet. This research evaluates how the pollens of some melon varieties respond both qualitatively and quantitatively to different storage treatments, which are at room temperature and low temperature during various storage duration. This study aimed to examine the effect of varieties and storage treatments on pollen viability.

2. Materials and methods
The research was conducted from April to July 2020 in Brajan Village, Mojosoongo District, Boyolali Regency that stretched from 110° 22’ N latitude to 110° 50’ S latitude and from 7° 7’ E longitude to 7° 36’ E longitude. This research used Randomized Complete Block Design with a split-plot design by using two treatment factors. The first factor was varieties as the main plot with two kinds of varieties: male golden melon variety (♂) A and male golden melon variety (♂) B. The second factor was storage treatment of the pollen as the subplot with eight paces, which are 6-days storage at low temperature (2-4°C), 6-days storage at room temperature (27-30°C), 4-days storage at low temperature (2-4°C), 4-days storage at room temperature (27-30°C), 2-days storage at low temperature (2-4°C), 2-days storage at room temperature (27-30°C), 0-day storage at low temperature (2-4°C), and 0-day storage at room temperature (27-30°C). The treatment was repeated three times. The 0-day storage meant that the pollen was kept for 12 hours. The equipment used during the research was: refrigerator (LG Japan) as the place to keep the pollen at low temperature, which was 2-4°C, container box as the place to keep the pollen at room temperature which was 27-30°C, binocular microscope to observe the pollen viability.

The materials used were golden melon A pollen variety and golden melon B pollen variety, variety C of golden melon plants 18 days after planting, and aluminium foil. The pollen was taken from golden melon in its 21 days after planting. It was then kept based on the research treatment planned and hybridized with the bud head of the golden melon plant in their 18 days after planting. The variables observed included: pollen viability was measured using the storage age treatment before hybridized to the bud head, then viable pollens and unviable pollens were calculated using the following formula:

\[
\text{Pollen viability} = \frac{\text{the number of viable pollens}}{\text{the number of pollens in one media}} \times 100
\]  

(1)

The percentage of fruits produced was observed for five days after the hybridization, the weight of fruits was measured 65 days after planting, and the level of sweetness of fruits was measured 65 days after planting by calculating the level of sugar by using a refractometer. The data were analyzed using ANOVA variance with a credibility level of 95% (α 5%). If the result was significant, it was further tested using the DMRT standard with a credibility level of 95% (α 5%). The application used was SPSS 2020 Application.

3. Results and discussion
Pollen viability is an important step in reproducing plants. This process is affected by some biotic and abiotic pressure, including low and high temperatures. The result of the study showed that the combination of the treatments between varieties and storage treatment highly affected the viability of the pollens (Table 1). The result of the study conducted by Mesnoua et al. [13] declares that the pollen viability is affected by temperature and storage duration, and variety. Golden Melon Variety B with a two-day storage period at low temperature showed the highest viability level that was 57.95% (Table
However, the pollen viability of gold melon variety B with two-day storage at room temperature was reduced compared to two-day storage at low temperature. The pollen viability of golden melon variety A with two-days storage at low temperature was 26.32% and was reduced in the amount of 14.58%in two-days storage at room temperature. This result showed that temperature plays an important role in pollen viability. The percentage of viability and the growth of pollen are increased significantly at the low temperature of 4°C to -20°C compared to room temperature storage [14]. It shows that low temperature lengthens the storage age of pollen [15]. It is because temperature affected the dehiscence of the pollen, and when the temperature is above 20°C, catkins would slowly have peeled off [16]. The life or viability of the pollen is susceptible because of its deployment in the air and its low ripeness [17]. However, the life sustainability or viability of pollen is not only determined by the atmosphere agent but also by internal factors, such as lipid and carbohydrate [18] as well as storage duration and condition as found in this research. Higher temperature causes a reduction in pollen viability related to a decrease of de-esterification of a phospholipid, which causes contraction of membrane integration [19].

The research results showed that a longer period of storage could decrease the viability of pollen. Storage of 6 days at low temperature resulted in reduced pollen viability compared to the storage of 4 days at low temperature. However, pollen viability with 4 days storage at low temperature decreased compared to 2 days storage at low temperature. This research result was compatible with Novara et al. [20] that the duration and storage temperature of the pollen affect the viability of the Corylus avellana plant. The longer the pollen is stored, the ability of the pollen to grow will be reduced [21]. The pollen viability of golden melon variety A was really different from the pollen viability of golden melon variety B (Table 1). The pollen viability of golden melon variety B was higher than golden melon variety A. The difference in pollen viability is caused by genetic viability [22].

**Table 1.** The effect of storage duration and temperature of two melon varieties toward pollen viabilities (%).

| Storage Treatment                  | Varieties | Average |
|------------------------------------|-----------|---------|
|                                    | A         | B       |
| 6-days storage at low temperature  | 28.74 bcd | 33.33 cd| 31.04   |
| 6-days storage at room temperature | 33.97 cd  | 39.68 d | 36.83   |
| 4-days storage at low temperature  | 30.37 bcd | 37.50 cd| 33.94   |
| 4-days storage at room temperature | 35.62 cd  | 20.51 ab| 28.06   |
| 2-days storage at low temperature  | 26.32 bc  | 57.94 e | 42.13   |
| 2-days storage at room temperature | 14.58 a   | 27.04 bcd| 20.81   |
| 0-days storage at low temperature  | 38.43 cd  | 32.78 bcd| 35.60   |
| 0-days storage at room temperature | 34.69 cd  | 36.57 cd| 35.63   |
| Average                            | 30.34     | 35.67 (+)|

Description: Numbers in the columns and rows that are followed by the same letters are not different based on DMRT 5% (+): interacting

The research result showed that pollen storage affected the percentage of fruits produced significantly (Table 2). The pollen storage for 0-days at room temperature was not different from 0-day pollen storage at low temperature. It showed that the percentage of the fruits produced was higher than other storage treatments. Different pollen storage method affects differently toward fruits production and quality [23]. However, 6-days storage at room temperature showed that the percentage of fruits produced was low (43.75%). This research result is compatible with Du et al. [24], showed that the pollens of pear plants, after 0-day storage, had fruits higher than the pollens, which are stored for 14-days. Based on the research conducted by Quinet and Jacquemart [25], it is concluded that the temperature of the pollen storage process affects fruit production and quality. Storing pollen at low temperatures increases pollen viability, improving the ability to form fruits and seeds of cucumber [26].
Table 2. The effect of storage duration and temperature of two melon varieties towards the percentage of fruits formation (%).

| Storage Treatment                        | Varieties | Average |
|-----------------------------------------|-----------|---------|
|                                         | A         | B       |         |
| 6-days storage at low temperature       | 58.33     | 58.33   | 58.33b  |
| 6-days storage at room temperature      | 45.83     | 41.67   | 43.75a  |
| 4-days storage at low temperature       | 62.50     | 58.33   | 60.42bc |
| 4-days storage at room temperature      | 62.50     | 62.50   | 62.50bc |
| 2-days storage at low temperature       | 70.83     | 79.17   | 75.00d  |
| 2-days storage at room temperature      | 75.00     | 66.67   | 70.83cd |
| 0-day storage at low temperature        | 91.67     | 83.33   | 87.50e  |
| 0-day storage at room temperature       | 95.83     | 91.67   | 93.75e  |
| **Average**                             | **70.31a**| **67.71a**|         |

Description: Numbers in the columns that are followed by the same letters are not definitely different based on DMRT 5%.

The research result showed that pollen storage affected fruits' weight formed significantly (Table 3). Pollen storage for 0-day at low temperature showed the most weight that was 2.2 kg. Fruits weight included of 0-day pollen storage at low temperature was not different from the one of 2-days pollen storage at low temperature and 4-days storage at low temperature. It showed that the temperature in pollen storage affected fruit production and quality. The study conducted by Fernández-gonzález et al. [23] shows that low temperature increases pollen viability, and the use of pollen kept at low temperature increases fruits' weight and size produced. This research showed that pollen viability was positively correlated to fruits' weight formed (Table 4). These results indicate that the higher the viability of the pollen, the higher the fruit weight. The results of this study are by Padureanu and Patras [27] that the higher of pollen viability increasing the ability of fruit formation, and the size of formed fruit. Storing pollen for one year at −18 °C reduced fruit weight only slightly compared to fresh pollen, whereas after two years of storage, fruit change significantly decreased. Fruit weight also decreased as pollen storage duration increased [28]. The difference in fruits weight, diameter, and the number of seed that is caused by the difference in pollen storage treatment is also observed in srikaya (Annona squamosal) [29].

Table 3. The effect of storage duration and temperature of two melon varieties towards fruits weight (kg).

| Storage Treatment                        | Varieties | Average |
|-----------------------------------------|-----------|---------|
|                                         | A         | B       |         |
| 6-days storage at low temperature       | 1.73      | 1.97    | 1.85 ab |
| 6-days storage at room temperature      | 1.87      | 2.10    | 1.98 abc|
| 4-days storage at low temperature       | 2.03      | 2.20    | 2.12 cd |
| 4-days storage at room temperature      | 1.83      | 1.80    | 1.82 a  |
| 2-days storage at low temperature       | 1.93      | 2.23    | 2.08 cd |
| 2-days storage at room temperature      | 1.80      | 1.73    | 1.77 a  |
| 0-day storage at low temperature        | 2.27      | 2.20    | 2.23 d  |
| 0-day storage at room temperature       | 2.27      | 2.00    | 2.07 bcd|
| **Average**                             | **1.95a** | **2.03a**|         |

Description: Numbers in the columns that are followed by the same letters are not different based on DMRT 5%.
Table 4. The correlation of pollen viabilities, percentage of fruits formed, fruits weight, and level of fruits sweetness.

| Pollen viability | Fruits weight |
|------------------|--------------|
| 1                | 0.485**      |

Description: ** Correlation is significant at the 0.01 level (2-tailed).

4. Conclusion
Pollen viability is influenced by climate change, one of which is temperature. The combination of varieties and pollen storage had a significant effect on pollen viability. Golden Melon Variety B with two days storage treatment at low temperature showed the highest pollen viability. Treatment of 0 days storage at low temperature showed optimum results.

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