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DRY-HEAT TREATMENT FOR RELEASING THE DORMANCY OF STORED OIL PALM (*Elaeis guineensis* Jacq.) SEEDS

VICHAI WONGVARODOM[1]; ROSSUKHON SOOKKARN[1]; NATANONG MUANGNAPHO[1]; THEERA EKSOMTRAMAGE[1]; SAITHIP THIPPAN[1] and LADAWAN LERSLERWONG[1]*

ABSTRACT

Seed dormancy of oil palms, after harvesting with low and non-uniform germination, is a significant problem for commercial seedling production. Practically, dry-heat treatments are performed to release the dormancy. This study is aimed to investigate the optimum heating requirement to remove the Sub-PSU1 oil palm seed dormancy, after different storage times while waiting for a commercial order and breeding program before the germination occurs. Abscisic acid levels, after the heat treatment of stored seeds were also studied concurrently. It was found that the dormancy of oil palm seeds had entirely declined naturally after 15 months of storage at 20°C, with germination of 85% within 49 days. A dry heat treatment at 40°C for 45 days, being shorter than the traditional practice (60-80 days), is found influential in promoting the germination of oil palm seeds after 15 months of storage. This treatment also effectively delayed the accumulated abscisic acid in stored seeds at higher levels, with a more extended storage period. In conclusion, the specific condition of the dry heat treatment for breaking the seed dormancy during long-term storage at a low temperature (20°C), will certainly benefit commercial seedling production, when used in the breeding programmes of sub-PSU1 oil palm.

Keywords: abscisic acid, germination, heat requirement, oil palm (*Elaeis guineensis* Jacq.), seed storage.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an important economic oil crop in the world, and its production has been increasing rapidly because of the high volume of global demand for edible oils and oil products, oleo-chemicals and clean transportation fuel (Tabi *et al.*, 2017). Currently, Thailand produces approximately a hundred million tonnes of oil palm at the third global trade rank, next to Indonesia and Malaysia (FAO, 2021). Thai export was valued at $3136 million in 2021. The growing area of 890 308.4 ha, or 85.50% of the total plantation area, is in the Southern part of the country (Office of Agricultural and Economics, 2022).

[1] Agricultural Innovation and Management Division (Plant Science), Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

* *Corresponding author e-mail: ladawan.l@psu.ac.th, ladawanllw@yahoo.com*

Recently, the Sub-PSU1, the new variety of oil palm, has been released through a breeding programme, based on selecting the local parental hybrids from the genetic base in Southern Thailand. The Sub-PSU1 oil palm is a high-yielding *tenera* variety, well adapted to poor environmental conditions (Junsawang *et al.*, 2020), *i.e.*, drought stress (Duangpan *et al.*, 2018), salt stress (Sukdee *et al.*, 2022), and some progeny hybrids resistance to *Curvularia* leaf spot disease (Kittimorakul *et al.*, 2019; 2020). Thus, the seedling of Sub-PSU1 oil palm is increasingly required for new planting and replanting, and is encouraged to grow in Southern Thailand.

Germinated oil palm seeds as planting materials for the commercial production of seedlings before field planting demonstrate a high dormancy level after harvesting with low and non-uniform germination (Jiménez *et al.*, 2008; Martine *et al.*, 2009). The physiological problem of seed dormancy is also found in oil palm var. Sub-PSU1. Oil palm seeds

generally combine morphological and physical dormancy (Baskin and Baskin, 2014; Norsazwan *et al*., 2016). Practically, poor, irregular, and delayed germination of oil palm seeds has traditionally been overcome by introducing 60 days of dry-heat treatment at a temperature of 40°C to release their dormancy (Fondom *et al*., 2010; Jiménez *et al*., 2008; Martine *et al*., 2019; Murugesan *et al*., 2005; Myint *et al*., 2010). In the case of oil palm seeds, a heat treatment for at least 30 days is necessary to break the dormancy (Wang *et al*., 2019).

Theoretically, abscisic acid (ABA) is a crucial hormone that plays a prominent role in maintaining seed dormancy and regulating germination (Ali *et al*., 2022; Wang *et al*., 2019). Wang *et al*. (2019) confirmed that in oil palm seeds, the accumulation of ABA to initiate seed dormancy occurs during seed maturation, while the ABA level decreases before the onset of the germination process. In addition, breaking the dormancy of oil palm seeds by heat treatment resulted in a sharp decrease in the ABA concentration (Jiménez *et al*., 2008). In oil palm, however, there are no data on ABA content after the breaking of dormancy by heat treatment of stored oil palm seeds.

There have been no previous reports on the dormancy released by heat treatment in the stored oil palm seeds. Therefore, this study is aimed to investigate the heating requirements to release the dormancy after storing oil palm seeds, while waiting for a commercial order and breeding program before germination occurs. These findings will be further applied to commercial seedlings for new planting or replanting. Concurrently, changes in the endogenous ABA contents after the heat treatment were also studied.

MATERIALS AND METHODS

Experiment I: Germination of Oil Palm Seeds after Storage

The experiment was conducted in the Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand. Seeds of the commercial oil palm variety, Sub PSU 1, were soaked with the fungicide, metalaxyl at 2 g L^{-1}, then surface air-dried and stored in 50.8 x 76.2 cm polyethylene plastic bags (about 5000 seeds per bag) in a cool room at 20°C, for 0, 3, 6, 9, 12 and 15 months, and later sampled and soaked in water for 5 days. After soaking, four replicates of 100 seeds were germinated in a plastic bag and placed in a germination room at 25°C-30°C. The first and final counts were conducted at 7 and 49 days. The number of normal and abnormal seedlings, disease-infected and dormant seeds were later counted and converted to percentages (Eksomtramage, 2011).

Experiment II: Effects of the Heating Period on the Germination of Stored Oil Palm Seeds

Seed materials. The experiment was conducted to study the effects of different heating periods on the germination of stored oil palm seeds. The seeds were stored under the same conditions as for experiment I for 5, 15 and 20 months then sampled and soaked in water for 5 days. They were then heated at 40°C for 0, 30 and 45 days and subjected to seed quality tests as listed below.

Seed Germination and Mean Germination Time (MGT)

After soaking for 5 days and subsequent heating, four replicates of 50 seeds were germinated in plastic bags and placed in the germination room. The first and final counts were conducted at 7 and 49 days, respectively. The number of normal and abnormal seedlings, diseased, decayed, non-viable, and dormant seeds was reported as percentages. The non-viable and dormant seeds were determined using the Tetrazolium test (TZ test) and were stained with 0.50% 2,3,5-triphenyl tetrazolium chloride, at 41°C for 4 hr (Murugesan *et al*. 2002). The number of normal seedlings was counted weekly, and the MGT was calculated according to the following formula of Ellis and Roberts (1981): $MGT = \sum nd / \sum n$, where n is the number of newly germinated seeds at time d, where d is days from the beginning of the germination test, and $\sum n$ is the final number of germinated seeds.

Experiment III: Effects of The Heat Treatment on ABA Contents of Stored Oil Palm Seeds

Experimental design and seed sampling. The experiment was performed in a completely randomized design (CRD), with three replications. Treatments from Experiment I and II include storage period and heat treatment. In other words, two treatments of heat drying at 40°C, for 45 days and unheated (control) were compared at 0, 3, 6, 9, 12 and 15 months after storage in the cool room (20°C). Endosperm and embryo were separately sampled and frozen with liquid nitrogen for further extraction.

ABA extraction. The samples of endosperm and embryo were freeze-dried and weighed (100 mg). The samples were then finely-ground in liquid nitrogen by mortar. Another 50 mL of 80.00% coal methanol was added to the seed sample in a bottle and kept at 4°C for 16 hr. The extract was then filtered with Grassinter-filter fixed to the round bottom flask. The solution was then evaporated at 40°C, using the rotary evaporator. The extract was washed with 0.01 M ammonium acetate (pH 7.5)

three times, at 4 mL each. The extract solution was later stored at -20°C for 16 hr. The extract was then thawed and centrifuged at 16 500 rpm for 10 min. The supernatant was purified using the Sep-Pak-C18 column method, in the next step.

ABA purification. The DEAE-Sephadex column comprised of four columns, which include: (1) sample supernatant (final volume after evaporation), (2) polyvinylpyrrolidone or PVP (10 mL), (3) DEAE-Sephadex (15 mL), and (4) Sep-Pak-C18. The PVP in the water was spun for 1 hr to prevent precipitation before packing to the column. The column of PVP and DEAE-Sephadex were left to precipitate for 1 hr. Then, another 15 mL of 0.1 M ammonium acetate (pH 7.5) was added to the sample supernatant column and the valve was opened to release the supernatant through the PVP column. The valve was closed when all the supernatant has depleted. The DEAE-Sephadex column was washed twice by adding 4 mL of 0.1 M acetic acid in absolute methanol. During running the column, the PVP and DEAE-Sephadex were not left dry. The PVP column was removed, while the DEAE-Sephadex column was connected to the sample column. The Sep-Pak-C18 column was connected to the end of the DEAE-Sephadex column. Another 15 mL of 0.75 M acetic acid (pH 7.5), was added to the sample column and let all the sample solutions go through the Sep-Pak-C18 column. The Sep-Pak-C18 column was later taken off and 4 mL of 0.1 M acetic acid was added to 50.00% methanol, to get the ABA solution.

ABA quantification. The quantification of ABA was performed using the Liquid Chromatograph-Mass Spectrometer (LC-MS), 2690, LCT (Waters, USA) (Hogge *et al*., 1992). Each sample was injected into a 20 mL LC/MS glass vial for LC-MS/MS analysis. The mobile phases and gradient were as follows: Mobile phase A, 0.1 M acetic acid in methanol; mobile phase B, 0.1 M acetic acid in water, 50:50 (v/v). The MS analysis was carried out using negative ion electrospray ionisation (ESI) detection modes. The mass spectrum parameters are as follows: Time of Flight Analyser (TOF); capillary voltage, 3.2 kV; desolvation temperature, 130°C; sample cone, 35 V; source temperature, 120°C; extraction cone, 5 V; con gas flow 50 L hr^{-1}; RF lens, 400 V; acquisition mode, scan 100-1000 m/z; desolvation gas flow, 279 L hr^{-1}. Each sample was extracted three times. The (+/-) ABA (CAS14375-45-2) was purchased from PhytoTech Labs, Inc. (USA) and was used as an internal standard.

Statistical analysis. Experiments I and II were arranged in CRD with four replications, and means were compared using Duncan's Multiple Range tests. Experiment III was arranged in a CRD with three replications, and means were compared using the least significant difference (LSD).

RESULTS AND DISCUSSION

The Release of Dormancy of Oil Palm Seeds after Storage

The dormancy of the oil palm seeds was progressively released during a storage period of 15 months at 20°C. None of the newly harvested seeds (0 month storage or without storage) had germinated at 49 days after being placed in the germination room, while the seeds stored for 3, 6, 9 and 12 months had 2.75%, 15.00%, 22.25% and 68.00% germination, respectively. The maximum germination of 85.00% was achieved with seeds stored for 15 months (*Figure 1*). For storage periods of 0, 3, 6 and 9 months, all or most of the non-germinated seeds were dormant with 100.00%, 96.25%, 84.75% and 77.25%, respectively. However, seeds stored for 12 and 15 months had significant proportions of abnormal seedlings of 13.75% and 8.75%, respectively. The seeds stored at a cool temperature of 20°C for 3-12 months had low disease infection levels, ranging from 0.00% to 1.25%.

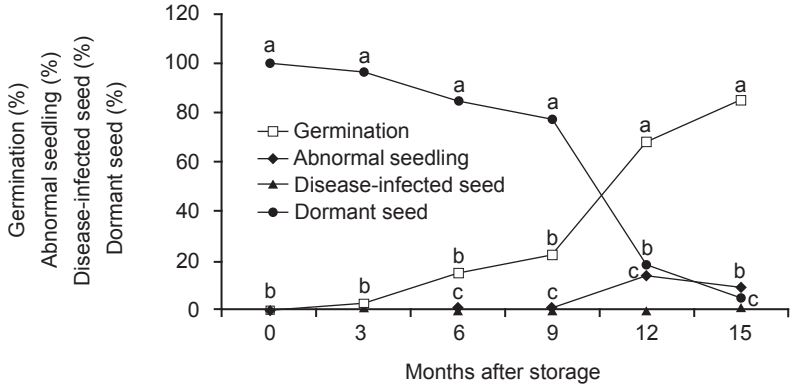


Figure 1. Germination, abnormal seedlings, disease-infected and dormant seeds after storage at 20°C for 0-15 months.

The germination percentage increased with storage periods and reached its maximum level after 49 days for most treatments. The germination of seeds stored for 12 months was 10.00% on the 21st day, then continuously increased and reached 68.00% after 49 days in the germination room. For seeds stored for 3, 6 and 9 months, germination only began on the 42nd day, and the percentages of germination were 4.00%, 12.00% and 22.00%, respectively. There was no germination after the 49th day in seeds that were not stored before germination. Finally, the germination of 12 and 15 month-stored seeds sharply increased between 35 and 49 days, whereas the germination of seeds stored for 3-9 months had only increased slightly at the end of 49 days (*Figure 2*).

The oil palm seeds, in natural conditions, entered a relatively long period of dormancy, which led to a very long period of germination (between 1 to 3 years) (Martine *et al*., 2019). The successful germination of oil palm seeds occurs during storage after harvesting, of which, heat treatment is required to break the dormancy (Norsazwan *et al*., 2020; Rees, 1962). Finch-Savage and Leubner-Metzger (2006) stated that seed dormancy release could be characterised by a loss of requirements or factors that stimulate germination which increases the rate of germination and germination velocity. In this study, the oil palm seeds dormancy seeds were successively released at 15 months of storage at 20°C with maximum germination of 85.00% after 49 days (*Figure 1* and *2*). Seeds stored for 12 months also produced high germination of about 70.00% without heating as a dormancy-breaking treatment. This study indicates that oil palm seeds gradually lose dormancy, which is completed after 12-15 months of storage. Additionally, Rees (1962) showed that the low-temperature stratification of temperate seeds has in some ways, become analogous to the heat treatment of oil palm seed, and it may be applied as a pre-treatment before the germination process. However, this entails a long germination process, causing an extended germination time of 49 days, as found in this study. Therefore, an appropriate heating period is deemed necessary to promote faster oil palm seed germination. Seeds must therefore be stored for a shorter period of 3-9 months due to their lower dormancy release or germinability after storage. Thus, the optimum heating period is vital, although some loss of germinability can be experienced, as reported by Martine *et al*. (2019).

Heat Requirement to Release the Dormancy of Stored Oil Palm Seeds

There were significant differences in the mean germination percentages of seeds stored for 5, 15 and 20 months, at 51.67%, 40.50% and 1.67%, respectively (*Table 1*). Seeds subjected to different heating periods also showed significant differences in the percentage of germination. Without heating, the seeds produced significantly lower germination (13.67%) than those heated for more extended periods. Seeds heated for 30 and 45 days had similar germination percentages of 38.17% and 42.00%, respectively.

Moreover, there were significant interactions between the seed storage time and heating duration. The highest germination percentages of 69.00% and 74.00% were found in the seeds heated for 30 to 45 days. However, the mean germination time decreased as the heating duration increased, and seeds heated for 0, 30 and 45 days had mean germination times of 29.01, 21.74 and 14.66 days, respectively. In the case of storage time, there was no significant difference in the mean germination times (23.83-28.76 days), neither for seeds stored between 5 and 15 months, nor the interaction between the seed storage and heating durations. In addition, a few abnormal seedlings were found, as shown in *Table 1*.

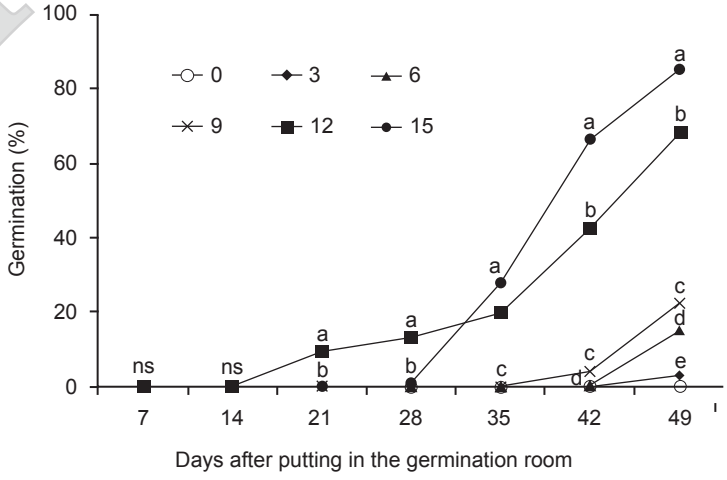


Figure 2. Cumulative germination between 7 and 49 days of oil palm seeds with storage at 20°C for periods 0, 3, 6, 9, 12 and 15 months.

TABLE 1. GERMINATION (%), MEAN GERMINATION TIME (DAYS), AND ABNORMAL SEEDLINGS (%) WITH DIFFERENT STORAGE AND HEATING DURATIONS OF OIL PALM SEEDS

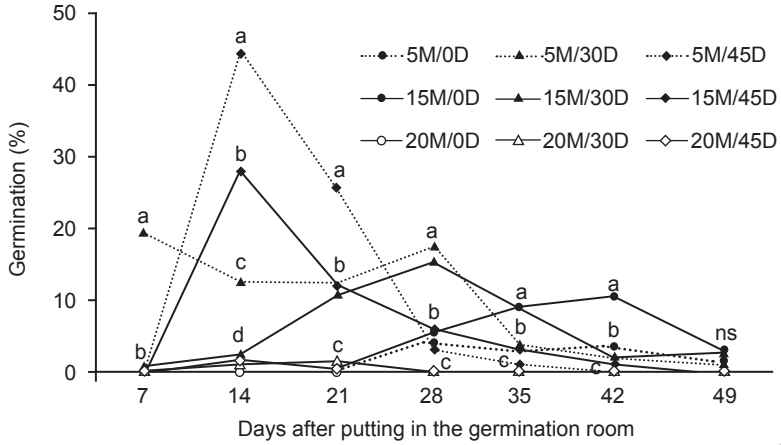
Storage time (months)	Germination (%)				Mean germination time (days)			
	Heating duration (days)				Heating duration (days)			
	0	30	45	Mean	0	30	45	Mean
5	12.00 d	69.00 a	74.00 a	51.67 A	35.97	18.61	16.91	23.83 A
15	28.50 c	43.00 b	50.00 b	40.50 B	38.82	28.24	19.20	28.76 A
20	0.50 d	2.50 d	2.00 d	1.67 C	12.25	18.38	7.87	12.83 B
Mean	**13.67 B**	**38.17 A**	**42.00 A**		**29.01 A**	**21.74 AB**	**14.66 B**	
Storage time (A)		**				**		
Heating duration (B)		**				**		
A×B		**				ns		
C.V. (%)		**21.57**				**39.75**		

Storage time (months)	Abnormal seedling (%)			
	Heating duration (days)			
	0	30	45	Mean
5	2.00	4.50	5.50	4.00 A
15	5.00	3.00	3.50	3.83 A
20	0.50	0.50	2.50	1.17 B
Mean	**2.50**	**2.67**	**3.83**	
Storage time (A)		*		
Heating duration (B)		ns		
A×B		ns		
C.V. (%)		**86.72**		

Note: ns - not significant; *, ** - difference significant at $p<0.05$ and 0.01, respectively. Means not sharing the same capital letters in the mean rows and columns are significantly different based on Duncan's Multiple Range Test.

The results illustrated in *Figure 3* show that heating at 40°C for 45 days promoted the highest early germination of 44.50% and 28.00%, at 14 and 21 days, respectively. After being stored for 5 and 15 months, seeds without heating exhibited low germination percentages with a slow germination rate, while seeds after 20 months produced very low germination rates.

There were significant differences in the number of decayed seeds among those stored for different times. The seeds stored for 5, 15 and 20 months had 9.00%, 38.33% and 35.50% decayed seeds, respectively (*Table 2*, *Figure 4*). However, heating seeds for different periods did not affect the percentage of decayed seeds. The percentage of dormant seeds had decreased, as both storage and heating duration increased. Seeds heated for 0, 30 and 45 days still exhibited dormancy rates of 25.67%, 9.17% and 2.67%, respectively. The dormancy rates in seeds heated for 0, 30 and 45 days were 29.00%, 6.00% and 2.50%, respectively. The number of non-viable seeds increased as storage time increased, with the highest percentage of non-viable seeds (56.83%), found in seeds after storage for 20 months (*Figure 4* and *5*).



Note: M - months; D - days.

Figure 3. Germination of oil palm seeds during 7-49 days of germination after different storage and heating durations.

TABLE 2. DECAYED (%), DORMANT AND NON-VIABLE (%) OIL PALM SEEDS AFTER DIFFERENT STORAGE AND HEATING DURATIONS

Storage time (months)	Decay seed (%)				Dormant seed (%)			
	Heating duration (days)				Heating duration (days)			
	0	30	45	Mean	0	30	45	Mean
5	14.00 c	6.00 c	7.00 c	9.00 B	61.00 a	10.50 bc	5.50 c	25.67 A
15	39.50 ab	38.50 ab	37.00 ab	38.33 A	19.00 b	6.50 c	2.00 c	9.17 B
20	29.00 b	35.50 ab	42.00 a	35.50 A	7.00 c	1.00 c	0.00 c	2.67 C
Mean	**27.5**	**26.67**	**28.67**		**29.00 A**	**6.00 B**	**2.50 B**	
Storage time (A)		**				**		
Heating duration (B)		ns				**		
A×B		**				**		
C.V. (%)		**18.02**				**40.64**		

Storage time (months)	Non-viable seeds (%)			
	Heating duration (days)			
	0	30	45	Mean
5	2.00	2.50	0.00	1.50 B
15	3.50	4.50	3.00	3.67 B
20	58.00	60.50	52.00	56.83 A
Mean	**21.17 AB**	**22.50 A**	**18.33 B**	
Storage time (A)		**		
Heating duration (B)		*		
A×B		ns		
C.V. (%)		**17.79**		

Note: ns - not significant; *, ** - difference significant at $p<0.05$ and 0.01, respectively. Means not sharing the same capital letters in the mean rows and columns are significantly different by Duncan's Multiple Range Test.

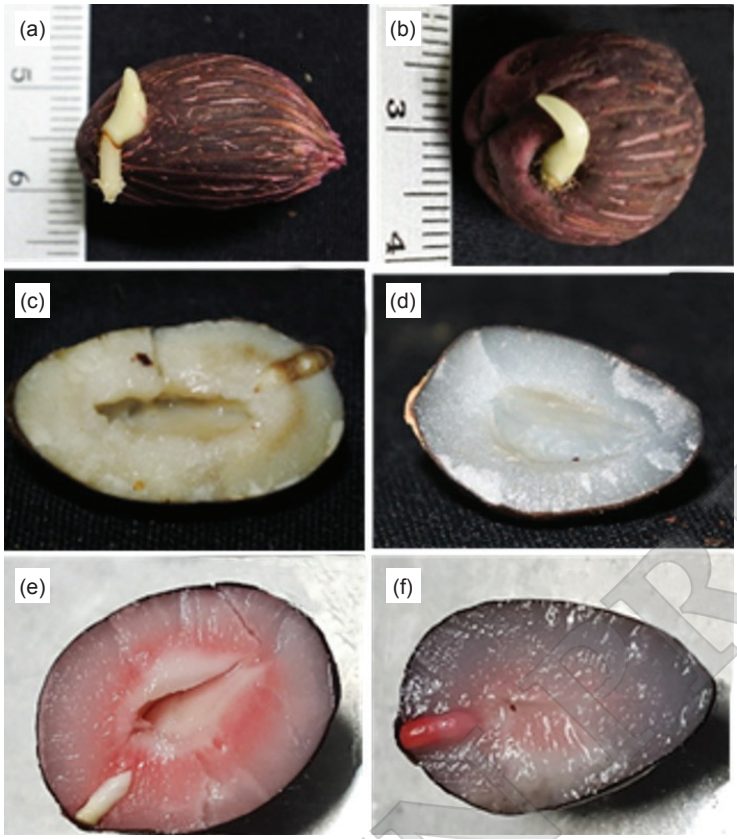
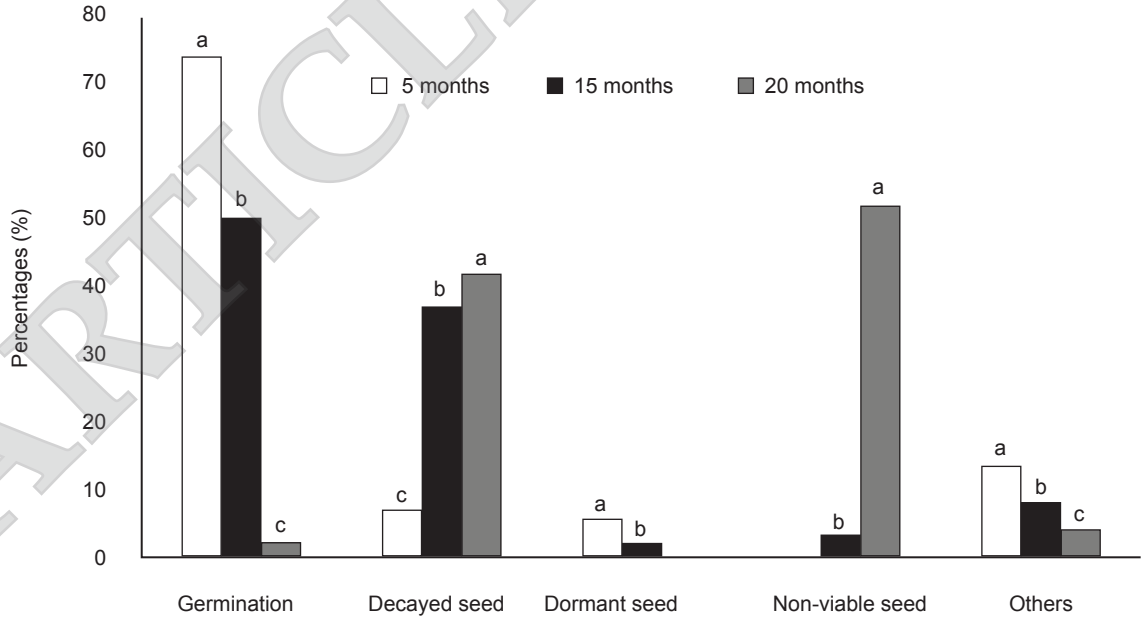


Figure 4. Stored oil palm seeds after germination: (a) normal seedling, (b) abnormal seedling, (c) decayed seed, (d) seed without the embryo, (e) non-viable seed and (f) dormant seed.



Note: Others include abnormal seedlings, seeds without an embryo, and diseased seeds.

Figure 5. Seed germination attributes of oil palm seeds after 5, 15 and 20 months of storage followed by heating at 40°C for 45 days.

Heat treatment at different temperatures and durations has been applied to promote the germination of oil palm seeds in commercial operations. Heating for too long can cause seeds to fail to germinate (Fondom *et al*., 2010; Martine *et al*., 2019), and heating seeds entails high energy costs for heating facilities, including being time-consuming (Myint *et al*., 2010) and labour-intensive. The results in *Table 1* show that seeds did not germinate when the stored seeds were not heated, in accordance with the previous report of Murugesan *et al*. (2015). Additionally, the results indicated that it is necessary to break the dormancy of oil palm seed by heat treatment to increase the germination rate (Green *et al*., 2013). However, heating for 45 days effectively enhanced the germination of oil palm seeds when stored for 5 and 15 months. Under these specific conditions, the seeds had reached their maximum germination potential of 74.00% and 50.00%, respectively, at 5 and 15 months of storage, with most of the remaining seeds being non-viable, decayed or deteriorated with rotten endosperms, and not having the potential to germinate due to the defects which arose during storage (Kok *et al*., 2015). The percentages of dormant seeds after storage for 5 and 15 months, followed by heating for 45 days, were very low at 5.50% and 2.00%, respectively (*Figure 5*). As shown in *Figure 4f*, the embryos of viable seeds with high vigour have a homogeneous red or pink colour (Maquiné *et al*., 2014).

Compared with the previous reports, the results suggest that there is no need to extend the heating period, such as 60 days, to accelerate the germination of stored oil palm seeds. A heating period of 45 days is already optimum for Sub-PSU1 oil palm in commercial cultivation and breeding programmes since it promotes physiological quality, reduces production costs by lowering energy needs and labour, and shortens the production period. Similarly, the maximum germination of commercial oil palm seeds in Brazil was achieved and varied based on the heat-treatment durations at 40°C. The heat treatment to break dormancy can be less than 80 days, *i.e*., 40-60 days, depending on the cultivars (Green *et al*., 2013). Recently, Dickson *et al*. (2021) have reported the application of heat treatment at 38°C-40°C for 60 days to increase the germination rate of *tenera* oil palm progenies, compared to the 70 days as a standard. According to the results and previous studies, it is confirmed that the heating period to break the dormancy of oil palm seed can be less than 80 days, which is currently the recommended time of the method.

Additionally, the germination capacity of oil palm seeds decreased with the length of storage, when the heating treatments are cumulated. Seeds incubated at 40°C for 60 days increased the germination rate, compared to the delay of 80 days of heating which is currently commercially applied (Martine *et al*., 2019). In the idolatrica palm (*Elaeis guineensis* var. idolatrica (Chevalier), it is highly recommended for the seeds to be stored at 21±1°C for up to four months, and the wet heat treatment at 39.5°C for 50 days, while the usual method of heating is at 38°C-40°C for 70-80 days (Addae-Kagyah *et al*., 1988). Interestingly, these findings demonstrated that the traditional germination techniques for oil palm seedlings production could be improved and can shorten the germination day by long-term storage at low temperatures, before applying the heat treatment. This study is the first to mention the mutual influence of low-temperature storage and heat treatment to release seed dormancy in commercial *tenera* oil palms.

However, seeds stored for shorter periods, such as 3 months, still require heat treatment at 40°C for 60 days, to overcome their dormancy (Martine *et al*., 2019). Seeds stored for extended periods require a shorter heating period (45 days), which might be due to their natural dormancy release. The natural dormancy release might be caused by the deterioration of the operculum of the seeds after a prolonged storage period, which is only more than 6-15 months. Kucera *et al*. (2005) stated that the seed dormancy release of oil palm species with physical dormancy is imposed by the balance of forces between the growth potential of the embryo, and the constraint exerted by the covering layers, *e.g*., the operculum. Myint *et al*. (2010) reported that oil palm seed germination was improved by removing the operculum without heat application. Ravichandran *et al*. (2016) also found that oil palm seeds exhibited an abridgement of the seed germination period from 110 days to 30-35 days when its operculum was chemically disintegrated. Moreover, there was a dislocation of the operculum during the heat treatment, as reported by Murugesan *et al*. (2015).

The 20°C storage condition maintained the viability of oil palm seeds for up to 15 months, with a germination rate of 50.00% (*Table 1*). However, the number of deteriorated seeds (*i.e*., those with rotten tissues) had also increased from 7.00% to 37.00% to 42.00%, as the storage period increased from 5 to 15-20 months, respectively (*Table 2*). Oil palm seeds also lost their ability to germinate entirely, after storage for up to 20 months, mainly due to a loss of viability (52.00% non-viable seeds) and the inner deterioration of the seeds (42.00% deteriorated seeds). These might be due to the oily endosperm, which readily decomposes and can be destroyed by fungi. Graeber *et al*. (2012) also reported that while the seed dormancy release occurs after ripening, the prolonged periods of seed storage and high oxidative stress can also lead to a gradual breakdown of proteins and nucleic acids, resulting in a loss of viability. In addition, treatment combination of storage and heat treatment significantly increased the germination capacity of the oil palm seeds and

drastically reduce the time, labour, and fuel used *vis-à-vis* the traditional 80-day germination campaigns usually carried out at the many oil palm research station (Addae-Kagyah *et al*., 1988).

Effect of Heat Treatment on ABA Contents of Stored Oil Palm Seeds

In this study, the ABA contents were analysed using the LC-MS method because it is effective for analysing trace samples in plant hormones (Wang *et al*., 2020). Previously, the application of LC-MS to analyse the endogenous hormones in oil palm seeds during fruit ripening has been first reported by Teh *et al*. (2014). They injected 0.3 mL sample into LC-MS, while our study used 10 mL sample injected into LC-MS. Later, Wang *et al*. (2019) determined the level of endogenous phytohormones (ABA, gibberellins, ethylene, cytokinins, auxins, and jasmonic acid) in the oil palm embryos during seed germination using LC-MS/MS system. They reported a rapid decrease of endogenous ABA to about 9.00% of oil palm embryos at 30 d and then stayed at deficient levels until germination. In addition, 2 mL sample injected into LC-MS was reported by Wang *et al*. (2019). It showed that the injection sample to LC-MS depends on the sample amounts, the extraction method, and the plant developmental stage. However, although LC-MS is a powerful tool, it has several limitations which include extra high costs, highly skilled operation, high time consumption, and multi-step sample preparation (Su *et al*., 2018).

Quantifying ABA contents in oil palm seed stored at 20°C for 0, 3, 6, 9, 12 and 15 months and then releasing the dormancy by heat treatment at 40°C for 60 days was successfully performed. The results showed increased ABA content in both endosperms and embryos when the storage time was increased. Heat treatment has directly delayed the increase of the ABA contents of embryos and endosperms during storage. The ABA content of embryos was found lower than endosperms (*Figure 6*).

ABA in oil palm seeds declines during germination, causing a natural dormancy release (Wang *et al*., 2019). Differently, the results in this study indicate that the accumulation of ABA occurred during long-term storage of 20°C and decreased by heat treatment to release the dormancy. For oil palm seeds, the temperature of 20°C is unfavourable for germination, due to the delay or inhibition of metabolism in the seed during storage. This condition suggests that the ABA contents also increased after the storage time progressed (*Figure 6*). The low temperature might have caused some stress to the seeds, which triggered the accumulation of ABA. Moreover, heat treatment to release the oil palm seed's dormancy after storage somewhat reduced the ABA content. Additionally, the increased germination of stored seeds without heat treatment (*Figure 1*) after 12 and 15 months has therefore indicated that the oil palm seed might also require a chilling period to release the natural dormancy or complete its germination. Similarly, Chen *et al*. (2010) have reported that the seeds of *Phellodendron amurense* var. *wilsonii* (Rutaceae), the medicinal tree, require chilling to break the dormancy and promote germination. They also found that ABA content is increased

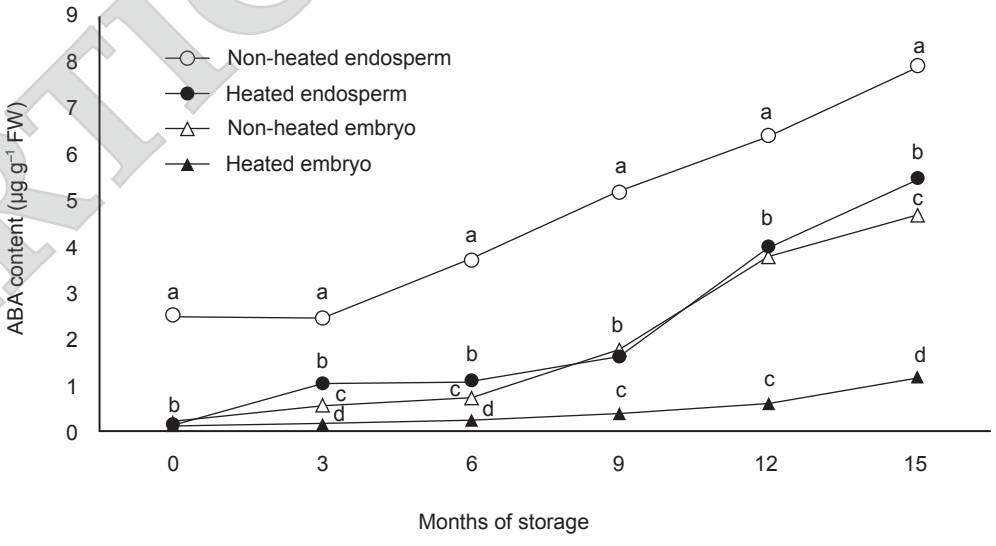


Figure 6. Changes of ABA contents in endosperm and embryo of oil palm seeds after heat treatment (40°C for 45 days) and stored at 20°C at different times.

when stored at low temperatures, incubated at 35°C/10°C, or cold stratification at 4°C for 12 weeks prior to incubation, hence breaking the seed's dormancy and promoting germination (Chien *et al*., 2006). Oil palm and *Phellodendron*, have oppositely different methods with regard to breaking the seed's dormancy. *Phellodendron* requires cold stratification, whereas the oil palm seed needs heat treatment. In addition, breaking dormancy by heat treatment is more effective for the oil palm seeds which need to be stored longer, indicating an orthodox storage behaviour. This phenomenon confirmed that the oil palm seeds are semi-recalcitrant, and the achievement of seed germination is complex and requires lengthy treatments and unique installations (Tristan and Cochard, 2005).

Finally, it should be emphasised that this research has established a germination protocol that has effectively improved the quantity and speed of the germination of stored oil palm seeds, hence offering a more practical and economical approach for commercial production. This study is the first report on breaking the dormancy of oil palm seeds by heat treatment to decrease the ABA content after long-term storage at low temperatures. However, future studies should include determining the period of the chilling requirement during the long-term storage, changes of ABA content during 49 days of germination, and comparison between non-dormant and dormant seeds to explain the precise role of ABA on dormancy of oil palm seeds.

CONCLUSION

The current study has demonstrated that the dry heat treatment at 40°C for 45 days has succeeded in releasing the dormancy of the sub-PSU1 oil palm seeds stored at 20°C for long-term storage, up to 15 months after harvesting. Long-term storage at 20°C had delayed the increase of ABA accumulation in the oil palm seeds, while heat treatment directly decreased the ABA content of oil palm seeds after storage. This method will be practically used for the sub-PSU1 oil palm seeds, after 5-15 months of storage, for awaiting commercial ordering or for use in breeding programmes. Further research is required to compare the effect of heat treatment at 40°C for 45 days towards breaking the dormancy of the long-term stored seeds at low temperatures, with other *tenera* oil palms on a commercial scale.

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REFERENCES

Addae-Kagyah, K A; Osafo, D M; Olympio, N S and Atubra O K (1988). Effect of seed storage, heat pretreatment and its duration on germination and growth of nursery stock of the idolatrica palm, *Elaeis guineensis* var. *idolatrica* (Chevalier). *Tropic. Agri., 65*: 77-83.

Ali, F; Qanmber, G; Li, F and Wang Z (2022). Updated role of ABA in seed maturation, dormancy, and germination. *J. Adv. Res., 35*: 199-214. DOI: 10.1016/j.jare.2021.03.011.

Baskin, J M and Baskin, C C (2014). What kind of seed dormancy might palms have?. *Seed Sci. Res. 24*: 17-22: DOI: 10.1017/S0960258513000342.

Chen, S-Y; Chien, C-T; Baskin, J M and Baskin, C C (2010). Storage behavior and changes in concentrations of abscisic acid and gibberellins during dormancy break and germination in seeds of *Phellodendron amurence* var. *wilsonii* (Rutaceae). *Tree Physiol., 30*: 275-284. DOI: 10.1093/treephys/tpp111.

Chien, C T; Chen, S Y; Chang, S H and Chung, J D (2006). Dormancy and germination in seeds of the medicinal Asian species *Phellodendron amurence* var. wilsonii (Rutaceae). *Seed Sci. Technol., 34*: 561-571. DOI: 10.15258/sst.2006.34.3.03.

Dickson, D A O; Daniel, A-D; Johnny, S O; Samuel, O A; Samuel, B and Claude, B (2021). Germination of oil palm (*Elaeis guineensis* Jacq.) seeds: A function of heat treatment and progeny. *Plant Cell Biotech. Mol. Biol., 22*: 59-66.

Duangpan, S; Buapet, P; Sujitto, S and Eksomtramage, T (2018). Early assessment of drought tolerance in oil palm D×P progenies using growth and physiological characters in seedling stage. *Plant Genet. Res., 16*: 544-554. DOI: 10.1017/s1479262118000151.

Ellis, R H and Roberts, E H (1981). The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol., 9*: 373-409.

Eksomtramage, T (2011). *Oil Palm Improvement*. O.S. Printing House, Bangkok. p. 153-179.

FAO (2021). *World Food and Agriculture - Statistical Yearbook 2021*. Rome. DOI: 10.4060/cb4477en.

Finch-Savage, W E and Leubner-Metzger, G (2006). Seed dormancy and the control of germination. *New Phytol., 171*: 501-523. DOI: 10.1111/j.1469-8137.2006.01787.x.

Fondom, N Y; Etta, C E and Mih, A M (2010). Breaking seed dormancy: Revisiting heat-treatment duration on germination and subsequent seedling growth of oil palm (*Elaeis guineensis* Jacq.) progenies. *J. Agric. Sci., 2*: 101-110. DOI: 10.5539/jas.v2n2p101.

Graeber, K; Nakabayashi, K; Miatton, E; Leubner-Metzger, G and Soppe, W J J (2012). Molecular mechanisms of seed dormancy. *Plant Cell Environ., 35*: 1769-1786. DOI: 10.1111/j.1365-3040.2012.02542.x.

Green, M; Lima, W A A; de Figueiredo, F F; Atroch, A L; Lopes, R; da Cunha, R N V and Teixeira, P C (2013). Heat-treatment and germination of oil palm seeds. *J. Seed Sci., 35*: 296-301. DOI: 10.1590/S2317-15372013000300004.

Hogge, L R; Abrams, G D; Abrams, S R; Thibault P and Plesance, S (1992). Characterization of abscisic acid and metabolites by combined liquid chromatography-mass spectrometry with ion-spray and plasma-spray ionization techniques. *J. Chromatogr. A., 623*: 255-263. DOI: 10.1016/0021-9673(92)80365-2.

Junsawang, N; Eksomtramage, T; Norsakul, P; Buapuean, C; Kaewpradab, T; Tongkum, P; Sae-Yang, R and Rungninrut, T (2020). Assessment of the Sub-PSU1 oil palm variety potential yield at the abandoned paddy field: A case study of Songkhla Province. *J. Agri. Product., 2*: 37-49.

Jiménez, V N; Guevara, E; Herrera, J; Alizaga, R and Bangerth, R (2008). Changes in hormone concentrations during dormancy release of oil palm (*Elaeis guineensis*) seeds. *Seed Sci. Technol., 36*: 575-587. DOI: 10.15258/sst.2008.36.3.07.

Kittimorakul, J; Eksomtramage, T; Sunpapao, A and Chairin T (2020). Indication of oil palm (*Elaeis guineensis* Jacq.) resistance to *Curvularia* leaf spot disease by PR-proteins productivity ability. *J. Oil Palm Res., 32*: 464-470. DOI: 10.21894/jopr.2020.0033.

Kittimorakul, J; Sunpapao, A; Duangpan, S; Anothai, J and Eksomtramage, T (2019). Screening of oil palm ('*Elaeis guineensis*' Jacq.) varieties for resistance to '*Curvularia*' leaf spot disease. *Aust. J. Crop Sci., 13*: 507-512. DOI: 10.21475/ajcs.19.13.04.p1276.

Kok, S Y; Ong-Abdullah, M; Ee, G C L and Namasivayam, P (2015). A histological study of oil palm (*Elaeis guineensis*) endosperm during seed development. *J. Oil Palm Res., 27*: 107-112.

Kucera, B; Cohn, M A and Leubner-Metzger, G (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res., 15*: 281-307. DOI: 10.1079/SSR2005218.

Maquiné, T M; Cysne A Q; de Lima, W A A; Abreu, S C; Green, M and de Almeida Rios, S (2014). Germination of seeds of interspecific hybrid Caiaué x oil palm submitted to the mechanical depulping. *Am. J. Plant Sci., 5*: 2965-2972. DOI: 10.4236/ajps.2014.520313.

Martine, B M; Laurent, K K; Pierre, B J; Eugène, K K; Hilaire, K T and Justin, K Y (2019). Effect of storage and heat treatments on the germination of oil palm (*Elaeis guineensis* Jacq.) Seed. *Afr. J. Agric. Res., 4*: 931-937.

Murugesan, P; Mathur, R K; Pillai, R S N and Babu, M K (2005). Effect of accelerated aging on seed germination of oil palm (*Elaeis guineensis* Jacq. var. *dura* Becc.). *Seed Technol., 27*: 108-112.

Murugesan, P; Ravichandran, G and Shareef, M (2015). Seed germination and ultrastructural changes in oil palm (*Elaeis guineensis*) hybrid seed influenced by heat treatments. *Indian J. Agric. Sci., 85*: 1419-1423.

Murugesan, P; Vanangamudi, K and Umarani, R (2002). Evaluation of the viability of oil palm (*Elaeis guineensis* Jacq.) seeds by tetrazolium test and comparison with germination and *in vitro* culture results. *Proc. of the 15th Plantation Crop Symposium Placrosym XV 2002*. Mysore, India. p. 246-250.

Myint, T; Chanprasert, W and Srikul, S (2010). Germination of seed of oil palm (*Elaeis guineensis* Jacq.) as affected by different mechanical scarification methods. *Seed Sci. Technol., 38*: 635-645. DOI: 10.15258/sst.2010.38.3.11.

Norsazwan, M G; Puteh, A B and Rafii, M Y (2016). Oil palm (*Elaeis guineensis*) seed dormancy type and germination pattern. *Seed Sci. Technol., 44*: 15-26. DOI: 10.15258/sst.2016.44.1.14.

Norsazwan, M G; Sinniah, U R; Puteh, A B; Namasivayam, P; Mohaimi, M and Aminuddin, I A (2020). Temperature fluctuation improves oil palm (*Elaeis guineensis*) *dura* x *pisifera* seed germination. *Seed Sci. Technol., 48*: 49-55. DOI: 10.15258/sst.2020.48.1.07.

Office of Agricultural Economics (2022). Agricultural Statistics of Thailand 2021. https://www.oae.go.th/assets/portals/1/files/jounal/2565/yearbook2564.pdf, accessed on 23 May 2022.

Ravichandran, G; Murugesan, P; Kumar, P N; Mathur, R K and Ramajayam, D (2016). Effect of chemicals on disintegration of the operculum in oil palm (*Elaeis guineensis*) seeds for early germination. *Seed Sci. Technol., 44*: 475-485. DOI: 10.15258/sst.2016.44.3.16.

Rees, A R (1962). High-temperature pre-treatment and the germination of seed of oil palm, *Elaeis guineensis* (Jacq.). *Ann. Bot., 26*: 569-581.

Su, Y; Li, W; Huang, Z; Wang, R; Luo, W; Liu, Q; Tong, J and Xiao, L (2018). Sensitive and high throughput quantification of abscisic acid based on quantitative real time immune-PCR. *Plant Methods, 14*: 104. DOI: 10.1186/s13007-018-0371-y.

Sukdee, T; Te-chato, S and Yenchon, S (2022). Effect of sodium chloride on growth and morphological responses of oil palm callus cv. SUP-PSU1. *Khon Kaen Agr. J., 50*: 810-818. DOI: 10.14456/kaj.2022.70.

Tabi, K M; Ebongue, G F N; Ntsomboh, G N and Youmbi, E (2017). Effect of dry heat treatment along with some dormancy breaking chemicals on oil palm seed germination. *South African J. Bot., 112*: 489-493. DOI: 10.1016/j.sajb.2017.06.023.

Teh, H F; Neoh, K; Wong, Y C; Kwong, Q B; Ooi, T E K; Mei Ng, T L; Tiong, S H; Low, J Y S; Danial, A D D; Ersad, M A; Kulaveerasingam, H and Appleton, D R (2014). Hormones, polyamines, and cell wall metabolism during oil palm fruit mesocarp development and ripening. *J. Agric. Food Chem., 62*: 8143-8152. DOI: 10.1021/jf500975h.

Tristan, D-G and Cochard, B (2005). Oil palm seed distribution. *OCL-Oilseeds Fats Crops Lipids, 12*: 148-153. DOI: 10.1051/ocl.2005.0148.

Wang, Y; Htwe, Y M; Li, J; Shi, P; Zhang, D; Zhao, Z and Ihase, L O (2019). Integrative omics analysis on phytohormones involved in oil palm seed germination. *BMC Plant Biol., 19*: 363. DOI: 10.1186/s12870-019-1970-0.

Wang, L; Zou, Y; Kaw, H Y; Wang, G; Sun, H; Cai, L; Li, C; Meng, L-Y and Li, D (2020). Recent developments and emerging trends of mass spectrometric methods in plant hormone analysis: A review. *Plant Methods, 19*: 54. DOI: 10.1186/s13007-020-00595-4.