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EVALUATION OF THE CONDITION FOR STORING POLLEN GRAINS OF JAPANESE MELON *Cucumis melo* L. (CUCURBITACEAE)

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ABSTRACT

In recent years, studies have been carried on the selection and breeding of melon to reduce its production cost. The F1 seeds of this species are usually created by parental lines. Notably, the parental lines are separated from each other to secure the breeding materials. However, there are no studies on establishing suitable conditions for storing pollen grains of Japanese melon *Cucumis melo* L. In this study, pollen grains of *C. melo* were dried at 38 ^{o}C temperature, for 1.5 hours, which served as the raw materials. After storage in acetone at 5 ^{o}C for 3, 5, 7 days, or in liquid nitrogen for 5 days, pollen grains were stained with triphenyl tetrazolium chloride (TTC) to evaluate their viability and pollination ability. Then, the following parameters including pollen grains' survival rate, morphology analysis of pollen grains, pollen grains' fruiting rate, fruit weight, firm seed rate, and germination of melon seeds were analyzed. The study results show that the grains' survival rates were 86.58%, 88.38%, 87.28% or 88.58% in the group stored in acetone for 3, 5, 7 days or liquid nitrogen for 5 days, respectively. The fertilized fruit rate was 100% in the group stored in liquid nitrogen for 5 days. But the rate of germination from firm seeds was highest, with 94.00% in the group stored in acetone for 3 days. The best method is storing the pollen grains in acetone for 3 days.

Keywords: Acetone, storage, *Cucumis melo* L., pollen grain, liquid nitrogen.

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INTRODUCTION

Japanese melon, *Cucumis melo* L., an important economic fruit in subtropical regions, is being grown in Vietnam, in recent years. The F1 seeds of this species are usually created by parental lines, which are separated from each other to secure the breeding materials. The viability of pollen grains depends on several factors, including the type of species, environmental factors, such as temperature, humidity, and preservation condition (Stanley & Linskens, 1974). The transport of pollen grains from the male anther of a flower (the male line) to the female stigma (the female line) takes a long time; this reduces their viability as well as their fertility and pollination ability (Stanley & Linskens, 1974). It has been shown that pollen grains are stored with organic solvents or liquid nitrogen to help their viability, and fertility (Bhat et al., 2014). *Camellia sasanqua* pollen soaked in organic solvent (acetone or diethyl ether) for 5 minutes grew three to four times longer than the unsoaked pollen (Iwanami, 1973). Another study also indicated that broccoli pollen which was stored in liquid nitrogen retained its survival rate of 54.2% compared to the control group whose survival rate was 53.4% (Crisp et al., 1984). Karipidis and Douma (2009) reported that tomato pollen grains stored at -20 ^{o}C for 10 months had the highest survival rate, but their ability was significantly decreased after 12-months of storage. Meanwhile, those stored at -196 ^{o}C still had high viability after 18 months of storage (Karipidis & Douma, 2009). Kumar et al. (2015) reported that the seed of *Elaeis guineensis* Jacq. stored in either diethyl ether or n-hexane at the temperature of 0−5 ^{o}C for 200 days was still viable. However, there are still limited studies on the relevance of preserving *C. melo* pollens. Therefore, in the current study, the appropriate conditions for storing *C. melo* pollen are reported for them to be able to adapt when being transported to the male line.

MATERIALS AND METHODS

Materials

The pollen grains of *C. Melo* were separated from the newly bloomed male flowers. They were dried at 38 ^{o}C for 1.5 hours in an incubator Memmert IF55, which served as the raw materials. For the storage evaluation, the pollen grains were stored in acetone at 5 ^{o}C for 3, 5, 7 days, or in liquid nitrogen for 5 days. The control group was selected as the blooming male flowers without drying and storage. The female flowers were directly pollinated.

Evaluation of the stored pollen grains' survival rate

The survival rate of the pollen grains, which were stored at different conditions was evaluated by 1% 2, 3, 5 -triphenyl tetrazolium chloride (TTC). Two drops of TTC solution and pollen grains were put on a microslide, then covered by cover glass in 2 hours. After that, pollen grains were observed by microscope Olympus CX23 × 40, by staining method (Beyhan & Serdar, 2008; Abdelgadir et al., 2012). Percentage of the survival grains was calculated according to the following formula: $P = (n \times 100)/N$, where P: Percentage of survival seed; n: number of survival pollen; N: total of counted pollen. The survival seed had pink colour, while the dead pollens had black colour or were colourless (Sheffield et al., 2005; Zeng-Yu et al., 2004).

Morphology analysis of pollen grains

The analysis of comparing the morphology of the pollen grains was done. The pollens were collected a day before the male flowers bloomed, and were stored at different conditions. The comparison was based on the following parameters: the morphology and size of pollen grains. Pollen grains were collected from 10 male flowers/vine and 5 replications/treatment.

Evaluation of the stored pollen grains' fruiting rate, and fruit weight

A total of 0.05 g of pollen grains stored in different conditions was used as the material

for pollination. The comparison was performed among the stored pollen grains and the control group based on their fruiting rate after 7 days of pollination. Two female flowers/vine were pollinated by stored pollen grains with randomized complete block design, 5 treatments and 5 replications. The percentage of fruiting rate was calculated according to the following ratio: percentage of fruiting/individual tree.

Evaluation of fertilized fruit

The weight of the fertilized melon from 0.05 g pollen grains in the experimental groups was calculated. The evaluation criteria were: weight of fruit/individual tree.

Evaluation of firm seed rate

The melon seeds were collected from fertilized melon from 0.05 g pollen in the experimental groups. The number of seeds including firm and floater seeds was counted. The rate of the firm seeds was calculated according to the following formula: $P = (n \times 100)/N$.

Evaluation of germination of melon seeds

The firm seeds were collected from the melon fruit. They were sterilized in 10% javel, two times (10 minutes/time), and cultured on MS 1/2 macronutrient. The flasks were placed in the dark for 3 days. Then, the flasks were placed under light at a cycle of morning/evening 12/12 hours, and light intensity 2,000 lux. After 7 days, the number of germinated seeds of each treatment was counted. The germination rate of the seeds was calculated according to the following formula: $P = (n \times 100)/N$.

Statistical analysis

All the results were analyzed by Statgraphics 3.0. Statistical significance was assumed as a two-sided p-value of $p < 0.05$ based on the Duncan classification. The positive group was the newly hatched flowers.

RESULTS

The survival rate of the stored pollen grains' survival rate stored in acetone and liquid nitrogen

The highest survival rate, 98.40%, was recorded in the control group. A lower rate was observed in each treatment. In detail, the survival rates were 86.58%, 88.38%, 87.28% or 88.58% in the group stored in acetone for 3, 5, 7 days or liquid nitrogen for 5 days, respectively (Figs. 1, 2).

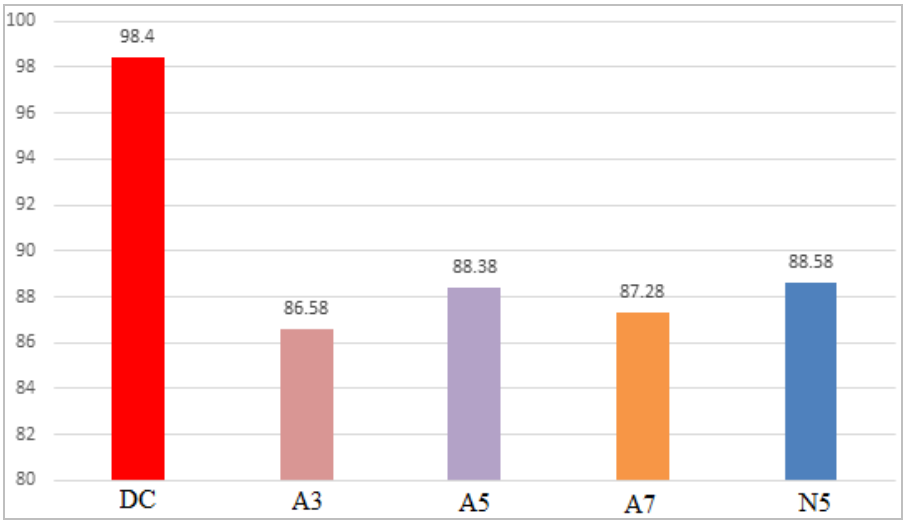

Figure 1. The survival rate (%) of (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

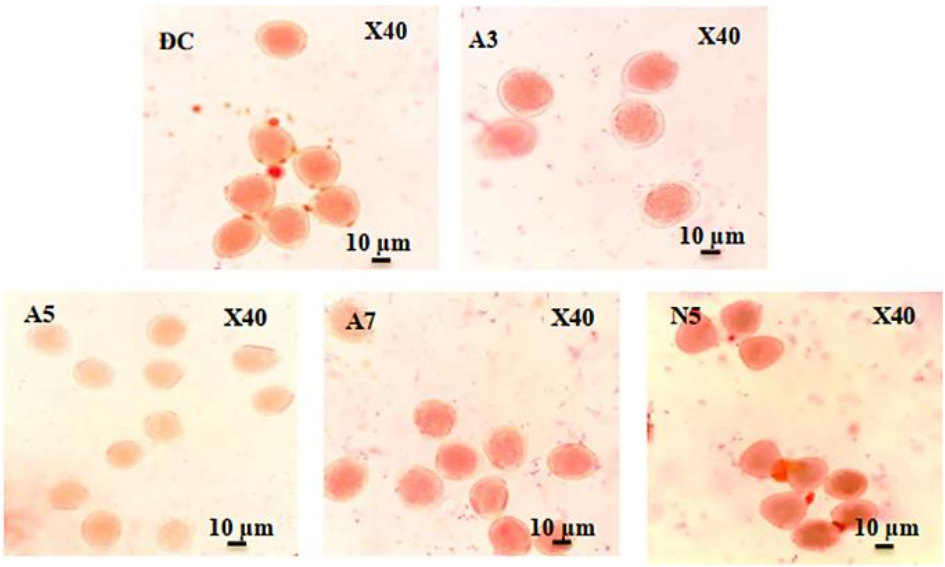

*Figure 2***.** The pollen grains stained by TTC 10% after (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days, (DC) control

Morphological analysis of pollen grains

The pollen grains had a spherical shape, were grey, had grid-surface with convex poles (Fig. 3). According to the study of Perveen and Qaiser (2008), melon pollen grains are spherical in shape with a size of about 56.5 µm. Their surface is meshy and has two membranes. The outer membrane has a thickness of 1.7−2.0 µm, thicker than the inner membrane, with convex poles of 5.8−10 µm diameters.

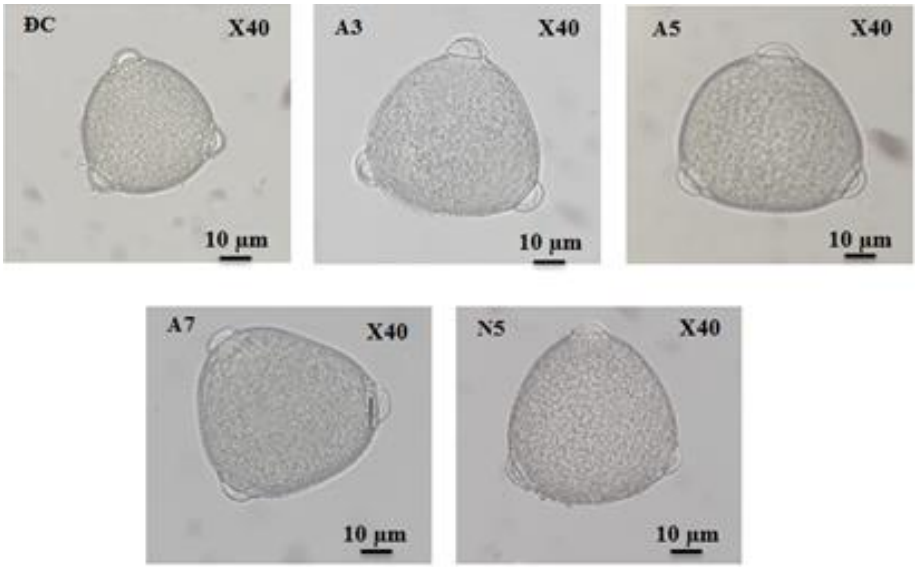

Figure 3. The morphology of pollen grains after (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

The rate of fertilized fruit of stored pollen grains

100% rate was recorded in the experimental group and control group stored in acetone for 5 days. The rate of fertilized fruit in the group stored in acetone for 3 days or liquid nitrogen for 5 days was the same, 96% (Fig. 4). There were no significant differences in each treatment and control group. The shape of the fruit obtained in each treatment was oval shape; the fruit had a small stalk, and not deformed, because the pollens were not affected at all or were slightly affected by the solvent, acetone or liquid nitrogen (Fig. 5).

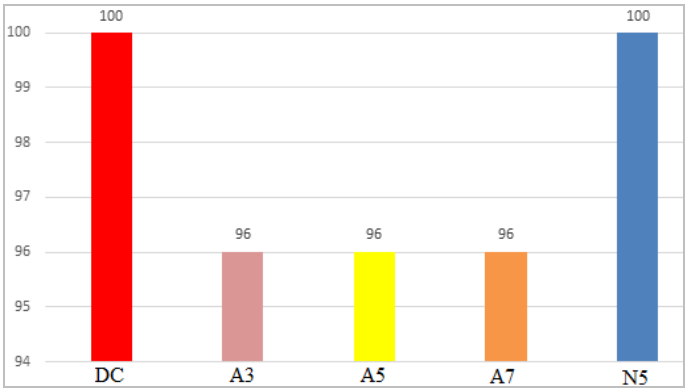

Figure 4. The rate of fertilized fruit of stored pollen grains (%) of (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

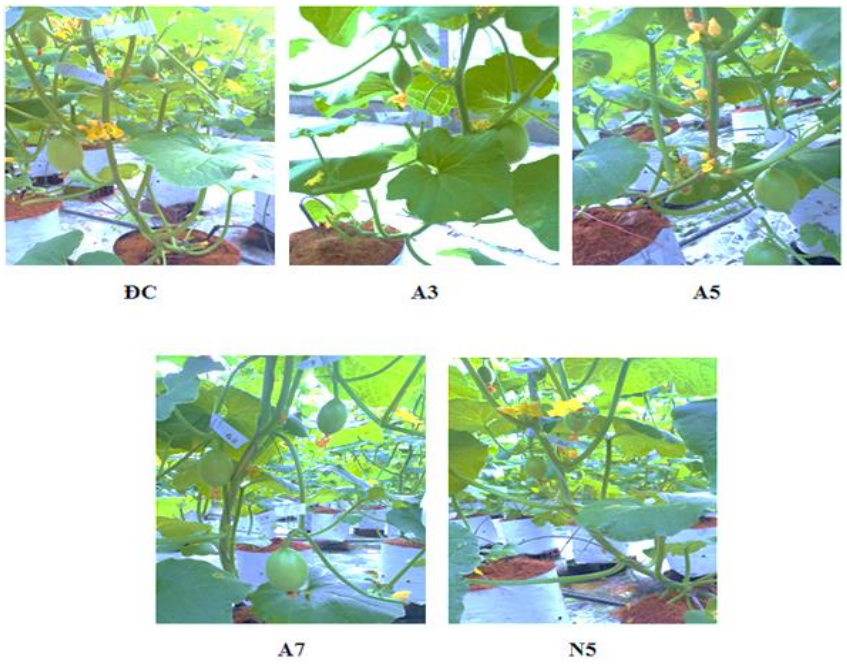

Figure 5. The morphology of fruit achieved in (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

The weight of *Cucumis melo* L.

There were no significant differences in each treatment and control group. The weight of the fruits was 1.64 kg; 1.58 kg; 1.50 kg; 1.54 kg; 1.50 kg in the control group, and those stored in acetone for 3,5, 7 days, or liquid nitrogen for 5 days, respectively (Table 1). The shape of the fruits in each treatment was oval and not deformed (Fig. 6).

Table 1. The weight, rate of firm seed and rate of germination of *Cucumis melo* L.

Treatment	Weight (kg)	Rate of firm seed (%)	Rate of germination (%)
DC	1.64	93.05	93.34[a]
A3	1.58	94.20	94.00[a]
A5	1.50	92.68	80.02[b]
A7	1.54	94.41	87.34[ab]
N5	1.50	89.08	83.32[b]
F test	Ns	ns	7.21

Notes: ns: no significant differences, (DC) control group; (A3) acetone-stored group for 3 days; (A5) acetone-stored group for 5 days; (A7) acetone-stored group for 7 days, (N5) liquid nitrogen-stored group for 5 days.

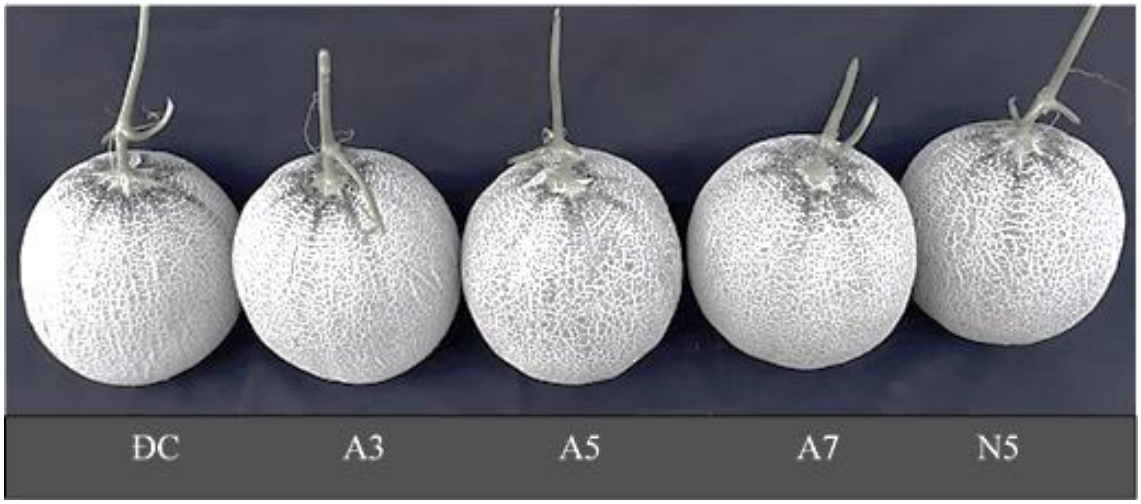

Figure 6. The morphology of fruit in the group of (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

The rate of *Cucumis melo* L firm seed

There were no significant differences in each treatment. The rate of firm seed was 93.05%, 94.20%, 92.68%, 94.41%, 89.08% in the control group and group stored in acetone for 3, 5, 7 days, or liquid nitrogen for 5 days (Table 1). The seeds grew normally, had an oval shape and were not deformed (Fig. 7).

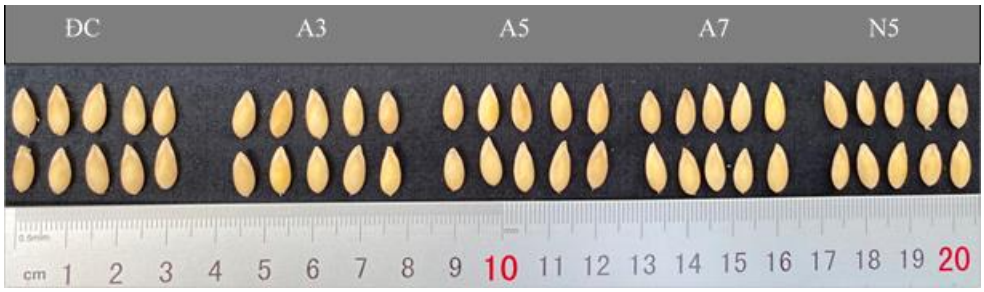

*Figure 7***.** The morphology of melon seeds achieved from the group of (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

The rate of germination

There were no significant differences in each treatment. The highest rates of germination, 93.34 %, 94.00 %, 80.02 % and 83.32 %, were recorded in the control group and group stored in acetone for 3, 5 days, or liquid nitrogen for 5 days, respectively. The rate of germination of the group stored in acetone for 7 days was lower than that of the control group (Fig. 8).

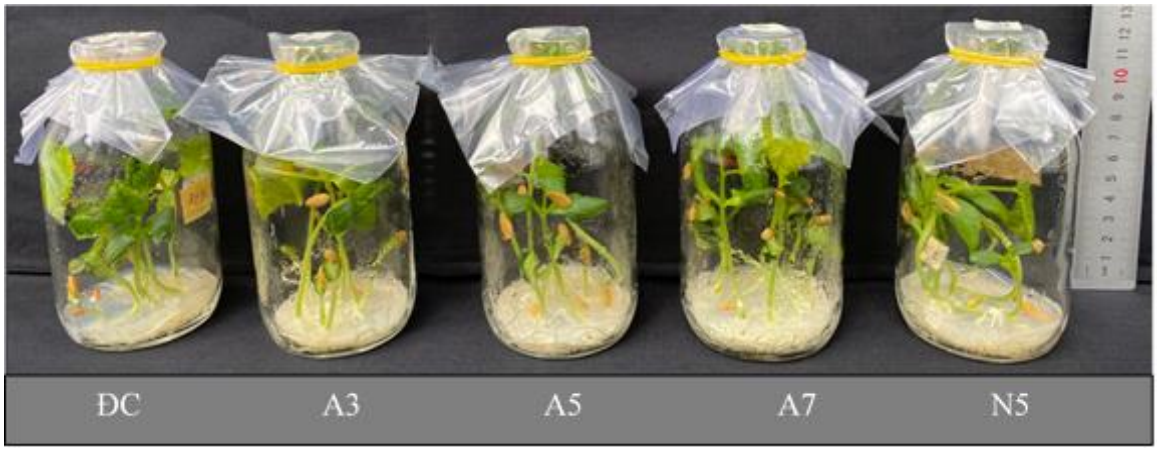

Figure 8. The tree of melon germinated from the group of (DC) control group; (A3) acetone-stored for 3 days; (A5) acetone-stored for 5 days; (A7) acetone-stored for 7 days, (N5) liquid nitrogen-stored for 5 days

DISCUSSION

When the volume of acetone was lower than water, acetone penetrated into the cell membrane, damaging the pollens. In preserving the melon pollens, acetone was used in its pure form and the pollen grains were dried at 38 °C before storage reduce the water content in the pollen. This resulted in the high survival rate of the pollens. The morphology of the pollen grains in this study is similar to that of Perveen and Qaiser (2008). Additionally, the fruit weights obtained in this study were similar to those of the control, 1.6 −1.7 kg. The fruits had an oval shape and were not deformed. These results indicate that acetone or liquid nitrogen had a less negative effect on the pollen grains, resulting in their normal development and lack of deformity. The rate of germination in the treatment group was not significantly different from that of the control group. It indicates that the seeds were not be affected by acetone or liquid nitrogen. According to

the research of Sakunnarak et al. (1990) performed on soybean plants, seeds were less affected when stored in acetone for 3 hours; the germination rate decreased from 95% to 90%. Hwakins et al. (2003) carried out a study on the seeds of Douglas conifer stored in liquid nitrogen. The study result showed that the Douglas conifer seeds were less affected by the liquid, and they had a high rate of germination.

CONCLUSION

In summary. All the treatment conditions, including the group stored in acetone at 5 ^{o}C for 3-days could be used for storing pollen grains. The best method for storing pollen grains is to store them in acetone at 5 ^{o}C for 3-days.

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